A review of Chinese *Cordyceps* with special reference to Nepal, focusing on conservation

Bikash Baral[1]*, Bhushan Shrestha[2,3], Jaime A. Teixeira da Silva[4]*****

[1]Faculty of Agriculture and Forestry, University of Helsinki, P.O. Box 27, Latokartanonkaari 7, 00014, Finland
[2]Institute of Life Science and Biotechnology, Sungkyunkwan University, Suwon 440-746, Republic of Korea
[3]Green Energy Mission/Nepal, Ghatte Kulo, Anam Nagar, Kathmandu, P.O. Box 10647, Nepal
[4]P.O. Box 7, Miki-cho post office, Ikenobe 3011-2, Kagawa-ken, 761-0799, Japan

*Corresponding author, E-mail: bikash.baral@helsinki.fi
**Corresponding author, E-mail: bhushanshrestha2004@yahoo.com
***Corresponding author, E-mail: jaimetex@yahoo.com

Abstract

The Chinese caterpillar fungus, *Ophiocordyceps sinensis* (syn. *Cordyceps sinensis*) is an endemic species to alpine sub-meadow habitats of Nepal Himalaya and the Tibetan Plateau. Owing to its crucial and elusive medicinal attributes and known rarity and availability in the high Himalayas, extraction and exploitation of this fungus has caused extreme biotic pressure on the Himalayan alpine meadows of Nepal. Changes in micro-climatic conditions, unprecedented collection intensity and profound economic dependence of rural communities certainly affect stages of the life-cycle of this fungus, which ultimately calls for sustainable resource management. Several studies strongly indicate that this fungus and its as yet unidentified relatives possess high amounts of potentially bioactive chemical compounds, endowing it with medicinal properties. These issues, together with its natural historical attributes, harvesting techniques, and an understanding of the genetic diversity and genesis of *O. sinensis* would provide important clues regarding its evolution as well as needed information for *in situ* and *ex situ* conservation of this fungus. Molecular analyses and the development of microsatellite markers would allow for the authentication of this medicinal fungus, differentiating it from several closely related *Cordyceps* species, thus preventing falsification and discouraging illicit trade and the marketing of available counterfeits. This review highlights the importance of this fungus through studies on its status, diversity, ecological niches, socio-economic, administrative and mycological perspectives, and practices that have been and should be performed for its effective sustainable management in the highly diverse Nepal Himalayas.

Key words: biodiversity conservation, *Cordyceps sinensis*, ecological niche, ecological constraints, endemic entomopathogenic fungi, fungus *cum* larvae, Himalaya, *Ophiocordyceps*, Yarsagumba.

Ecology

Ophiocordyceps sinensis (Berk.) G.H. Sung, J.M. Sung, Hywel-Jones & Spatafora [syn. *Cordyceps sinensis* (Berk.) Sacc.] is colloquially known as caterpillar fungus (Winkler 2005; Winkler 2008) or Chinese caterpillar fungus (Shrestha et al. 2010) in English and Yarsagumba (fungus *cum* larvae) in Nepali (Shrestha et al. 2010; Shrestha 2011). Indigenous people in some parts of Nepal simply call it Bu (fungus *cum* larvae). Besides the name Yarsagumba, it is known in different parts of Nepal such as Bhu-Sanjivani, Jivan Buti, Jingani, Kira Chhyau, Kira Jhar, Saram Buti Jadi and Saram Buti (Shrestha et al. 2010). This fungus infects and eventually kills Lepidopteran larvae of about 60 different species (Chu et al. 2004; Wang, Yao 2011), usually that of the Himalayan bat moth *Hepialus armonicanus* (Gao et al. 2003; Holliday et al. 2005; Wang, Yao 2011). *O. sinensis* is rare and endemic to the high Himalayas and Tibetan Plateau and is immune to extreme cold alpine pastures (Kinjo, Zang 2001; Boesi 2003; Winkler 2008), and very thin oxygen (50 to 60% thinner than in oceans). It is an entomophagous fungus (Stone 2008; Zhao et al. 2013) and belongs to the Ophiocordycipitaceae family in the order Hypocreales of the Ascomycota (Sung et al. 2007).

Harboring enormous medicinal properties with promising bioactive compounds that are in high demand (Wasser 2002), *O. sinensis* has and continues to be harvested at an alarming rate (Negi et al. 2006). Since the Himalayan ecosystems are fragile, being surrounded by and interspersed with towering mountain ranges, the prompt conservation measures for wild populations of *O. sinensis* and also an effective, abundant and sustainable supply of this natural resource are required (Yang 1999; Negi et al. 2006). This review highlights the status, diversity and conservation of *O. sinensis* under natural and laboratory conditions along with its sustainable management, with the objective of conservation (*in situ* and *ex situ*) and proper utilization of this resource.

Uses and ethno-mycological practices

The caterpillar-fungus complex has been used traditionally, but not exclusively, for decades to design various pharmaceutical products (Winkler 2008) and their derivatives (Zhu et al. 1998a, 1998b). Because of its highly nutritive and medicinal properties, it is considered as the single-most expensive raw material used in Oriental Medicine around the world (Holliday, Cleaver 2008). It can be consumed cooked with aged duck to treat patients suffering from cancer and asthenia, or cooked with hen's meat to treat hyposexuality and male impotence, especially emission (Jiang 1994). Moreover, it is cooked with pork, sparrow and turtle to treat fatigue (Miller 2009). The hand-collected caterpillar-fungus complex is valued by herbalists as a source of libido and a genuine specimen is used as an aphrodisiac and also as an elixir (Boesi 2003; Winkler 2010). It is widely considered as "Himalayan Viagra". The tonic and powders made from Yarsagumba are sold as remedies for conditions ranging from asthma and chronic bronchitis to impotence. Wild *Cordyceps* and laboratory produced mycelial formulations have enormous uses in Oriental medicines due to the higher range of bioactive compounds, ranging from aphrodisiac to immune-modulating properties (Table 1). As a result of its high efficacy and potency in curing various diseases, this fungus has been recommended by medicinal practioners as a "Panacea of all ills". Moreover, medicinal products of *Cordyceps* origin are not commercially available and not commonly used in Nepal, even today. The accumulation of bioactive metabolites like cordycepin and cyclosporine (Fig. 1) by insects is well documented (Brown, Trigo 1994). Cordycepin has also been isolated from the aqueous extract of *O. sinensis* (Yoshikawa et al. 2004; Wu et al. 2007) and is similar to adenosine (Yue et al. 2008). Cyclosporin is an immunosuppressant and may cause nervous system issues and promote the build-up of harmful lipids like cholesterol in the vascular system, while *O. sinensis* may counteract some of cyclosporin's negative effects (Xu et al. 1995).

O. sinensis has thus a rich and documented repertoire of medicinal properties.

Life cycle and growth

In nature, *O. sinensis* is strictly endemic to the alpine shrub-meadow zone and grasslands of the high Himalayas or the highlands of Northern Nepal, ranging from 3 000 m above sea level, depending on the location, up to the snowline (Li et al. 2002; Yao 2004; Winkler 2008; Shrestha et al. 2010), where average annual precipitation is at least 350 mm and usually more than 400 mm (Namgyel, Tshitila 2003; Winkler 2008). Recent studies have indicated 27 mountain districts of Nepal as having rich ecological diversity enriched with this caterpillar fungus (Shrestha, Bawa 2013). It exhibits a fascinating life cycle in which it parasitizes various root-boring *Thitarodes* (*Hepialus*) caterpillars (Wang, Yao 2011). The choice of *O. sinensis* host depends on the altitude at which they are found and include 57 recognizable potential host species (Wang, Yao 2011). This fungal spore, after adhering to the insect's surface, invades and proliferates as the caterpillar hibernates underground during winter (Hajek, Leger 1994). Were the caterpillars not forestalled by the fungus, they would produce ghost moths, also known as bat moths or swift moths. The normal life cycle lasts from 2 to 6 years, mostly as a caterpillar, depending on the species and environmental conditions, and the whitish adult moth lives for hardly a few days in order to mate (Chen et al. 2002; Winkler 2008). Uninfected larvae generally hibernate deeper in the soil than infected larvae, which become less active in 6 to 10 days; the fungus drives the infected host to 2 – 5 cm beneath the soil surface leading to and making conditions favorable for fruiting (Li et al. 2002; Cannon et al. 2009; Zhang et al. 2012b).

Hyphal development occurs inside the body of the caterpillar immediately after infection, eventually mummifying the complete host (Stone 2008; Xing, Guo 2008) by circulating mycotoxins such as enniatin, HC-toxin (a cyclic tetrapeptide) and gliotoxin, especially in *Cordyceps militaris* (Zheng et al. 2011), throughout the larval body. The infected larvae die in 15 to 25 days. Over time, the interior of the infected caterpillar (organs along with other parts) converts into a sclerotium surrounded by an exoskeleton, allowing it to survive the upcoming winter (Wang, Yao 2011). Fruiting generally starts in early spring (April-July), rupturing the host body and developing directly from the dorsal surface of the prothorax, which is the corpse of a parasitized ghost moth larva, or a larva's fontanel (Winkler 2008), following over-wintering. It now forms sexual structures known as stroma that emerge from the dead host (Paterson 2008; Wang, Yao 2011). The slender, greyish, club-shaped fruiting body emerges from the ground with a total length (5 to 16 cm) approximately twice as long as its host (Winkler 2008; Chen et al. 2013; Lo et al. 2013). After maturation, the fungal fruiting body, which develops from the fontanel part of the larva, then forcefully discharges its spores to the surroundings with the purpose of infecting

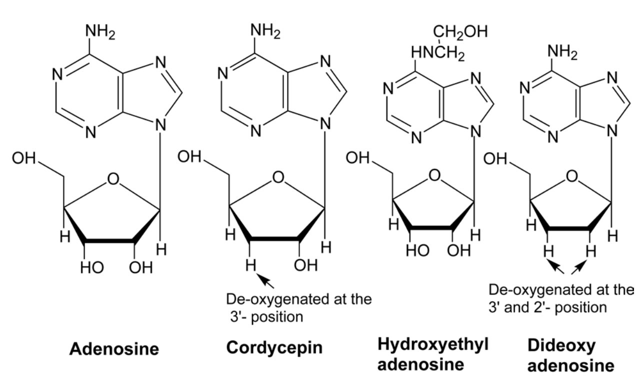

Fig. 1. Different bioactive chemicals found in *Cordyceps*.

Table 1. Potential pharmaceutical uses of *Ophiocordyceps sinensis*

Biological activity	Reference
Anti-aging, pro-sexual, anti-cancer, hyperlipidaemia, pharmacokinetic effect	Borel 2002; Shin et al. 2003; Wang et al. 2005; Nakamura et al. 2006; Negi et al. 2006; Liu et al. 2008; Khan 2010; Ding et al. 2011; Liu et al. 2010b; Pao et al. 2012
Virility (consumed usually in conjunction with other medicines)	Devkota 2006; Winkler 2008
Anti-viral activity, anti- leukemic	Chen et al. 1997; Yoshikawa et al. 2011
Immuno-modulating, antioxidant, and anti-fibrotic activities, cholesterol-reducing	Wang et al. 2005; Kuo et al. 2007; Dong, Yao 2008; Miller 2009; Zhang et al. 2011; Zhang et al. 2012a; Yao et al. 2014
Improves blood circulation and stabilization of blood sugar metabolism	Berne 1980; Winkler 2008; Zhou et al. 2008
Potential to increase stamina and libido	Canney 2006; Holliday, Cleaver 2008; Seth et al. 2014
Sterols and proteins (prevent chronic kidney inflammation, lupus erythematosus and asthma, and increase the contractile ability of the atrium and ventricle); stimulate corticosteroids production	Wang et al. 1998; Mizuno 1999; Kondrashov et al. 2012; Qian et al. 2012
Fungus has a diuretic effect (crucial for nephralgia)	Chiou et al. 2000
Has potential to adjust protein metabolism, inhibit lung carcinoma, replenish kidneys, soothe lungs	Xing, Guo 2008
Increases superoxide dismutase (functions as a free radical scavenger, reduces oxidation and aging of cells, and anti-inflammation) activity by reducing the formation of free radicals	Mizuno 1999
Delays cognitive decline	Kawas et al. 1997; Sherwin 1999
Protective effect against ischemia-induced brain infarction (modulating 17β-estradiol production)	Liu et al. 2010a
Increases ATP levels in the body (almost 28%)	Miller 2009
Chronic coughs, chronic bronchitis, insomnia, hypertension, and provides endurance, vitality and longevity	Zhu et al. 1998b
Treats pulmonary emphysema, anemia, night sweats	Zhu et al. 1998a; Xing, Guo 2008; Yue et al. 2008
Myocardial mitochondrial ATP generation	Paterson 2008; Jia et al. 2009
Health boosters, debilitating asthma, respiratory diseases	Xing, Guo 2008
Consumptive coughs with haematemesis, prevention of cardiac arrhythmias, heart and liver diseases	Pelleg, Porter 1990; Zhu et al. 1998b
Impotence, spermaturia, lumbar and knee pains	Chinese Pharmacopoeia Commission 2005
Hypocholesterolemic	Koh et al. 2003
Potent hypoglycemic activity in genetic diabetic mice (after intraperitoneal administration)	Kiho et al. 1996; Zhang et al. 2006
Blood-lipid lowering effects	Wasser, Weis 1999
Aqueous extract of *O. sinensis* is used to prevent tumour metastasis in mice as an adjuvant agent in cancer chemo-therapy	Nakamura et al. 2003
Effective against tuberculosis and leprosy	Miller 2009
Improves the internal balance mechanism (enhances oxygen utilization)	Miller 2009
Preventive remedy	Kuo et al. 2005
Prevent memory impairment related to Alzheimer's disease	Chiba 2010

continued

Table 1. *continued*

Biological activity	Reference
Ethanolic extract fraction is known to possess anti-tumourous activity of four cancer cell lines (MCF-7 breast cancer, B16 mouse melanoma, HL-60 human premyelocytic leukemia and HepG2 human hepatocellular carcinoma	Wu et al. 2007
Effects on coronary and cerebral circulation	Chen et al. 1997
Anti-neoplastic therapeutic agent	Khan 2010
Cerebroprotective effect (boosting defense against cerebral ischemia by increasing the activity of antioxidants related to lesion pathogenesis, antioxidant changes reflect an altered redox balance	Miller 2009; Liu et al. 2010a
Hyposexuality (especially emission)	Jiang 1994
Treating fatigue	Miller 2009
Male impotence, and hyposexuality	Jiang 1994
Water extract cures for remedy from taxol-induced leukopenia	Liu et al. 2008
Accelerates osteogenic and haematopoietic differentiation	Liu et al. 2008
Hyptotensive and vasorelaxant activities	Chiou et al. 2000
Possess tumour growth inhibitors	Kuo et al. 1994; Zhang et al. 2005; Wong et al. 2010; Jeong et al. 2011
Cytotoxic activities	Jing et al. 2005; Jia et al. 2009

nearby caterpillars as hosts for the next generation (Winkler 2008). Choice and shift in host preferences may occur due to overlap in feeding habitat or microhabitat (Shaw 1988). Furthermore, the medicinal properties of this fungus are supposed to be enhanced by close habitation of highly-valued plants like *Aconitum* spp., *Rhododendron setosum*, *Rhododendron anthopogon*, *Delphinium* spp., *Nardostachys* sp., *Neopicrorhiza* sp., *Primula* spp., *Lagotis* sp., Kutki (*Neopicrorhiza scrophulariiflora*), Jatamansi (*Nardostachys grandiflora*), *Pedicularis* spp., Bukiphool (*Anaphalis* sp.), *Rheum alexandrae, Meconopsis horridula* (Wu 1997; Zang, Kinjo 1998) and Pennsylvania smartweed (*Polygonum pensylvanicum*) (Chee-Sanford 2008). As plants along with their seeds occur in the fungal colonized zone, it might be speculated that fungus has roles in relation to plant preservation and that it ultimately contribute to seed bank longevity (Chee-Sanford 2008).

Harvesting techniques and season

The main harvesting period ranges from April to July in Nepal every year as high elevation grasslands spring to life, with slight variations in different geographic and microclimatic conditions (Adhikari 2008; Amatya 2008; Chhetri, Lodhiyal 2008; Winkler 2008). *O. sinensis* at higher elevation gradients tends to fruit later than at lower gradients (Winkler 2008). During the monsoon season, there is a seasonal migration of local human populations to the high Himalayas, where they search and forage for this unique fungus (Weckerle et al. 2010). Children are astute gatherers of *Cordyceps* because of their sharp eyes and proximity to the ground (Winkler 2008). Being tiny in size, and restricted to a very small area, the fungus is extremely difficult to find and harvest (Holliday et al. 2005). As this fungus does not fruit in groups and is highly dispersed, finding it becomes much more difficult. No sophisticated technology or capital are required for harvesting this fungus (Winkler 2005).

Economic value and production restraints

Cordyceps is a vital source of income and economic commodity, and commercial collection of *O. sinensis* is a crucial income-generating activity among communities living in Dolpa (53.3% of the total household; Shrestha, Bawa 2014a), Darchula and other districts of Nepal, mainly districts lying on the northern belt within the high Himalayas (Amatya 2008; Chhetri, Lodhiyal 2008). The major distribution of *O. sinensis* in Nepal is Dolpa (40% of the annual supply in Nepal), Darchula, Manang, Mustang, Humla, Gorkha, Rasuwa, Dolakha, Lamjung, Sindhupalchowk, Solukhumbu and Rukum (Adhikari 2008), while other places lying at high-altitudes await discovery. The fungus is being increasingly explored for commercial purposes, and thus faces the threat of rapid extinction (Winkler 2008).

The fungus is more valuable prior to sporulation or during early sporulation (Winkler 2010). In the final stages of fruiting, i.e. sporulation, the mushroom's upper part loses its robustness and weight (Buenz et al. 2005). Pricing is achieved by inspecting the size and firmness of the larval host (posterior part of the specimen), which is often tested by squeezing between two fingers; the stiffer it is, the higher the price (NRs 500; US$ 5.56 per specimen; Winkler 2008). Colours are also observed when pricing: a saturated yellowish-brown colour is preferred to paler colours (Chakraborty et al. 2014). Other physical characteristics taken into account when assessing quality are size, weight, smell, taste and robustness (Boesi 2003; Chakraborty et al. 2014). The odour of freshly collected specimens is relatively fleshy while the taste is bitter (Au et al. 2012). Persons who had collected 150 to 200 pieces (fungus with caterpillar as a whole) in 2011 could only find 2 to 3 pieces in 2012 in Darchula, and some could collect only 1 to 6 pieces of the herb a day in Dolpa, accentuating the alarming rate at which natural populations are disappearing (Chhetri, Gotame 2010). The legal trade was 3.1 kg in 2003, which peaked to 872 kg in 2008, and during this time the increase in royalty rate was from NRs 62 000 (US$ 800) to NRs 8 723 000 (US$ 112 554.83) (Chhetri, Gotame 2010). The total collected amount increased from 872 kg in 2008 to 1 560 kg in 2009 in Dolpa alone. Ironically, harvest is even more prevalent in the Gaurishankar Conservation area, Dolakha, Lamabagar. Moreover, in the period 1998 and 2008, the rate had increased dramatically, ranging from US$ 3 000 to 18 000 per kg for top quality material (up to 10-fold increment; Paul et al. 2009; Winkler 2013).

Nepal's Forest Act (1993) and Forest Regulations (1995) are the main legislative documents in place on protection of medicinal plants, including Yarsagumba. Due to heavy demand, this fungal specimen is transported at an alarming rate abroad. According to the Forest Act (1993), collection, marketing and distribution, carriage and export of *O. sinensis* was totally banned (penalty rate: NRs. 500 per piece; Forest Regulation Act 1995) until 2001. In addition, due to its wide popularity and conservation needs, the Government of Nepal issued a Yarsagumba stamp as the first wild mushroom series stamp in 1994 for the institutional strengthening of this species (Fig. 2). Furthermore, alarmed by the high harvest and illicit trade of this medicinal fungus in the Tibetan plateau, it was categorized as an endangered species under the second class of state protection by the Chinese Government (State Forestry Administration and Ministry of Agriculture of the People's Republic of China 1999). The HMGN (2001) endorsed it for trade abroad only in a processed form (with the approval from the Department of Forests; royalty rate: NRs. 20 000 per kg). Moreover, the next Gazette (HMGN 2004) endorsed it for trade abroad without processing (royalty rate: NRs. 20 000 per kg), although, due to continuous pressure, the rate was halved (royalty rate of NRs. 10 000 per kg; HMGN 2007). However, the laws were not amended thereafter.

The dependence on collection has now been reduced by a substantial amount through new biotechnological interventions, such as growing *O. sinensis* in artificial medium in the laboratory, allowing a unique profile of bioactive metabolites to be produced by employing fermentation technology (Sharma 2004; Siddique et al. 2011; Yan et al. 2014). Moreover, there are efforts underway in China to establish genetic databases for all *O. sinensis* strains (Dong, Yao 2012). Although *O. sinensis* possesses different phenotypes at different periods of its life cycle, its genotype remains unique during various growth stages, which can be identified by employing molecular biological tools such as RAPD or nrDNA sequence diversity (Buenz et al. 2005), RAPD-PCR (to study the relationship between *H. sinensis* and *O. sinensis*; Chen et al. 1999; Li et al. 2000) and probes based on ITS-region nrDNA sequences (ITS1, 5.8s and ITS2 nrDNA sequences; Chen et al. 2001). This topic is beyond the scope of this review.

Fig. 2. Stamp of *Cordyceps sinensis*.

Harvest permits, quantity and royalties

The annual collection of *O. sinensis* in Nepal during 2004 was 50 000 kg. Harvesting of *O. sinensis* decreased from 260 to only seven pieces per person in 2006 then increased to 125 pieces in 2010 (Awasti 2012). According to the data provided by Dolpa district, only 3.1 kg was marketed in 2002, reaching 872.4 kg in 2009, then plummeting to 473.8 kg in 2011. The national trade volume of *Cordyceps* throughout Nepal was 2 442.4 kg in 2009, which declined to 1 170.8 kg in 2011 (Shrestha, Bawa 2013). No data is available from 2012 onwards. This decline in populations may be attributed to various factors, including over-harvesting, decrease in moth and larval populations, microhabitat modifications, upsurge in grazing intensity, and to some extent climate change (Shrestha, Bawa 2013). Also, premature harvest, overgrazing, unavailability of host larvae and the impact of climate change at the Himalayan apex accelerate the worsening conditions (Gupta, Negi 2010; Shrestha, Bawa 2014b). All people, including children, venture out to collect this rare fungus, men making up two out of every three collectors (Weckerle et al. 2010), inducing increased competition and reduced individual harvest (Winkler 2008). In many places of Nepal, even outsiders are allowed to harvest the fungus, provided that they pay the collecting fee (Shrestha, Bawa 2013). In China, harvesters spend around 20 RMB (US$ 2.919) per person per season to hire guards to safeguard boundaries of their collection area and, at the end of the season when money becomes scarce, guards are organized by villagers on a rotational basis (Weckerle et al. 2010). Owing to lucrative economic return, poor rural communities have increased their gathering activities substantially, thus posing serious ecological consequences (Chakraborty et al. 2014) and threatening its existence. An increasing and unprecedented flow of harvesters, socio-economic strains creating tussles and conflicts between collectors, continuous theft at sites, haphazardous collection of the prized herb, and climate change are all contributing to the loss of Himalayan biodiversity with an increased scarcity of Yarsagumba (Shrestha, Bawa 2013). Recently, several incidences claiming the lives of two people took place involving severe disputes among local communities and the police (or National Park Buffer Zone Management Committee) in Dolpa, which serves as a major warehouse of *Cordyceps* (The Record 2014). Also, in China, exact information on harvesting frequency and management system is blurred, but Bhutan has been able to effectively address and institutionalize, at a national level, a management strategy for harvesting *Cordyceps* (Cannon et al. 2009). Moreover, due to the encroachment of brokers at collection sites, research on consequences of intensive harvest of this rare species is noticeably lacking and, if present, remains understandably concealed (Thapa et al. 2014). About six years ago, a committee of community leaders in Dolpa instituted a taxation system on harvesters in an effort to control numbers, ensure that the local communities remain resilient amidst environmental transformations, and that these groups continue to take care of harvesting areas for the sustainable management and conservation of *Cordyceps*. The Nepalese Government endorses the control of harvesting practices, and the generated income is collected at the government level and is used for the benefit of local communities (Shrestha, Bawa 2013).

On occasion, there may be upheaval and uprising in local economics due to the harvesting of *Cordyceps,* which may lead to negative consequences, as observed in Domkhok and its neighbors in China, where local nomadic pastoralists became more prosperous and sold their entire flock of sheeps (Sulek 2011). This certainly leads to a decrease in the dispersal of spores and consequently a decrease in *Cordyceps* populations in subsequent years (Sulek 2011).

Market channels, trade and supply chains of local collections

Commercial collection of natural resources is strongly influenced by regional and global markets as well as fluctuations in demand and supply (Weckerle et al. 2010). Thus, unsustainable wild life trade seems to be a major driver of biodiversity loss (Broad et al. 2003). In indigenous medicinal practices, wild plants (including fungi) are considered to have wider therapeutic benefits and thus command higher prices (Olsen, Helles 1997; Holliday, Cleaver 2008). The trading season generally starts from the beginning of April and lasts until the end of July (Boesi 2003). Market price, trade and channels of *O. sinensis* collection are not so clear in the Nepalese context, as commercial trading takes place illegally because of fear of being charged higher taxes (Banjade, Paudel 2008). In many scenarios, the local pastoralists underreport their harvesting intensity and willingly underrate their insignificant income. In general, collectors are mostly local inhabitants who deliver their collected products to nomadic brokers (often the villagers acts as brokers) and then to contractors, national brokers (mostly Tibetan) and finally to international brokers, and pharmaceutical companies (Shrestha et al. 2010; Shrestha, Bawa 2013).

Economics

There is no single explanatory factor attributed to the skyrocketing prices of Chinese *Cordyceps*. High altitudinal indigenous tribes depend on this fungus as the only income-generating and profitable natural resource, rendering a good earning for their sustainable livelihood. *O. sinensis* is by far the most profitable mushroom on the Nepalese Himalaya and Tibetan plateau. It is found in alpine meadows, which are used for livestock herding (Boesi 2003; Garbyal et al. 2004; Zhang et al. 2012b). In Darchula alone, the collection of revenue from the export of Yarsagumba in the 2011 fiscal year was around NRs. 7.3 million (US$ 81 111.11; Awasti 2012). Pricing of specimens generally differs according to the place, and superiority of samples collected, i.e., color and stiffness of larvae (Hsu et al. 2002; Li et al. 2006). The price of Chinese *Cordyceps* has risen dramatically in the Tibetan plateau (900% between 1998 and 2008; Winkler 2008) and in Kathmandu, the capital of Nepal (Li et al. 2006; Winkler 2008). In the 1980s, one kg of Yarsagumba cost US$ 1 800.00; in 1997 ca. 8 400 RMB (US$ 1 225.81) with an increment of 467% over the previous year; in 2004, ca. 36 000 RMB (US$ 5253.49) with an increment of 429% over the previous year; in 2005 its cost ranged from 10 000 to 60 000 RMB (US$ 1 459.30 to 8 755.80). The price increased dramatically from

1 800 RMB per kg (US$ 262.67; Chen, Chu 1996) in the Tibetan capital Lhasa, to 8 400 RMB (US$ 1 225.81; for average quality) in 1997 (a 366% increase), then to 36 000 RMB (US$ 5 253.48) in 2004 (a further increase of 1900%). In June 2005, prices in Tibet ranged from 10 000 to 60 000 RMB (US$ 1 459.30 to 8 755.80) per kg. The best quality fungus was marketed at up to Singapore Dollar (SG$) 130 000 per kg (RMB 599 918.5 per kg) in Singapore in March 2012 (Leng 2012), while in China the gold standard *Cordyceps* rate skyrocketed to US$ 140 000 per kg (RMB 936 446 per kg; Xuan 2012). Thereafter, there are no new statistics in the scientific literature. In Nepal, during 2007 and 2008, the price rose to NRs 800 000.00 (US $10 000; for average quality) and NRs 600 000.00 (US$ 7 500; http://www.kantipuronline.com/capsule.php?&nid=186165; last accessed on June 16, 2015; Daurio 2009), while at present, it costs ca. NRs. 600 000.00 (US$ 6 667) per kg, in China 40 000.00 RMB (US$ 6 296) and in the USA, US$ 32 000. One pound (0.454 kg) of the biggest Yarsagumba consists of 800 to 900 clean specimens (Winkler 2010). According to Thapa et al. (2014), in 2011, the market price of *Cordyceps* rose to US$ 25 000 per kg in Kathmandu. In Dolakha, a single piece of Yarsagumba costs NRs 50 (US$ 0.56) at the collection site, while in Lamabagar it yields NRs 100 (US$ 1.10) or even more. Dried specimens of Chinese *Cordyceps* are considered to be more expensive, especially during winter (Winkler 2005).

Laws related to the export of herbs and medicinal products like caterpillar fungus from Nepal are very complicated, forcing local people to sell these products illegitimately (Olsen, Helles 1997). Businessmen and highlanders very close to collection sites buy most of the products collected in the area because of the open borders with no obstacles, allowing flourishing of this business (Zhang et al. 2012b). Furthermore, due to strict rules, the collectors purposely hide the harvest intensity. In a Nepalese context, owing to the lack of sufficient awareness, estimated tons of herbs are being sold for the price of peanuts while the remaining products are being wasted without use because of the fear of being taxed (Olsen, Helles 1997; Kunwar et al. 2006). Thus, brokers usually prefer the Tibetan border to sell the herbs. Following harvest, the specimens should be immediately dried within three days to prevent the loss of vigour, colour, odour and taste, which otherwise would become totally useless (Gupta, Negi 2010). However, at alpine altitudes, it is rather difficult to dry the samples, and thus harvesters prefer to sell immediately after collection. Furthermore, there is little systematic research that has been conducted on ecological aspects of fungus harvesting and extraction, which threatens the long-term ecological sustainability of Yarsagumba (Shashidhar et al. 2013).

In situ and *ex situ* conservation issues of *O. sinensis*

Prevention of loss of natural habitat by designating a buffer zone helps to maintain the suitable environment for the fungal propagation and reduces chances of them being extinct (Varghese, Ticktin 2008). In harsh microbial ecosystems like alpine pastures, sustainable maintenance of the fungal larvae and spores is a major challenge and of utmost importance for sustainable harvest of *O. sinensis* (Devkota 2010; Zhang et al. 2012b). Due to increasing demand for this rare fungus, collectors are increasing exponentially, in sharp contrast to previous years, which makes the locations more vulnerable to various conflicts and inhumane activities (Garbyal et al. 2004). Diverse conservation strategies concentrating on the impact of harvesting for a short period in a year, collection of revenues and taxes, and management of buffer zone areas would provide economic benefits for not harvesting (Boesi 2003; Sharma 2004; Varghese, Ticktin 2008; Weckerle et al. 2010) while the use of more scientific techniques for proper harvesting, including spore dispersal immediately after collection or burying pits after uprooting, could aid sustainable management of the wild resources (Shrestha, Bawa, 2013). The majority of collectors (95.1%) believe that the intensity of *Cordyceps* has been greatly reduced each year (Shrestha, Bawa, 2014a), which warrants its immediate conservation. Creating rest areas may also help to maintain a stable level of *Cordyceps* populations. Also, knowledge of fungal reproduction and establishing an end-date to the collection season might allow for sufficient spore dispersal to maintain sustainable populations. However, lagging behind economically, Nepal is struggling to cope amid the economic challenges. There is increasing longing for high-value commodities like Yarsagumba, while maintaining, and sustainably guaranteeing it for future generations. In such a scenario, it is wise to enforce a law to provide local inhabitants with control over this resource by means of community-based conservation approaches.

With breathtaking leaps in the progress of modern science, several techniques and safeguards have been developed to shield rapidly declining species. Nevertheless, such techniques may be expensive, so the best way is to conserve the species in its natural habitat (*in situ* conservation of these natural wonders and their allies) is by protecting the original habitat. In such a scenario, scientific conservation and modern techniques with the assistance of scientists' expertise may help to manage the preservation of these habitats, thus preventing an ecological disaster. Also, the use of molecular approaches such as molecular markers, including ISSR-TAIL-PCR, may help in the study of population genetics and the conservation biology of *O. sinensis* (Wang et al. 2011).

Microbial species composition and diversity are often neglected, and there is a need to understand how these systems function. The medicinal properties of this rare fungus pique the interest of specialists and society alike and thus call for its effective conservation. Local collectors can be the best stewards of their resources, if they are well-versed, so they understand what is at stake. In Nepal, many of the medicinal fungi, including the caterpillar fungus, are under threat due mainly to habitat destruction, degradation and fragmentation, and overharvesting for commercial trade (Winkler 2008; 2010). These highly threatened species require immediate *ex situ* conservation.

The sustainability of current collecting practices remains undocumented; however, ecological concerns include, among others, the impact of harvesting on caterpillar fungus reproduction in nature, the improper disposal of waste and the removal of top soil during collection. Occasionally, deliberate fire is set to promote vegetation in spring, which ultimately kills both the host and fungal spores. Habitat destruction by uprooting of grasses during foraging, break-down of immature *Cordyceps* stroma, and prevention of spore dispersal are the main issues while harvesting natural *Cordyceps* (Garbyal et al. 2004). The extreme Himalayan apex receives intense UV-radiation, an inadequate oxygen supply and is a frigid environment, which adds to the complexity of life even for Himalayan species (Dong, Yao 2012). Moreover, proper harvesting plans, policies, adaptation, and their mitigation measures should be carefully planned, formulated and strictly adopted for harvesting *Cordyceps* resources. In a Nepalese context, data related to Chinese *Cordyceps* harvested annually seems to be insufficient, because of immediate selling of a bulk amount of the herb collected from the alpine meadows. Thus, potential locations of *Cordyceps* should be perfectly traced, mapped and well managed, in order to know the actual and potential changes in its ecological behavior and also preventing it from depletion. Scientific study of spore dispersal mechanisms in suitable localities and developing means of dispersing fungal spores should also be planned for proper management of this resource (Roy et al. 2006). Also, as legal harvesting is relatively new in Nepal, there is a dire need to formulate immediate plans and produce definite maps of potential areas for expansion of the caterpillar fungus, which may help to understand how harvesting areas cross social or cultural boundaries or various tenure regimes. The Nepalese Government still possesses a very poor monitoring and management stratagem for on-site (*in situ*) conservation of this species, even though a large tax (discussed above) is levied for the collection of this herb. Ironically, there is no definite set of laws under the jurisdiction of the Department of Forests for standardization of the licensing system for this species, which the government should have implemented to prevent it from exhaustion. Thus, the licensing system works better through the implementation of state-led rules and community-based management.

Modern *ex situ* conservation (a method of safeguarding genetic pools) techniques have so far been developed to preserve fungal spores either in dry (lyophilized) or in liquid (broth) forms (Verma 2011). Mass production of mycelia by bioreactors will also reduce stress on natural populations and provide material in a season-independent manner (Yan, Wu 2014). These techniques may help to relocate, to some extent, endangered species to their original habitats through culture techniques (if they disappear from their original habitats). Though these techniques have not yet been employed in Nepal, such conservation methods await discovery. *Ex situ* conservation may help to reduce the human dependency on valuable herbs such as Chinese *Cordyceps* in natural habitats, thus preventing it from becoming extinct. In developing countries like Nepal where natural biological resources are being heavily exploited, *ex situ* conservation is a promising goal.

Conclusions and future perspectives

Even though this species has contributed significantly to the domestic market industry, most of the collection intensity and trade channels remain fairly unknown. The depth and breadth of harvest of *Cordyceps* now taking place are unprecedented, and research is needed to determine what sort of fundamental management measures, if any, are required to ensure that this fungus remains bountiful for generations to come. The elucidation of *O. sinensis* host insects may impart a basic understanding for effective management strategies of insect resources and for the conservation and sustainable use of the fungus. Thus, when there is a decline in the moth's population, they should be promptly propagated by any means and reintroduced to the environment. Construction, agricultural cultivation, cattle grazing, human migration in high numbers and other activities rendering an imbalance to the ecosystem should be discouraged. Furthermore, increasing harvest pressure and the absence of reliable basal data clearly need more research to devise sound management strategies to secure the long-term survival of this fungus. Alternative income sources that are clearly defined for the support of indigenous people relying on *Cordyceps* should be formulated. Government-issued regulations and directives have only a reasonable chance of successful implementation when local communities are integrated into decision-making and their implementation. Due to its restricted localization, genetic erosion of this species is likely to occur (accelerated habitat fragmentation leading to shrinking gene pools, the major cause being the human activity), which needs to be addressed. More collaborative studies (domestic and international) are needed to ensure its socio-economic and ecological values. The ability of this fungus to restrain tumor growth, suppress cancerous cells and augment aspects of the immune system is established. More mechanism-based, disease-oriented pharmacological studies are required to ensure clinical applications, while other avenues of research on this important medicinal fungus could promote its conservation. Furthermore, adjuvant therapy of *O. sinensis* in immune function disturbances, cancer, and renal failure is a real possibility. More extensive and thorough studies of this fungus may result in the discovery of new medicinal strategies, although recent studies have provided new vistas on the biology and artificial cultivation of this fungus-insect partnership (Barseghvan et al. 2011; Yue et al. 2013; Zhang et al. 2014; Zhou et al. 2014).

Acknowledgements

The authors declare no conflicts of interest, financial or other.

References

Adhikari M.K. 2008. The diversity of Cordycepioid fungi (Ascomycotina: Clavicipitales) reported from Nepal. *Bull. Dept. Plant Resourc.* 30: 1–7.

Amatya G. 2008. Trade and socioeconomic attribution of *Cordyceps sinensis* (Berk) Saccardo in Darchula district, Nepal. In: Jha P.K., Karmacharya S.B., Chetri M.K., Thapa C.B., Shrestha B.B. (eds) *Medicinal Plants in Nepal: an Anthology of Contemporary Research.* Ecological Society, Kathmandu, pp. 195–203.

Au D., Wang L., Yang D., Mok D.K.W., Chan A.S.C., Xu H. 2012. Application of microscopy in authentication of valuable Chinese medicine I – *Cordyceps sinensis*, its counterfeits, and related products. *Microsc. Res. Tech.* 75: 54–64.

Awasti K. 2012. Yarchagumba mission comes unglued. The Kathmandu Post. http://www.ekantipur.com/the-kathmandu-post/2012/06/05/nation/yarchagumba-mission-comes-unglued/235703.html (last accessed: June 16, 2015).

Banjade M.R., Paudel N.S. 2008. Economic potential of non-timber forest products in Nepal: myth or reality? *J. Forest Livelihood* 7: 36–48.

Barseghvan G.S., Holliday J.C., Price T.C., Madison L.M., Wasser S.P. 2011. Growth and cultural-morphological characteristics of vegetative mycelia of medicinal caterpillar fungus *Ophiocordyceps sinensis* G.H. Sung et al. (Ascomycetes) isolates from Tibetan plateau (P.R. China). *Int. J. Med. Mushrooms* 13: 565–581.

Berne R.M. 1980. The role of adenosine in the regulation of coronary blood flow. *Circ. Res.* 47: 807–813.

Boesi A. 2003. The dbyar rtswa dgun'bu (*Cordyceps sinensis* Berk.): an important trade item for the Tibetan population of the Li Thang County, Sichuan Province, China. *Tibet J.* 28: 29–42.

Borel J.F. 2002. History of the discovery of cyclosporin and of its early pharmacological development. *Wien. Klin. Wochenschr.* 114: 433–437.

Broad S., Mulliken T., Roe D. 2003. The nature and extent of legal and illegal trade in wildlife. In: Oldfield S. (ed) *The Trade in Wildlife: Regulation for Conservation.* Earthscan Publications, London, UK, pp. 3–22.

Brown K.S.Jr., Trigo J.R. 1994. Multi-level complexity in the use of plant allelochemicals by aposematic insects. *Chemoecology* 4: 119–126.

Buenz E.J., Bauer B.A., Osmundson T.W., Motley T.J. 2005. The traditional Chinese medicine *Cordyceps sinensis* and its effects on apoptotic homeostasis. *J. Ethnopharmacol.* 96: 19–29.

Canney S. 2006. *Cordyceps sinensis* – animal, vegetable or both? *J. Chinese Med.* 80: 43–49.

Cannon P.F., Hywel-Jones N.L., Maczey N., Norbu L., Tshitila, Samdup T., Lhendup P. 2009. Steps towards sustainable harvest of *Ophiocordyceps sinensis* in Bhutan. *Biodivers. Conserv.* 18: 2263–2281.

Chakraborty S., Chowdhury S., Nandi G. 2014. Review on Yarsagumba (*Cordyceps sinensis*) – an exotic medicinal mushroom. *Int. J. Pharmacog. Phytochem. Res.* 6: 339–346.

Chee-Sanford J.C. 2008. Weed seeds as nutritional resources for soil Ascomycota and characterization of specific associations between plant and fungal species. *Biol. Fertil. Soils.* 44: 763–771.

Chen P.X., Wang S., Nie S., Marcone M. 2013. Properties of *Cordyceps sinensis*: a review. *J. Funct. Foods* 5: 550–569.

Chen S.J., Yin D.H., Zhong G.Y., Huang T.F. 2002. On the biology of adult parasite of *Cordyceps sinensis, Hepialus biruensis. Zhongguo Zhongyao Zaxhi* 27: 893–895.

Chen S.Z., Chu J.Z. 1996. NMR and IR studies on the characterization of cordycepin and 2'deoxyadenosine. *Zhongguo Kangshengsu Zaxhi* 21: 9–12.

Chen Y., Zhang Y.P., Yang Y., Yang D. 1999. Genetic diversity and taxonomic implication of *Cordyceps sinensis* as revealed by RAPD markers. *Biochem. Genet.* 37: 201–213.

Chen Y.J., Shiao M.S., Lee S.S., Wang S.Y. 1997. Effect of *Cordyceps sinensis* on the proliferation and differentiation of human leukemic U937 cells. *Life Sci.* 60: 2349–2359.

Chen Y.Q., Wang N., Qu L.H., Li T.H., Zhang W.M. 2001. Determination of the anamorph of *Cordyceps sinensis* inferred from the analysis of the ribosomal DNA internal transcribed spacers and 5.8s rDNA. *Biochem. Syst. Ecol.* 29: 597–607.

Chhetri R., Gotame B. 2010. Employment generation and economic up scaling from collection and trade of Yarsagumba (*Ophiocordyceps sinensis*) (Berk.) in Nepal. *Proceeding: Forest-People Interaction*. Pokhara, Nepal, UMB/ NUFU Project, Institute of Forestry, Pokhara, Nepal, pp. 13–19.

Chhetri R., Lodhiyal L.S. 2008. Collection of *Cordyceps sinensis* (Berk.) Sacc. (Yarsagumba) and its implications in rural livelihood and biodiversity conservation: a case of Darchula district, Nepal. In: Jha P.K., Karmacharya S.B., Chhetri M.K., Thapa C.B., Shrestha B.B. (eds) *Medicinal Plants in Nepal: An Anthology of Contemporary Research*. Ecological Society, Kathmandu, Nepal, pp. 214–223.

Chiba T., Yamada M., Torii K., Suzuki M., Sasabe J., Ito M., Terashita K., Aiso S. 2010. Effects of extracts from *Cordyceps sinensis* on MI muscarinic acetylcholine receptor *in vitro* and *in vivo. J. Receptor Ligand Channel Res.* 3: 97–104.

Chinese Pharmacopoeia Commission 2005. Herba Epimedii. In: Chinese Pharmacopoeia Commission (ed) *Chinese Pharmacopoeia, Part I*. Chemical Industry Press, Beijing, China, pp. 229.

Chiou W.F., Chang P.C., Chou C.J., Chen C.F. 2000. Protein constituent contributes to the hypotensive and vasorelaxant activities of *Cordyceps sinensis. Life Sci.* 66: 1369–1376.

Chu H.F., Wang L.Y., Han H.X. 2004. *Fauna Sinica.* Vol. 38, Lepidoptera: Hepialidae, Epiplemidae. Science Press, Beijing, pp. 291.

Daurio C.M. 2009. Exploring perspectives on landscape and language among Kaike speakers in Dolpa, Nepal. Master of Arts thesis. The University of Montana, Missoula, MT.

Devkota S. 2006. Yarsagumba (*Cordyceps sinensis* (Berk.) Sacc.); traditional utilization in Dolpa district, Western Nepal. *Our Nature* 4: 48–52.

Devkota S. 2010. *Ophiocordyceps sinensis* (Yarsagumba) from Nepal Himalaya: Status, Threats and Management Strategies. In: Zhang P.H.-W. (ed) *Cordyceps Resources and Environment*. Grassland Supervision Center, Ministry of Agriculture, People's Republic of China, pp. 91–108.

Ding C., Tian P.X., Xue W., Ding X., Yan H., Pan X., Feng X., Xiang H., Hou J., Tian X. 2011. Efficacy of *Cordyceps sinensis* in long term treatment of renal transplant patients. *Frontiers Biosci.* 3: 301–307.

Dong C.H., Yao Y. 2012. Isolation, characterization of melanin derived from *Ophiocordyceps sinensis*, an entomogenous fungus endemic to the Tibetan Plateau. *J. Biosci. Bioeng.* 113: 474–479.

Dong C.H., Yao Y.J. 2008. *In vitro* evaluation of antioxidant activities of aqueous extracts from natural and cultured mycelia of *Cordyceps sinensis*. *LWT Food Sci. Technol.* 41: 669–677.

Forest Act 1993. (2049 BS) Ministry of Forest and Soil Conservation. Forestry Development Project; HMGN/USAID.

Forest Regulation 1995. (2051 BS) Ministry of Forest and Soil Conservation, Forestry Development Project, HMGN/USAID.

Gao H.C., Gao J.Q., Xi Q.Y., Li X.H. 2003. Research and development for *Cordyceps sinensis. J. Microbiol.* 23: 50–55.

Garbyal S.S., Aggarwal K.K., Babu C.R. 2004. Impact of *Cordyceps sinensis* in the rural economy of interior villages of Dharchula sub-division of Kumaon Himalayas and its implications in the society. *Indian J. Trad. Knowledge* 3: 182–186.

Gupta R.C., Negi P.S. 2010. *Cordyceps sinensis* (Yarsa gamboo): a high value medicinal mushroom. Chapter 7. In: Maheshwari M.K., Dubey R.C., Saravanamuthu R. (eds) *Industrial Exploitation of Microorganisms*. International Publishing House Pvt. Ltd. New Delhi, India, pp. 107–128.

Hajek A.E., Leger R.J.C. 1994. Interaction between fungal pathogens and insect hosts. Annu. Rev. Entomol. 39: 293–322.

His Majesty's Government of Nepal, Ministry of Forests and Soil Conservation (HMGN). 2001. Nepal Gazette, 12th Feb. 2001 (Falgun 1, 2057).

His Majesty's Government of Nepal, Ministry of Forests and Soil Conservation (HMGN). 2004. Herbs and Non-Timber Forest Products Development Policy. Singha Durbar, Kathmandu, Nepal.

His Majesty's Government of Nepal, Ministry of Environment, Science and Technology (HMGN). 2007. Notice of the government. Nepal Gazette 2007, Khanda 57(19), 2064/5/3.

Holliday J., Cleaver M., Wasser S.P. 2005. *Cordyceps*. In: Coates P.M., Blackman M.R., Cragg G., Levine M., Moss J., White J. (eds) *Encyclopedia of Dietary Supplements*. New York, Marcel Dekker, pp. 1–13.

Holliday J.C., Cleaver M.P. 2008. Medicinal value of the caterpillar fungi species of the genus *Cordyceps* (Fr.) Link (Ascomycetes): a review. *Int. J. Med. Mushrooms.* 10: 219–234.

Hsu T.H., Shiao L.H., Hsieh C., Chang D. M. 2002. A comparison of the chemical composition and bioactive ingredients of the Chinese medicinal mushroom DongChonXiaCao, its counterfeit and mimic, and fermented mycelium of *Cordyceps sinensis*. *Food Chem.* 78: 463–469.

Jeong J.W., Jin C.Y., Park C., Hong S.H., Kim G.Y., Jeong Y.K., Lee J.D., Yoo Y.H., Choi Y.H. 2011. Induction of apoptosis by cordycepin via reactive oxygen species generation in human leukemia cells. *Toxicol. In Vitro* 25: 817–824.

Jia J., Tao H., Feng B. 2009. Cordyceamides A and B from the culture liquid of *Cordyceps sinensis* (Berk.) Sacc. *Chem. Pharm. Bull.* 57: 99–101.

Jiang S.J. 1994. A tonic application of *Cordyceps sinensis* in medicinal diets. *J. Chinese Medic. Mater.* 17: 43–47.

Jing M., Xiao C., Chun F.W., Li J.W., Gao H. 2005. Cordycedipeptide A, a new cyclodipeptide from the culture liquid of *Cordyceps sinensis* (Berk.) Sacc. *Chem. Pharm. Bull.* 53: 582–583.

Kantipur Staff Writer, "Yarsagumba Sale Takes a Nosedive," Kantipur, March 24, 2009, http://www.kantipuronline.com/capsule.php?&nid=186165 (last accessed on June 16, 2015).

Kawas C., Resnick S., Morrison A., Brookmeyer R., Corrada M., Zonderman A., Bacal C., Lingle D., Metter E. 1997. A prospective study of estrogen replacement therapy and the risk of developing Alzheimer's disease: the Baltimore longitudinal study of aging. *Neurology* 48: 1517–1521.

Khan M.D.A., Tania M., Zhang D., Chen H. 2010. *Cordyceps* mushroom: a potent anticancer nutraceutical. *Open Nutraceuticals J.* 3: 179–183.

Kiho T., Yamane A., Hui J., Usui S. , Ukai S. 1996. Polysacharides in fungi. XXXVI. Hypoglycemic activity of a polysaccharide (CS-F30) from the cultural mycelium of *Cordyceps sinensis* and its effect on glucose metabolism in mouse liver. *Biol. Pharm. Bull.* 19: 294–296.

Kinjo N., Zang M. 2001. Morphological and phylogenetic studies on *Cordyceps sinensis* distributed in Southwestern China. *Mycoscience* 42: 567–574.

Koh J.H., Kim J.M., Chang U.J., Suh H.J. 2003. Hypochlesterolemic effect showed hot-water extract of mycelia of *Cordyceps sinensis. Biol. Pharma. Bull.* 26: 84–87.

Kondrashov A., Meijer HA, Barthet-Barateig A, Parker HN, Khurshid A, Tessier S, Sicard M, Knox AJ., Pang L, de Moor CH. 2012. Inhibition of polyadenylation reduces inflammatory gene induction. *RNA* 18: 2236–2250.

Kunwar R.M., Nepal B. K., Kshhetri H.B., Rai S. J., Bussmann R. W. 2006. Ethnomedicine in Himalaya: a case study from Dolpa, Humla, Jumla and Mustang districts of Nepal. *J. Ethnobiol. Ethnomed.* 2: 27.

Kuo C.F., Chen C.C., Luo Y.H., Huang R.Y., Chuang W.J., Sheu C.C., Lin Y.S. 2005. *Cordyceps sinensis* mycelium protects mice from group A streptococcal infection. *J. Med. Microbiol.* 54: 795–802.

Kuo M., Chang C., Cheng T., Wu M. 2007. Immunomodulatory effect of exopolysaccharides from submerged cultured *Cordyceps sinensis*: enhancement of cytokine synthesis, CD11b expression, and phagocytosis. *Appl. Microbiol. Biotechnol.* 75: 769–775.

Kuo Y.C., Lin C.Y., Tsai W.J., Wu C.L., Chen C.F., Shiao M.S. 1994. Growth inhibitors against tumor cells in *Cordyceps sinensis* other than cordycepin and polysaccharides. *Cancer Invest.* 12: 611–615.

Leng W. 2012. Will lemon law affect herb sellers? http://www.channelnewsasia.com/stories/singaporelocalnews/view/1193893/1/.html (last accessed: June 16, 2015).

Li S.P., Su Z.R., Dong T.T., Tsim K.W. 2002. The fruiting body and its caterpillar host of *Cordyceps sinensis* show close resemblace in main constituents and anti-oxidation activity. *Phytomedicine* 9: 319–324.

Li S.P., Zhang G.H., Zeng Q., Huang Z.G., Wang Y.T. Dong T.T.X., Tsim K.W.K. 2006. Hypoglycemic activity of polysaccharide, with antioxidation, isolated from cultured *Cordyceps* mycelia. *Phytomedicine* 13: 428–433.

Li Z.Z., Huang B., Zhen F.M. 2000. Molecular evidence for anamorph determination of *C. sinensis* (Berk) Sacc. I. Relation between *Hirsutella sinensis* and *C. sinensis. Mycosystems* 19: 60–64.

Liu W.C., Chuang W.L., Tsai M.L., Hong J.H., McBride W.H., Chiang C.S. 2008. *Cordyceps sinensis* health supplement enhances recovery from taxol-induced leucopenia. *Exp. Biol. Med.* 233: 447–455.

Liu Y., Xu F., Chen B., Zhang J., Yao S. 2010a. CZE determination of nucleosides and nucleobases from natural *Cordyceps sinensis* and cultured *Cordyceps militaris*. *Yaowu Fenxi Zazhi* 30: 24–29.

Liu Z., Li P., Zhao D., Tang H., Guo J. 2010b. Protective effect of extract of *Cordyceps sinensis* in middle cerebral artery occlusion-induced focal cerebral ischemia in rats. *Behav. Brain Funct.* 6: 61–68.

Lo H.C., Hsieh C., Lin F.Y., Hsu T.H. 2013. A systematic review of the mysterious caterpillar fungus *Ophiocordyceps sinensis* in DongChongXiaCao and related bioactive ingredients. *J. Trad. Complement. Med.* 3: 16–32.

Miller R.A. 2009. The *Cordyceps sinensis* medicinal mushroom. *Nexus*, pp. 23–28.

Mizuno T. 1999. Medicinal effects and utilization of *Cordyceps* (Fr.) Link (Ascomycetes) and Isaria Fr. (mitosporic fungi) Chinese caterpillar fungi, "Tochukaso". *Int. J. Med. Mushrooms* 1: 251–261.

Nakamura K., Konoha K., Yamaguchi Y., Kagota S., Kazumasa S., Kunitomo M. 2003. Combined effects of *Cordyceps sinensis* and methotrexate on hematogenic lung metastasis in mice. *Receptors Channels* 9: 329–334.

Nakamura K., Yoshikawa N., Yamaguchi Y., Kagota S., Shinozuka K., Kunitomo M. 2006. Antitumor effect of cordycepin (3'-deoxyadenosine) on mouse melanoma and lung carcinoma cells involves adenosine A3 receptor stimulation. *Anticancer Res.* 26: 43–48.

Namgyel P., Tshitila. 2003. Rare, endangered, over-exploitation and extinction of plant species. Putting *Cordyceps* – a high-value medicinal plant – to test. CORE- BTFEC Report. Thimphu: Bhutan Trust Fund for Environmental Conservation and Council of Research and Extension. RNR- RC Yusipang, Thimphu, Bhutan.

Negi C.S., Koranga P.R., Ghinga H.S. 2006. Yar tsa Gumba (*Cordyceps sinensis*): A call for its sustainable exploitation. *Int. J. Sust. Dev. World Ecol.* 13: 165–172.

Olsen C.S., Helles F. 1997. Medicinal plants, markets, and margins in the Nepal Himalaya: trouble in paradise. *Mount. Res. Dev.* 17: 363–374.

Pao H., Pan B., Leu S., Huang B. 2012. Cordycepin stimulated steroidogenesis in MA-10 mouse Leydig tumor cells through the protein kinase C pathway. *J. Agr. Food Chem.* 60: 4905–4913.

Paterson R.R.M. 2008. *Cordyceps* – a traditional Chinese medicine and another fungal therapeutic biofactory? *Phytochemistry* 69: 1469–1495.

Paul F.C., Nigel L.H.J., Norbert M., Lungten N., Tashi S., Phurba L. 2009. Steps towards sustainable harvest of *Ophiocordyceps sinensis* in Bhutan. *Biodiver. Cons.* 18: 2263–2281.

Pelleg A., Porter R.S. 1990. The pharmacology of adenosine. *Pharmacotherapy* 10: 157–174.

Qian G., Pan G.F., Guo J.Y. 2012. Anti-inflammatory and antinociceptive effects of cordymin, a peptide purified from the medicinal mushroom *Cordyceps sinensis. Nat. Prod. Res.* 26: 2358–2362.

Roy H.E., Steinkraus D.C., Eilenberg J., Hajek A.E., Pell J.K. 2006. Bizzare interactions and endgames: entomopathogenic fungi and their arthropod hosts. *Annu. Rev. Entomol.* 51: 331–357.

Seth R., Haider S.Z., Mohan M. 2014. Pharmacology, phytochemistry and traditional uses of *Cordyceps sinensis* (Berk.) Sacc: a recent update for future prospects. *Indian J. Tradit. Knowl.* 13: 551–556.

Sharma S. 2004. Trade of *Cordyceps sinensis* from high altitudes of the Indian Himalaya: conservation and biotechnological priorities. *Curr. Sci.* 86: 1614–1619.

Shashidhar M.G., Giridhar P., Sankar K. U., Manohar B. 2013. Bioactive principles from *Cordyceps sinensis*: a potent food supplement – a review. *J. Funct. Foods* 5: 1013–1030.

Shaw S.R. 1988. Euphorine phylogeny: the evolution of diversity in host-utilization by parasitoid wasps (Hymenoptera: Braconidae). *Ecol. Entomol.* 13: 323–335.

Sherwin B.B. 1999. Can estrogen keep you smart? Evidence from clinical studies. *J. Psychiatry Neurosci.* 24: 315–321.

Shin K.H., Lim S.S., Lee S., Lee Y.S., Jung S.H., Cho S.Y. 2003. Anti-tumour and immuno-stimulating activities of the fruiting bodies of *Paecilomyces japonica*, a new type of *Cordyceps* spp. *Phytother. Res.* 17: 830–833.

Shrestha B. 2011. Diversity of Cordyceps fungi in Nepal. *Nep. J. Sci. Technol.* 12: 103–110.

Shrestha B., Zhang W.M., Zhang Y.J., Liu X.Z. 2010. What is Chinese caterpillar fungus (Ophiocordycipitaceae)? *Mycology* 1: 228–236.

Shrestha U.B., Bawa K.S. 2014a. Harvesters' perceptions of population status and conservation of Chinese caterpillar fungus in the Dolpa region of Nepal. *Reg. Environ. Change* DOI 10.1007/s10113-014-0732-7.

Shrestha U.B, Bawa KS. 2014b. Economic contribution of Chinese caterpillar fungus to the livelihoods of mountain communities in Nepal. *Biol Conser.* 177: 194–202.

Shrestha UB., Bawa K.S. 2013. Trade, harvest, and conservation of caterpillar fungus (*Ophiocordyceps sinensis*) in the Himalayas. *Biol. Conserv.* 159: 514–520.

Siddique S.S., Arif M., Babu R., Shamim M. 2011. Wonder drug – an insect and fungus relationship (*Hepialus-Cordyceps*) through biotechnological approaches. *Biol. Med.* 3: 70–75.

State Forestry Administration and Ministry of Agriculture of the People's Republic of China, (Order No. 4). 1999. The list of the wild plants under the state emphasized protection. http://www.gov.cn/gongbao/content/2000/content_60072.htm. (last accessed: June 16, 2015).

Stone R. 2008. Last stand for the body snatcher of the Himalayas? *Science* 322: 1182.

Sulek E. 2011. Disappearing sheep: the unexpected consequences of the emergences of the caterpillar fungus economy in Golok, Qinghai, China. *Himalaya J. Assoc. Nepal Himal. Stud.* 30: 9.

Sung G.H., Hywel-Jones N.L., Sung J.M., Luangsa-ard J.J., Shrestha B., Spatafora J.W. 2007. Phylogenetic classification of *Cordyceps* and the clavicipitaceous fungi. *Stud. Mycol.* 57: 5–59.

Thapa B, Panthi S., Rai R.J., Shrestha U.B., Aryal A., Shrestha S., Shrestha B. 2014. An assessment of Yarsagumba (*Ophiocordyceps sinensis*) collection in Dhorpatan hunting reserve, Nepal. *J. Mount. Sci.* 11: 555–562.

The Record. 2014. Police brutality on Dolpo's "Black Day" under investigation. The Record, June 10, 2014. www.recordnepal.com/wire/police-brutality-dolpo's-"black-day"-under-investigation (last accessed: June 16, 2015).

Varghese A., Ticktin T. 2008. Regional variation in non-timber forest product harvest strategies, trade, and ecological impacts: the case of Black Dammar (*Canarium strictum* Roxb.) use and conservation in the Nilgiri biosphere reserve, India. *Ecol. Soc.* 13: 11.

Verma A.K. 2011. Potential of marine-derived fungi and their enzymes in bioremediation of industrial pollutants. PhD thesis. National Institute of Oceanography, Goa University, Dona Paula, Goa-403004, India. pp. 297.

Wang B.J., Won S.J., Yu Z.R., Su C.L. 2005. Free radical scavenging and apoptotic effects of *Cordyceps sinensis* fractionated by supercritical carbon dioxide. *Food Chem. Toxicol.* 43: 543–552.

Wang S.M., Lee L.J., Lin W.W., Chang C.M. 1998. Effects of a water-soluble extract of *Cordyceps sinensis* on steroidogenesis and capsular morphology of lipid droplets in cultured rat adrenocortical cells. *J. Cell Biochem.* 69: 483–489.

Wang X.L., Yang R.H., Yao Y.J. 2011. Development of microsatellite markers for *Ophiocordyceps sinensis* (Ophiocordycipitaceae) using an ISSR-TAIL-PCR method. *Amer. J. Bot.* e391–e394.

Wang X.L., Yao Y.J. 2011. Host insect species of *Ophiocordyceps sinensis*: a review. *ZooKeys* 127: 43–59.

Wasser S.P. 2002. Medicinal mushrooms as a source of antitumor and immunomodulating polysaccharides *Appl. Microbiol. Biotechnol.* 60: 258–274.

Wasser S.P., Weis A.L. 1999. Therpeutic effects of substances occurring in higher Basidiomycetes mushrooms: modern perspective. *Crit. Rev. Immunol.* 19: 65–96.

Weckerle C.S., Yang Y., Huber F.K., Li Q. 2010. People, money and protected areas: the collection of the caterpillar mushroom *Ophiocordyceps sinensis* in the Baima Xueshan Nature Reserve, Southwest China. *Biodivers. Conserv.* 19: 2685–2698.

Winkler D. 2005. Yartsa Gunbu – *Cordyceps sinensis*: Ecology, ecology and ethno-mycology of a fungus endemic to the Tibetan plateau. In: Boesi A., Cardi F. (eds) *Wildlife and Plants in Traditional and Modern Tibet: Conceptions, Exploitation and Conservation*. Memorie della Società Italiana di Scienze Naturali e del Museo Civico di Storia Naturale di Milano, Vol. 33.1; http://www.mushroaming.com/Yartsa_Gunbu_Cordyceps. (last accessed on June 16, 2015).

Winkler D. 2008. Yartsa Gunbu (*Cordyceps sinensis*) and the fungal commodification of Tibet's rural economy. *Econ. Bot.* 62: 291–305.

Winkler D. 2010. Caterpillar fungus (*Ophiocordyceps sinensis*) production and sustainability on the Tibetan plateau and in the Himalayas. *Asian Medic.* 5: 291–316.

Winkler D. 2013. Steps towards sustainable harvest of Yartsa Gunbu (caterpillar fungus, *Ophiocordyceps sinensis*). 7th International Medicinal Mushroom Conference, Beijing, China. Aug. 26–29, pp. 635–644.

Wong Y.Y., Moon A., Duffin R., Barthet-Barateig A., Meijer H.A., Clemens M.J., de Moor C.H. 2010. Cordycepin inhibits protein synthesis and cell adhesion through effects on signal transduction. *J. Biol. Chem.* 285: 2610–2621.

Wu J. Y., Zhang Q. X., Leung P.H. 2007. Inhibitory effects of ethyl acetate extract of *Cordyceps sinensis* mycelium on various cancer cells in culture and B16 melanoma in C57BL/6 mice. *Phytomedicine* 14: 43–49.

Wu N. 1997. Ecological situation of high-frigid rangeland and its sustainability – A case study on the constraints and approaches in pastoral Western Sichuan / China. *Abh. Anthropogeo.* 55: 1–281.

Xing X.K., Guo S.X. 2008. The structure and histochemistry of sclerotia of *Ophiocordyceps sinensis. Mycologia* 100: 616–625.

Xu F., Huang J.B., Hiang L., Xu J., Mi J. 1995. Amelioration of cyclosporine nephrotoxicity by *Cordyceps sinensis* in kidney transplanted recipients. *Nephrol. Dialysis Transplant.* 10: 141–143.

Xuan C. 2012. How long can the caterpillar fungus craze last? China Dialogue. http://www.chinadialogue.net/article/show/single/en/5143-How-long-can-the-caterpillar-fungus-craze-last (last accessed: June 16, 2015).

Yan J.K., Wang W.Q., Wu J.Y. 2014. Recent advances in *Cordyceps sinensis* polysaccharides: mycelial fermentation, isolation, structure, and bioactivities: a review. *J. Funct. Foods* 6: 33–47.

Yan J.K., Wu J.Y. 2014. Submerged fermentation of medicinal fungus *Cordyceps sinensis* for production of biologically active mycelial biomass and exopolysaccharides. In: Paek K-Y., Murthy H.N., Zhong J-J. (eds) *Production of Biomass and Bioactive Compounds Using Bioreactor Technology.* Springer Science+Business Media, Dordrecht, pp. 93–120.

Yang D.R. 1999. Regional and ecological distribution of *Cordyceps sinensis. J. Anhui. Agri. Univ.* 26: 143–148.

Yao X., Meran S., Fang Y., Martin J., Midgley A., Pan M.M., Liu B.C., Cui S.W., Phillips G.O., Phillips A.O. 2014. *Cordyceps sinensis*: *In vitro* anti-fibrotic bioactivity of natural and cultured preparations. *Food Hydrocoll.* 35: 444–452.

Yao Y.J. 2004. Conservation and rational use of the natural resources of *Cordyceps sinensis. Sci. News* 15: 28–29.

Yoshikawa N., Nakamura K., Yamaguchi Y., Kagota S., Shinozuka K., Kunitomo M. 2004. Antitumor activity of cordycepin in mice. *Clin. Exp. Pharmacol. Physiol.* 31: S51–S53.

Yoshikawa N., Nishiuchi A., Kubo E., Yamaguchi Y., Kunitomo M., Kagota S., Shinozuka K., Nakamura K. 2011. *Cordyceps sinensis* acts as an adenosine A3 receptor against on mouse melanoma and lung carcinoma cells, and human fibrosarcoma and colon carcinoma cells. *Pharmacol. Pharm.* 2: 266–270.

Yue G.G.L., Lau C.B.S., Fung K.P., Leung P.C., Ko W.H. 2008. Effects of *Cordyceps sinensis, Cordyceps militaris* and their isolated compounds on ion transport in Calu-3 human airway epithelial cells. *J. Ethnopharmacol.* 117: 92–101.

Yue K., Ye M., Zhou Z. 2013. The artificial cultivation of medicinal caterpillar fungus, *Ophiocordyceps sinensis* (Ascomycetes): a review. *Int. J. Med. Mushrooms* 15: 425–434.

Zang M., Kinjo N. 1998. Notes on the alpine *Cordyceps* of China and nearby nations. *Mycotazon* 66: 215–229.

Zhang G., Huang Y., Bian Y., Wong J.H., Ng T.B., Wang H. 2006. Hypoglycemic activity of the fungi *Cordyceps militaris, Cordyceps sinensis, Tricholoma mongolicum*, and *Omphalia lapidescens* in streptozotocin-induced diabetic rats. *Appl. Microbiol. Biotechnol.* 72: 1152–1156.

Zhang J., Yu Y., Zhang Z., Ding Y., Dai X., Li Y. 2011. Effect of polysaccharide from cultured *Cordyceps sinensis* on immune function and anti-oxidation activity of mice exposed to ^{60}Co. *Int. Immunopharmacol.* 11: 2251–2257.

Zhang Q.X., Leung P.H., Wu, J.Y., Hu, Z.D. 2005. Comparison of antitumor effect of extracts in cultivated *Cordyceps sinensis* fungus HK-1 and natural *Cordyceps sinensis. Chin. Trad. Herbal Drugs* 36: 1346.

Zhang X., Liu B., Al-Assaf S., Phillips G.O. 2012a. *Cordyceps sinensis* decreases TGF- β1 dependent epithelial to mesenchymal trans-differentiation and attenuates renal fibrosis. *Food Hydrocolloids* 28: 200–212.

Zhang Y., Li E., Wang C., Li Y., Liu X. 2012b. *Ophiocordyceps sinensis*, the flagship fungus of China: terminology, life strategy and ecology. *Mycology* 3: 2–10.

Zhang Y., Zhang S., Li Y., Ma S., Wang C., Xiang M., Liu X., An Z., Xu J., Liu X. 2014. Phylogeography and evolution of a fungal-insect association on the Tibetan Plateau. *Mol. Ecol.* 23: 5337–5355.

Zhao J., Xie J., Wang L.Y., Li S.P. 2013. Advanced development in chemical analysis of *Cordyceps*. *J. Pharm. Biomed. Anal.* 87: 271–289.

Zheng P., Xia Y., Xiao G., Xiong C., Hu X., Zhang S., Zheng H., Huang Y., Zhou Y., Wang S., Zhao G.P., Liu X., Leger R.J., Wang C. 2011. Genome sequence of the insect pathogenic fungus *Cordyceps militaris*, a valued traditional Chinese medicine. *Gen. Biol.* 12: R116.

Zhou X.W., Li L.J., Tian E.W. 2014. Advances in research of the artificial cultivation of *Ophiocordyceps sinensis* in China. *Crit. Rev. Biotechnol.* 34: 233–243.

Zhou X.X., Luo L., Dressel W., Shadier G., Krumbiegel D., Schmidtke P., Zepp F., Meyer C.U. 2008. Cordycepin is an immunoregulatory active ingredient of *Cordyceps sinensis. Am. J. Chin. Med.* 36: 967–980.

Zhu J.S., Halpern G.M., Jones K. 1998a. The scientific rediscovery of an ancient Chinese herbal medicine: *Cordyceps sinensis*: Part I. *J. Alter. Compl. Med.* 4: 289–303.

Zhu J.S., Halpern G.M., Jones K. 1998b. The scientific rediscovery of an ancient Chinese herbal medicine: *Cordyceps sinensis*: Part II. *J. Alter. Compl. Med.* 4: 429–457.

Received 7 February 2015; received in revised form 11 May 2015; accepted 1 June 2015