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Notes on *Cordyceps* species Collected from the Central Region of Nepal

Bhushan Shrestha[1] and Jae-Mo Sung*

Entomopathogenic Fungal Culture Collection (EFCC), Department of Applied Biology, Kangwon National University, Chuncheon 200-701, Korea

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The present study was carried out to explore the *Cordyceps* species and other entomopathogenic fungal flora around Kathmandu Valley and a few high altitude locations of Nepal. In this paper, we report eight *Cordyceps* species as new to Nepal: *C. gracilis*, *C. ishikariensis*, *C. liangshanensis*, *C. martialis*, *C. militaris*, *C. pruinosa*, *C. sphecocephala* and *C. tricentri*. We also mention a few allied genera such as *Beauveria*, *Hirsutella* and *Paecilomyces* from Nepal. Further collections from different ecological regions of Nepal will show the richness of entomopathogenic fungal floral diversity of Nepal.

KEYWORDS: ***Cordyceps*, Entomopathogenic fungi, Kathmandu valley, Langtang area, Manang village**

Cordyceps species have been reported from many parts of the world. The genus *Cordyceps* belongs to the family Clavicipitaceae in the order Hypocreales of Ascomycota. *Cordyceps* species mostly infect different stages of their host insects from larva to adult, kill them and eventually grow out of dead bodies of insects, except for a few species which grow on hypogeal *Elaphomyces* species. Very few *Cordyceps* species, including *C. sinensis*, have been reported from Nepal. However, scientific research on these species has been lacking although knowledge on their medicinal properties such as tonic and aphrodisiac values are common in the Nepalese society. Scientific studies on *Cordyceps* species started about 300 years ago, when *Cordyceps militaris* was described under the generic name, *Clavaria*, due to its *Clavaria*-like stromata (Linnaeus, 1753). Linnaeus followed the same generic name *Clavaria* and mentioned few *Cordyceps* species in his great work Species Plantarum (Linnaeus, 1753). Since then, it has attracted attention of great mycologists such as Persoon (1799), Fries (1823), Link (1833), Berkeley (1843), Tulasne Brothers (1865), Saccardo (1883) and Massee (1895) and was described under different generic names. Old literature proposed different generic names for *Cordyceps* such as *Clavaria*, *Sphaeria* and *Torrubia*, before Link (1833) finally erected *Cordyceps* as a new generic name. During last hundred years or more, regional exploration of *Cordyceps* species continued in many parts of the world such as Australia (Olliff, 1895; Willis, 1959), North America (Seaver, 1911; Mains, 1958), New Zealand (Dingley, 1953), Ceylon (present day Sri Lanka) (Petch, 1924), Great Britain (Petch, 1932, 1948), Japan (Kobayasi, 1939a, b, 1941; Kobayasi and Shimizu, 1983), Congo (Moureau, 1962), Norway (Eckblad, 1967), Ghana (Samson *et al.*, 1982), Taiwan (Tzean *et al.*, 1997), Amazonia (Evans and Samson, 1982, 1984; Samson and Evans, 1985), Thailand (Hywel-Jones 1994, 1995a, b, c, 1996; Hywel-Jones and Sivichai, 1995), Korea (Sung, 1996), China (Zang and Kinjo, 1998), and Mexico (Guzman *et al.*, 2001). Apart from the work of Petch, very little work has been carried out on *Cordyceps* in South Asian region. To date, a few species of *Cordyceps* have been reported from Nepal. *C. sinensis* is one of them, which has been reported from high altitude areas (Balfour-Browne, 1955; Kobayasi, 1981; Adhikari and Durrieu, 1996). This species is the most popular species in Nepal due to its high medicinal values, thus regarded as a natural gift for humans as in other East Asian countries. Another *Cordyceps species*, *C. nutans* has been reported in Manang Region (Shrestha, 1985). The third *Cordyceps* species, *C. nepalensis* was reported as a new *Cordyceps* species from Mt. Kangchenjunga of Nepal (Zang and Kinjo, 1998). Recently, a glimpse of *Cordyceps* diversity of Nepal was presented (Shrestha and Sung, 2005).

A Few *Cordyceps* exploration trips were carried out in Nepal during last several years to explore entomopathogenic fungal diversity of Nepal. Detailed taxonomic studies of *Cordyceps* species collected from Korea and other parts of the world, including Nepal, are undergoing at Entomopathogenic Fungal Culture Collection (EFCC), Kangwon National University, Korea (Shrestha *et al.*, 2004; Sung *et al.*, 2005). This paper intends to provide information on *Cordyceps* species collected from Nepal during last few years in order to highlight the entomopathogenic fungal diversity of Nepal (Table 1).

Collection trips were made around Kathmandu Valley (Fig. 1) from 1997 to 2001 and in 2003. One trip was

*Corresponding author <E-mail: jmsung@kangwon.ac.kr>
[1]Present Address: Green Energy Mission/Nepal, Anam Nagar, Kathmandu P.O. Box 10647, Nepal

Table 1. *Cordyceps* species collected from Nepal

Cordyceps species	EFCC number[1]	Location	Collection date	Host
C. gracilis	EFCC 10528, 10529	Nagarkot Hill	July 2003	Lepidopteran larva, usually *Hepialus* sp.
C. ishikariensis	EFCC 10407	Godawari Hill	July 2003	Larva of cicadae
C. liangshanensis	EFCC 1520, 1521, 1522	Shivapuri Hill	July 1997.	Lepidopteran larva
C. martialis	EFCC 3193, 3194	Shivapuri Hill	July 1999	Coleopteran larva
C. militaris	EFCC 7338, 7339	Nagarkot Hill	July 2001	Lepidopteran pupae and larva
C. nutans	EFCC 7206, 7207	Nagarjun Hill and Gokarna	June 2001	Hemipteran bugs
C. pruinosa	EFCC 10468, 10909	Shivapuri Hill	July 2003	Limacodidae cocoon (Lepidoptera)
C. sinensis	EFCC 7282, 7283, 7284	Manang and Langtang Villages	May 1999, May 2001	Larva of *Hepialus* sp.
C. sphecocephala	EFCC 7236, 7237, 7238	Nagarjun Hill	June 2001	Bees and wasps
C. tricentri	EFCC 7251, 7252	Gokarna	June 2001	Aadults of Vespidae (Homoptera)
Allied species				
Beauveria sp.	EFCC 7270	Shivapuri Hill	July 2001	Hemipteran bug
Hirsutella sp.	EFCC 3240, 3241	Shivapuri Hill	July 2001	Lepidopteran larva
Paecilomyces cicadae	EFCC 7170, 7171	Nagarjun Hill	June 2001	Larva of cicadae
Paecilomyces sp.	EFCC10570, 10571	Shivapuri Hill	July 2003	Lepidopteran larva

[1]EFCC; Entomopathogenic Fungal Culture Collection, Kangwon National University, Chuncheon 200-701, Korea.

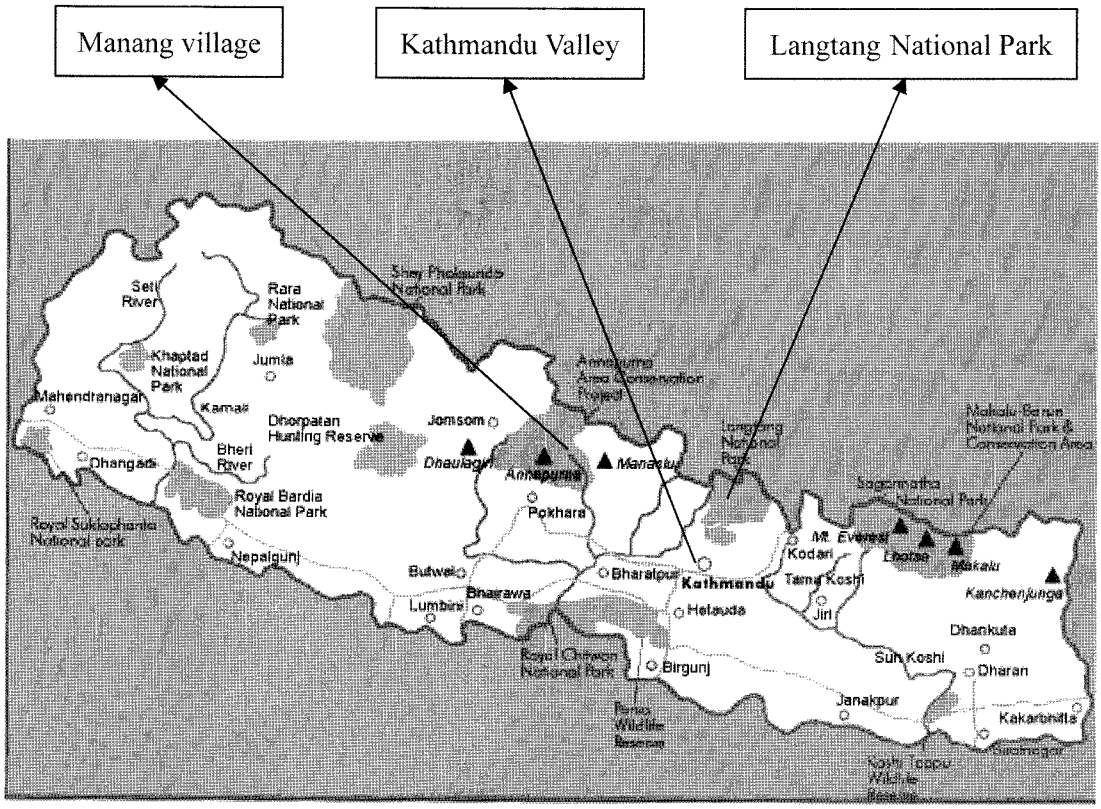

Fig. 1. Geographical map of Nepal.

made to Langtang National Park in 2001, from where the specimens of *C. sinensis* were purchased from local collectors. *C. sinensis* specimens collected from local people of Manang Area were also observed. Specimens were air-dried and have been preserved in Entomopathogenic Fungal Culture Collection (EFCC), Kangwon National University, Chuncheon, Korea. Morphological characters of *Cordyceps* species given by Mains (1958), Kobayasi (1941, 1982), Kobayasi and Shimizu (1983), Shimizu (1997), Tzean *et al.* (1997) and Zang and Kinjo (1998) were referred to for the identification of *Cordyceps* species (Fig. 2).

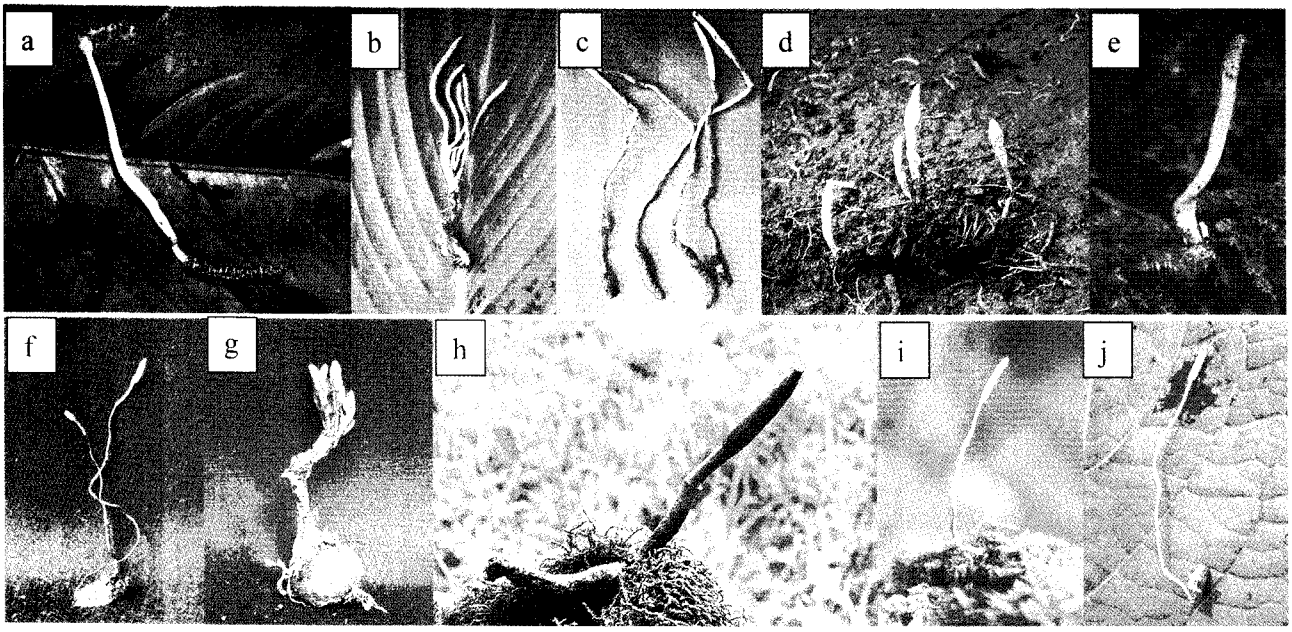

Fig. 2. *Cordyceps* species collected from Nepal. a, *C. gracilis*; b, *C. ishikariensis*; c, *C. liangshanensis*; d, *C. martialis*; e, *C. militaris*; f, *C. nutans*; g, *C. pruinosa*; h, *C. sinensis*; i, *C. sphecocephala*; j, *C. tricentri.*

***Cordyceps gracilis* Durieu & Montagne.** Stroma consists of a stout cylindrical stipe, with a white to light brown, and ovoid to subglobose, brown-colored head. Stipe ranges from 30~49 mm in length and 1~2 mm in diameter. Head is 3~6 mm long and 3~4 mm wide. Stromata are produced in solitary per host, usually from head or thorax region. Both stalk and head are smooth in surface. Head is dotted with ostioles of perithecia. Perithecia are completely immersed in head and range in size from 500~600 × 150~200 μm. Perithecia are ovoid with a slightly long neck. Size of ascus is 325~350 × 5 μm. Size of ascus cap is 6~6.5 × 4~4.5 μm.

***Cordyceps ishikariensis* Kobayasi & Shimizu.** It produces five to six brown-yellow colored stromata per host. Stromata arise from the head region of the host. Stipe is 30~35 mm long, whereas head is 15~20 mm long. Stromata are 1.5~2 mm wide, stipe being more slender than head. Stipe is rough due to torn outer epidermal layer. Perithecia are semi-immersed and 500~570 × 240~300 μm in size. Perithecia are ovoid to broadly ovoid. Size of ascus is 250~360 × 4 μm. Size of ascus cap is 3~4 × 2 μm.

***Cordyceps liangshanensis* M. Zang, D. Liu & R. Hu.** Stromata are light to dark brown and are produced in solitary. Stromata arise from the head region of the host. Surface of stipe is longitudinally striate. Stipe ranges from 40~60 mm in length and 1.5~2 mm in diameter. Head is about 15 mm long and slightly wider than stipe. Perithecia are semi-immersed and 400~450 × 200~250 μm in size. Perithecia are ovoid to oval in shape. Size of ascus is 175~210 × 6~7 μm. Size of ascus cap is 4~5 × 3 μm.

***Cordyceps martialis* Speg.** It produces brownish orange stromata, one to a few per host. Stipe varies in length from 25 to 45 mm, while head ranges from 10 to 20 mm. Stipe and head are 0.5~1 and 1~2 mm wide, respectively. Surface of the head is rough due to presence of neck of immersed perithecia. Surface of stipe is sometimes covered by short spine-like structures. Upper parts of head are sometimes branched. Perithecia are ovoid or flask-shaped with long conical neck and almost immersed in head. They range in size from 500~530 × 150 μm. Size of ascus is 225~260 × 5~6 μm. Size of ascus cap is 6~7 × 4~5 μm.

***Cordyceps militaris*. (L. ex Fr.) Link.** Stromata are usually orange, club or clavate shaped. Stipe and head are 25~40 mm and 20~30 mm long, respectively. Stipe is 1~1.5 mm wide, head being slightly broader than stipe. Stromata are usually solitary, but sometimes a few per host. Surface of head is rough due to apices of perithecia. Stipe is sometimes slightly twisted and the surface is irregularly furrowed. Perithecia are semi-immersed in head and are 650~770 × 300~500 μm in size. Perithecia are broadly ovoid in shape. Size of ascus is 350~450 × 3~4 μm. Size of ascus cap is 3~4 × 3~3.5 μm. Size of part-spore is 3~3.5 × 1 μm.

Cordyceps nutans Patouillard. It produces black stipe with yellow to orange or red head. The uppermost part of stipe is usually similar to head in color. Stipe and head are

42~60 × 1 mm and 5~10 × 1~1.5 mm in size, respectively. The head is oval, obtuse or cylindrical and is erect. Surface of head is smooth and that of stipe is smooth, but longitudinally furrowed. Stipe is sometimes twisted. Stromata vary from solitary to a few per host. Perithecia are completely immersed and are obliquely vertical in head. They are 800~900 × 300 μm in size and elongated flask shaped. Size of ascus is 525~550 × 4~7 μm. Size of ascus cap is 10~12 × 6~8 μm. Size of part-spore is 7~8 × 1~1.4 μm.

***Cordyceps pruinosa* Petch.** It produces red colored stromata, single or few per host. Stipe is 10~15 × 1 mm in size and head is 5~10 mm long and 1.5 mm wide. The head is narrow-clavate. Surface of head is rough due to ostioles of semi-immersed perithecia. Stipe has irregular but short furrows on its surface. Perithecia are semi-immersed in head and are 350~500 × 240~290 μm in size. Perithecia are ovoid, with ostiole sometimes directed to one side. Size of ascus is 185~200 × 2 μm. Size of ascus cap is 3 × 1.5~2 μm. Part-spores are joined at both ends in a thin thread-like structure.

***Cordyceps sinensis*. (Berk.) Sacc.** Stromata are black with cylindrical stipe and slightly swollen head, usually with sterile apex. Stipe ranges from 40~45 mm in lengths and 1.5 mm wide. Head is slightly wider than stipe, usually 2 mm wide and 20~25 mm long. Perithecia are slightly projecting from the surface of the head. Surface of stipe is smooth with irregularly furrowed. Stipe is slightly twisted. Perithecia are 330~370 μm long and 170~270 μm wide. Perithecia are ovoid and have thick perithecial wall. Size of ascus is 200~210 × 7~8 μm.

***Cordyceps sphecocephala* (Berk.) Sacc.** It produces pale-yellow to brownish-yellow slender stipe with ovoid to cylindrical yellow head. Stipe is 30~55 mm long and 1 mm wide. Head is 5~10 mm long and wider than stipe, about 1.5~2 mm wide. Each host produces single stroma. Stipe is smooth and longitudinally striate. Head is dotted with ostioles of perithecia on apex of ridges. Perithecia are immersed in head and are 620~780 × 200~230 μm in size. Perithecia are obliquely vertical in head, elongated flask-shaped or conoid in shape. Size of ascus is 350 × 6~7. Size of ascus cap is 8~9 × 5~6 μm. Size of part-spore is 9~10.5 × 1~1.3 μm and is fusiform in shape with pointed ends.

***Cordyceps tricentri* Yasuda.** Stromata are produced in solitary per host and are yellow in color. Stipe is 33~43 mm long and very slender, 0.25~0.3 mm wide. Head is ovoid, 5~6 mm long and 1~1.5 mm wide. Both stipe and head are smooth in surface. Perithecia are obliquely immersed in head and range from 550~650 × 110~120 μm in size. Perithecia are ovoid in shape. Ascus size is 300~320 × 5 μm. Ascus cap is 5~6 × 3~4 μm.

The present study has explored *Cordyceps* species in Nepal. Additionally, this study has also explored allied species of *Cordyceps*, such as *Beauveria*, *Hirsutella*, *Paecilomyces*, etc. Among *Paecilomyces* species, *P. cicadae* (Miquel) Samson was found growing on larva of cicadae in Nagarjun Area of Kathmandu valley.

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