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South American *Cordyceps* s.l. (Hypocreales, Ascomycota): first assessment of species diversity in Argentina

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With 4 figures and 1 table

Abstract: In recent years *Cordyceps* s.l. has been the subject of many studies which have enabled a breakthrough in our understanding of the diversity of species, their distributions, systematics, ecology and mechanisms of fungus-host interaction. Knowledge of the group in Argentina is based on relatively few records that are scattered throughout the literature. In this work, we developed a baseline on the knowledge of *Cordyceps* s.l. in Argentina through a search of bibliographic records, analysis of existing specimens kept in herbaria and new collections made by the authors. A total of 22 species are recorded from the country, inclunding first records of *C. pseudomilitaris*, *Ophiocordyceps amazonica*, and *O. melolonthae*. Descriptions and illustrations of the new records and an annotated dichotomous key of the known species in Argentina are presented.

Key words: Cordycipitaceae, entomopathogenic fungi, Hypocreales, Ophiocordycipitaceae, taxonomy.

Introduction

Cordyceps Fr. sensu lato is a large group of ascomycetes, known for its diversity of interactions with plants, animals and fungi (Sung et al. 2007, Kepler et al. 2012b, Quandt et al. 2014). *Cordyceps* s.l. encompasses more than 400 species distributed around the world reaching its highest diversity in the tropical and subtropical zones.

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Nearly 60% of the species of *Cordyceps* s.l. are recorded on insects belonging to Coleoptera and Lepidoptera (Shrestha et al. 2016). Other insect orders such as Hymenoptera, Hemiptera and Orthoptera exhibit a variety of *Cordyceps* species infecting them. Spiders are also infected by *Cordyceps* s.l., although the taxonomy of those species is unclear and some species have already been transferred to *Ophiocordyceps* (Sung et al. 2007). *Torrubiella*, a genus within Cordycipitaceae is well-known to exhibit several species infecting exclusively spiders, which includes its anamorphs such as *Gibellula*, *Granulomanus*, *Akanthomyces* and *Lecanicillium* (Evans 2013). Some entomopathogenic species are known to control populations of insect pests, regulating the impact of these arthropods in both forest and agricultural systems (Sosa-Gomez et al. 2010).

More than 25 anamorphic genera have been linked to sexual (teleomorphic) state of *Cordyceps* s.l., including *Beauveria* Vuill., *Hirsutella* Pat., *Hymenostilbe* Petch, *Isaria* Pers., *Lecanicillium* W.Gams & Zare, *Metarhizium* Sorokīn, *Nomuraea* Maubl., *Paecilomyces* Bainier, *Polycephalomyces* Kobayasi and *Verticillium* Nees (Hodge 2003, Sung et al. 2007). Given the evidence provided by molecular phylogenetic analyses (Sung et al. 2007) and in accordance with changes of the International Code of Nomenclature for algae, fungi and plants (ICN) (McNeill et al. 2012), the taxonomy and nomenclature of the group is being constantly updated (Kepler et al 2014).

Taxonomy and systematics of the group has had a major breakthrough based on molecular evidence. Phylogenetic analyses in Sung et al. (2007) revealed that *Cordyceps* does not constitute a monophyletic group within Clavicipitaceae Earle, and several genera are currently recognized, i.e. *Cordyceps* s.s., *Ophiocordyceps* Petch and *Tolypocladium* G.H.Sung & Spatafora segregated from *Cordyceps* s.l. (Quandt et al. 2014). More recently, studies have demonstrated the monophyly of *Tyrannicordyceps* Kepler & J. W.Spatafora (Kepler et al. 2012b) and *Polycephalomyces* Kobayasi emend. Kepler & Spatafora (Kepler et al. 2013). Three families are currently recognized taxonomic concepts recognize Cordycipitaceae, Clavicipitaceae, and Ophiocordycipitaceae G.H.Sung, J.M.Sung, Hywel-Jones & Spatafora. Molecular phylogenetic analysis of *Cordyceps* s.l. is not in line with the classification based on the traditional morphological characters used in the past, i.e. perithecium arrangement, ascospore fragmentation, shape and color of stromata, in contrast the host affiliation is more correlated with the phylogeny (Sanjuan et al. 2014, Sanjuan et al. 2015, Araújo et al. 2015). In some species the anamorphic stage is an integral part of the stroma; in others it develops separately (as synnemata) or directly on the body of the host (Evans 1982).

Despite the various recent breakthroughs to occur in our understanding of cordycipitaceous fungi (Sosa-Gómez et al. 2010, Sanjuan et al. 2015, Shrestha et al. 2016), the situation in Argentina is quite different. Knowledge of the group is based on relatively few (15) records that are scattered throughout the literature. The last scientific report of a *Cordyceps* s.l. species (with description and notes) from Argentina dates back to 25 years ago (Mueller & Rajchenberg 1991). In this work, we aim to establish a baseline of knowledge of *Cordyceps* s.l. in Argentina, through a search of bibliographic records, the study of herbarium specimens stored in the country and the study of new collections made by the authors.

Materials and methods

An intensive search of *Cordyceps* s.l. records in Argentina in literature was performed. We also revised all collections kept at the herbaria CORD, LPS, LIL, CTES and BAFC. Herbarium acronyms follow Thiers (2016). In addition, new specimens were collected in different localities including protected and non-protected areas of north-eastern and north-western Argentina: Parque Sierra San Javier (Tucumán Province), Parque Provincial Cochuna (Tucumán Province) and Parque Nacional Baritú (Salta Province) corresponding to the Yungas Mountain rain forest phytogeographic region in the northwest; and Reserva Natural Estricta and Tobuna (Misiones Province) in the northeast corresponding to the Atlantic rain forest phytogeographic region.

Studied specimens are kept at BAFC, CORD, CTES and LIL, and were studied macroscopically using a stereoscope (Leica MZ6). For microscopical analysis, free-hand sections of fungal fruiting bodies were mounted in water, 5% KOH, 1% phloxine and cotton blue, and observed under the light microscope (Olympus CX31). Microscopical structures (perithecia, asci and ascospores) were measured. The hosts were identified by specialists whenever possible, either to order, family or genus according to the condition of the specimen.

Results

A total of 17 new collections were made during the study, and with the literature records, we recorded a total of 22 species in Argentina, three of them are new records for the country (Table 1). Descriptions often selected species which constitute distributional novelties or have an undetermined taxonomic status, are listed below in alphabetical order.

Cordyceps polyarthra Möller, Bot. Mitt. Trop. 9: 213 (1901) Fig. 1 A–E

Stromata cespitose, narrowly clavate, long slender cylindric, 30–44 mm long, yellow-orange, covered with a white powder, apically paler yellow. Fertile parts cylindric to narrowly clavate, covered with semi-immersed perithecia, reddish brown, 12–14 mm long. Stipe light yellow, up to 27 mm long, 1–1.5 mm wide. Perithecia ovoid, orientated at right angles in relation to the stromal surface, 220–300 × 180–200 µm. Asci cylindric 167–217.5 × 3.5–4.5 µm, apical apparatus 2.0–3.0 µm thick. Ascospores filiform, hyaline, multiseptate, part-spores cylindrical, 4.5–11 × 0.5–1.0 µm. Synnemata long, with slightly acuminate apices, brown to light yellow, up to 30 mm long., 1.0–1.5 mm thick. Conidia hyaline, ellipsoid, 2–2.5 × 1–1.5 µm.

Host: larvae of Lepidoptera.

Specimens examined: ARGENTINA: MISIONES Province, Iguazú National Park, near Macuco path, 8 April 1984, Rajchenberg & Job (BAFC 29708, BAFC 29709); ibid., 7 April 1984, Wright, Rajchenberg & Job M-3635 (BAFC 30058); General Manuel Belgrano, Selva Paranaense, Reserva Nacional Estricta, San Antonio, 26°08'00" S 53°43'00" W, 16 April 2011, O.Popoff 4212 (CTES 0574415).

Known distribution: Argentina (Wright & Wright 2005), Brazil, Costa Rica, Panama, Russia (Kobayasi 1981a, Mains 1958), Ghana (Samson et al. 1982), Mexico (Rubio Bustos et al. 1999).

Remarks: *Cordyceps polyarthra* was proposed from material collected by Möller in Blumenau (Brazil), on chrysalid of Sphingideae, Lepidoptera (Möller 1901). Our

Table 1. Species of *Cordyceps* s.l. present in Argentina.

Species	Host	Geographic distribution (Provinces)	References
C. australis (Speg.) Sacc.	Ants	Misiones	Mains (1959)
C. curculionum (Tul. & C.Tul.) Sacc.	On beetles	Tucumán	Mains (1959)
C. dipterigena Berk. & Broome	adult flies	Tucumán	Yasen de Romero (1984)
C. gracilis (Grev.) Durieu & Mont.	Nonidentified	Tucumán	Mains (1959)
C. ignota Marchion.	Spider	Buenos Aires	Marchionatto (1945)
C. aff. *incarnata* Möller	N/d	Misiones	Wright & Wright 2005)
C. militaris (L.) Link	Lepidopteran larva	Neuquén	Mueller & Rajchenberg (1991)
C. nutans Pat.	*Edessa* sp. (Pentatomidae)	Misiones	Mains (1959)
C. polyarthra Möller	N/d	Misiones	Wright & Wright (2005)
	Lepidopteran larva	Misiones	This work
C. pseudomilitaris Hywel-Jones & Sivichai	Lepidopteran larva	Tucumán	This work
C. singeri Mains	Trapdoor spider	Tucumán	Mains (1954)z
	Spider (Araneae)	Salta	This work
C. sobolifera (Hill ex Watson) Berk. & Broome	*Proarna bergi* nymph (Hemiptera-Cicadidae)	Jujuy	López Lastra (1989)
C. sphecocephala (Klotzsch ex Berk.) Berk. & M.A.Curtis	Wasp	Salta	Mains (1959)
C. submilitaris Henn.	Coleoptera larva	Tucumán	Spegazzini (1909)
C. takaomontana Yakush. & Kumaz	Lepidopteran pupa	Tucumán	This work
C. unilateralis (Tul. & C.Tul.) Sacc.	formicidae	Misiones	Spegazzini (1912)
Metarhizium martiale (Speg.) Kepler, G.H.Sung & Spatafora	Coleoptera larva	Misiones	This work
Ophiocordyceps amazonica (Henn.) G.H.Sung et al.	Grasshopper (Acrididae, Orthoptera)	Misiones	This work
O. australis (Speg.) G.H.Sung et al.	*Pachycondyla striata* (Hymenoptera, Formicidae, Ponerinae)	Misiones	This work
O. melolonthae (Tul. & C.Tul.) G.H.Sung et al.	Scarabaeidae larva (Coleoptera)	Misiones	This work
O. nutans (Pat.) G.H.Sung et al.	Adult stinkbug (Hemiptera, Pentatomidae)	Misiones	This work
Purpureocillium atypicola (Yasuda) Spatafora et al. (anamorph)	*Actinopus trapdoor*	Salta, Tucumán	Coyle et al. (1990)
P. atypicola (Yasuda) Spatafora et al. (anamorph)	Spider (Nemesiidae, Araneae)	Misiones	This work

specimens agree with the original description of *C. polyarthra* differing only in having shorter stromata, i.e. up to 44 mm vs 55 mm long in the original description (Möller 1901). There are also small differences in perithecial size of our specimens when compared with more recent descriptions: 220–300 µm long in our material, slightly shorter than those described by Mains (1958) as 300–400 µm long, and significantly shorter than in Kobayasi (1981a) as 450–500 µm long. These differences in perithecial size and the records of this species parasitizing larvae of Coleoptera (Ramírez Arias 1985) would suggest that the current concept of this species involves more than one taxon. Our specimens would represent the species in a sensu stricto, since they were collected relatively near to the type locality (Blumenau, Brazil), and in the same ecosystem, i.e. Atlantic rain forest, and associated with the same host. Unfortunately, the type specimen of *C. polyarthra* seems to be lost and the species needs to be neotypified. We would suggest designating a neotype from the type locality where the species is relatively common (Mafalda Freire & Drechsler-Santos, pers. comm.). It is worth noting that the species is epizootic, fruiting typically in "blooms" and several specimens could be observed within a few meters (Fig. 1 A). This species was previously recorded in a list of fungi of Misiones Province (Wright & Wright 2005), but was not accompanied by a description.

Cordyceps pseudomilitaris Hywel-Jones & Sivichai, Mycol. Res. 98 (8): 940 (1994)
Fig. 1 F–I

Stromata simple, one to three per host, cylindrical to clavate, 12–25 mm long, yellowish-orange. Fertile parts 2.5–9(–10) × 1.5–2.5 mm. Stipe 1–2 mm wide. Perithecia ovoid to ovoid elongate, semi-immersed, 320–500 × 225–350 µm. Asci cylindric, 210–395 × 5–6 µm, apex 3 x 2 µm. Ascospores filiform, hyaline, multiseptate, not breaking into part-spores, 200–380 × 1 µm. No conidial state observed.

Host: larvae of Lepidoptera attached to leaf litter.

Specimen examined: ARGENTINA: TUCUMÁN Province, Dpto. Yerba Buena, Parque Sierra San Javier, Horco Molle, 14 May 2010, V.Casanova Jesús & M.Catania 3189 (LIL).

Additional specimen examined: ***Cordyceps militaris*** (L.: Fr.) Link: ARGENTINA: NEUQUÉN Province, Los Lagos, 4 km del paso Puyehye, 20 March 1987, M.Rajchenberg & G.Mueller (BAFC 31033).

Known distribution: Argentina (new record), Thailand (Hywel-Jones 1994).

Remarks: *Cordyceps pseudomilitaris* is only known from the type locality in Thailand, parasitizing larvae of Lepidoptera (Hywel-Jones 1994). Our specimens resemble *C. pseudomilitaris* in the color of the stromata, the morphology of the perithecia and asci, and have non disarticulating ascospores. The infected larva is surrounded by a white mycelium and a loose network of rhizomorph-like structures.

The Argentinian specimen agrees with the original description given by Hywel-Jones (1994), but we have observed slightly smaller stromata (15–25 × 1–2 mm vs 15–30 × 0.9–3 mm), as well as smaller asci (210–395 µm vs 290–410 µm long).

Until now, the known distribution of *Cordyceps pseudomilitaris* has been restricted to Thailand but this study shows that it seems to have a broader range in the tropics.

Cordyceps pseudomilitaris represents a new record for the Yungas Region in the northwest of Argentina. However, molecular studies are necessary to confirm the identification of this taxon in Argentina.

There are a number of macroscopically similar species which had been considered conspecific with *Cordyceps militaris*, of which *C. pseudomilitaris* is but one, but differences between the two species have been amply noted (Kobayasi 1941, Hywel-Jones 1994, Sung & Spatafora 2004). *Cordyceps militaris* has been recorded from Patagonian Andes forest in Southern Argentina (Table 1).

Cordyceps singeri Mains, Bull. Torrey Bot. Club 81: 499 (1954) Fig. 1 J–M

Stromata one to two, capitate, simple, 10–20 mm long, light brown to red orange. Fertile parts cylindrical to clavate, 2–2.5 mm wide. Stipe 0.5–1.5 mm wide. Perithecia ovoid, embedded in a soft loose stroma, upper portions more or less projecting, 325–520 × 220–475 µm. Asci cylindrical, (187–)425–475 × 3–4(–4.5) µm, apical apparatus 2.5–3.5 µm thick. Ascospores filiform, hyaline, multiseptate, breaking up into one-celled cylindrical segments, 3.0–4.0 × 1.0 µm. No conidial state observed.

Host: spider (Araneae).

Known distribution: the species remains so far endemic from the Yungas ecosystem in NW Argentina. The type specimen was described from Tucuman province on a trapdoor spider (Mains 1954) and is now recorded in Salta province, also on a spider.

Specimen examined: ARGENTINA: SALTA Province, Santa Victoria. Parque Nacional Baritú, 22°26'52.0"S, 64°44'39.1"W, 21 March 2011, Niveiro et al. 2164 (CTES 0575038).

Remarks: The specimen examined agrees with all morphological characters of *C. singeri*, based on the original description. It also was found on a spider and in the same ecosystem. The type material was unavailable to study, as it appears to be lost (pers. comm. from curator of MICH).

Cordyceps takaomontana Yakush. & Kumaz, Sci. Rep. Tokyo Bunrika Daig., Sect. B 5: 108 (1941) Fig. 1 N–P

= *Isaria tenuipes* Peck, Ann. Rep. N.Y. St. Mus. nat. Hist. 31: 44 (1878)

≡ *Paecilomyces tenuipes* (Peck) Samson, Stud. Mycol. 6: 49 (1974).

Colonies on the natural substrate with a white mycelium enveloping the insect from which several erect synnemata arise. Synnemata simple, furcate or irregularly branched,

Fig. 1. A–E: *Cordyceps polyarthra*. A. Epizootic on lepidoptera larvae in natural habitat (photo by O. Popoff). B. Stromata on larva of Lepidoptera. Note synnema (arrow). C. Part of stromata showing semi-immersed perithecia. D. Asci showing different stages of maturity. E. Asci and multiseptate ascospores. F–I: *Cordyceps pseudomilitaris*. F. Stromata on larvae of Lepidoptera, arising from the leaf litter. G. Lepidopteran larva surrounded by white mycelium and rhizomorph-like structures (arrow). H. Apices of perithecia, detail. I. Asci. J–M: *Cordyceps singeri*. J. Stromata on spider. K. Apices of perithecia. L. Asci, apical apparatus. M. Asci, partspores. N–P: *Cordyceps takaomontana*. N. Synnemata on pupa of Lepidoptera. O. Synnema, detail of upper part. P. Conidia.


20 mm
A
10 mm
B
1 mm
C
10 µm
D
5 µm
E
5 mm
G
200 µm
H
10 µm
I
5 mm
F
5 µm
L
5 µm
10 µm
M
10 mm
N
5 mm
J
200 µm
K
1 mm
O
5 µm
P

5.0–20 mm long. Stipe cylindrical, sterile, yellow to yellow-greenish, 0.4–0.8 mm wide; terminating in slightly broader fertile heads, white or pale-yellow. Conidiophores up to 110 µm long, smooth-walled, hyaline, densely branched and swollen, terminating with a group of 2–4 divergent phialides. Phialides with a subglobose basal portion and a thin neck, 6.0–6.5 × 2.5 µm. Conidia hyaline, smooth-walled, cylindrical or slightly curved, 3.5–4.5 × 1.5–2.0 µm. Only anamorphic state observed.

Host: pupa of Lepidoptera.

Known distribution: widespread; Europe (Belgium, Brazil, France, Germany, Netherlands, UK; Samson 1974), Asia (Indonesia, Japan; Nikoh & Fukatsu 2000), North America (Bissett 1979), Australia, Africa (Ghana). South America: Argentina (new record), Brazil (D'Alessandro et al. 2013), Colombia (Castro-Pérez et al. 2013), Bolivia, Ecuador and Paraguay (http://mushroaming.com/).

Specimen examined: ARGENTINA: TUCUMAN Province, Chicligasta, Cochuna, Cuesta del Clavillo, 27°50'56"S 65°57'45"W, 16 April 2010, Robledo 2279 (CORD).

Remarks: *Isaria tenuipes* is a parasite of several Lepidoptera species; characterised by synnematous growth of the anamorphic state.

Samson (1974) considered all entomogenous *Isaria* with flask-shaped phialides tapering to thin long necks and catenate conidia as *Paecilomyces* and proposed several further combinations that generally have been accepted. Based on recent phylogenetic studies, most of the *Paecilomyces* Bainier species have been re-assigned in the genus *Isaria* (Luangsa-Ard et al. 2005, Gams et al. 2005, Hodge et al. 2005) and placed in the family Cordycipitaceae (Sung et al. 2007).

Spegazzini (1899) described two species of *Isaria* for Argentina: *I. argentina* Speg. (Buenos Aires and Chaco Provinces) and *Isaria arachnicida* Speg. (Buenos Aires Province). Samson (1974) studied Spegazzini's material and both species were synonymized with *Paecilomyces tenuipes* (Peck) Samson. This new record of *Cordyceps takaomontana* has been collected in the north-west of Argentina.

Metarhizium martiale Kepler, Rehner & Humber, Mycologia 106(4):811−829 (2014) Fig. 2 A–E

≡ *Cordyceps martialis* Speg., Bol. Acad. Nac. Cienc. Córdoba 11 (4): 535 (1889).

≡ *Metacordyceps martialis* (Speg.) Kepler, G.-H.Sung, & Spatafora, Mycologia 104 (1): 182–197 (2012).

Stromata cylindrical to clavate with acute apices, 14–20 mm long, red orange to ochre. Fertile parts rough due to presence of protruding dark ostioles, 1–2.5 mm wide. Stipe 1–1.5 mm thick. Perithecia ovoid, obclavate, immersed except for the apices, in an oblique arrangement, 500–750 × (200–)250–400 µm. Asci cylindric, 220–400 × 3–4.5 µm, apical apparatus 2.0–3.0 µm thick. Ascospores filiform, hyaline, multiseptate, partspores cylindrical, not easily separated, 6.0–9.0 × 1.0–1.5 µm. No conidial state observed.

Host: on larvae of Coleoptera.

Known distribution: Brazil (Spegazzini 1889). Brazil, Belize, Guyana, China, Russia (Mains 1958, Kobayasi & Shimizu 1982), Bolivia (http://mushroaming.com/

Rainforest_Cordyceps), China, Guyana, Japan, Russia (Sung & Owensby 2015). (G.-H.Sung, A.Owensby in *Cordyceps*.us/species/*Cordyceps*/martialis). Argentina: Previously known in the NW Yungas forests on Coleoptera larvae [as *C. submilitaris* Spegazzini (1909)].

Specimen examined: ARGENTINA: MISIONES Province, San Pedro (BAFC 32855); ibid., Gral. Belgrano, R.N.E. San Antonio. Margen Arroyo Pesado, 26°1'16"S 53°48'4.1"W, 490 m snm 16 April 2011, Niveiro & Popoff 2355 (CTES 0574921).

Remarks: Spegazzini (1889) originally described *Cordyceps martialis* from Brazil on larva of Cerambycidae (Coleoptera) and it has been commonly recorded on larvae of Coleoptera in the Neotropics (Mains 1959, Mushroaming.com; www.mycokey.com/Fungiecuador.html). Later, with the synonymy of *Cordyceps submilitaris* Henn., it has been accepted that this species can also parasitize larvae of Lepidoptera (Kepler et al. 2012a).

The material studied differs from the original description of *C. martialis* (Spegazzini 1889) in having shorter stromata (14–20 mm long. vs 40–50 mm long) and asci (220–400 µm vs 450–500 µm long). The material of *M. martiale* studied presents some differences when compared with the description by Kobayasi & Shimizu (1982). Our specimen has smaller stromata (14–20 mm vs 50 mm long), larger perithecia (500–750 µm vs 570–630 µm long), and has a sparse mycelial layer covering the host instead of covering it completely. Kobayasi & Shimizu (1982) suggested *Cephalosporium* Corda as the anamorphic state.

Ophiocordyceps amazonica (Henn.) G.H.Sung, J.M.Sung, Hywel-Jones & Spatafora, Stud. Mycol. 57: 40 (2007) Fig. 2 F–I

≡ *Cordyceps amazonica* Henn., Hedwigia 43: 247 (1904).

Stromata single, gregarious, capitate, 20–32 mm long. Fertile head globose, rough by protruding ostioles, brownish red, 2.0–3.5 mm diam. Stipe slightly reddish to yellowish, 0.5–1.0 mm wide. Perithecia immersed with dark red ostioles, narrowly ovoid, in perpendicular arrangement, 600–870 × 210–350 µm. Asci cylindrical, 200–350 × 3.0–5.0 µm, apical apparatus 3.0–3.5 × 4.5–5.0 µm. Ascospores filiform, hyaline, multiseptate, partspores cylindrical, 7.0–10 × 1.0–1.5 µm. No conidial state observed.

Host: grasshopper (Acrididae, Orthoptera).

Known distribution: Argentina (**new record**), Brazil, Colombia, Ecuador, Guyana (Sanjuan et al. 2015); Belize (Mains 1940, 1958); Bolivia (Mains 1959); Costa Rica (Ramirez Arias 1985).

Specimen examined: ARGENTINA: MISIONES Province, Bosque de Palo Rosa, 5 March 1980, Capelli M-3342 (BAFC 27023).

Remarks: The species was originally described on *Locusta* sp. (Orthoptera) from Brazil (Hennings 1904). Our material fits well with the current concept of the species (Sanjuan et al. 2015). Some differences were observed in comparison with the description given by Sanjuan et al. (2015); the material studied has slightly smaller structures, i.e. smaller stromata (20–32 mm vs 20–45 mm long), fertile head (2.0–3.5 mm vs 3–5.5 mm diam.), perithecia (600–870 µm vs (760–) 800–1100 µm long), asci (200–350

vs 325–450 µm long) and part-spores (7.0–9.9 µm vs 9–15 µm long). These differences suggest the possibility that the Argentinian specimen represents another taxon, but deeper studies including molecular data are needed to elucidate the situation. The material studied shares the host described by Sanjuan et al. (2015), namely imagos and adults of grasshoppers from families Acrididae and Romelidae, which are buried in the leaf litter. *Ophiocordyceps amazonica* is reported for the first time from Argentina, and constitutes the southernmost record of the species.

Ophiocordyceps australis (Speg.) G.H.Sung, J.M.Sung, Hywel-Jones & Spatafora, Stud. Mycol. 57: 40 (2007) Fig. 2 J–N

≡ *Cordyceps unilateralis* var. *australis* Speg., Anales Soc. Cient. Argent. 12: 215 (1881).

≡ *Cordyceps australis* (Speg.) Sacc., Syll. Fung. 2: 571 (1883).

Stromata solitary, simple or branched, bicolored, 34–42 mm long. Fertile head ovoid-subglobose, surface slightly rugose due to the ostioles, 2.5–3.0 × 1.5–2.0 mm. Stipe 0.4–0.7 mm wide, black to dark brown, becoming yellow orange to cream yellow in end portion of the stipe and fertile region. Perithecia immersed, cylindrical-conoid, inclined to the stromal surface, 420–750 × 170–250 µm. Asci cylindrical, 280–450 × 4.0–5.0 µm, apical apparatus 5.5–6.0 µm. Ascospores filiform, hyaline, multiseptate, breaking up into one-celled cylindrical-fusoid segments, 8.0–10 × 1.0–1.5(–2.0) µm. No conidial state observed.

Host: ant, *Pachycondyla striata* Fr.Smith (Hymenoptera, Formicidae, Ponerinae).

Ophiocordyceps australis is usually found parasitizing *Pachycondyla striata* in the Amazon region of Ecuador and Brazil; and also *Paltothyreus tarsatus* and *Bothroponera pachyderma* in West African forests (Samson et al. 1982). Sanjuan et al. (2001) found this species of fungus parasitizing *Paraponera clavata* and *Pachycondyla crassinoda* in the Amazon region of Colombia.

Known distribution: Argentina: Misiones (Mains 1959); Brazil (Spegazzini 1881, 1889, Möller 1901, Mains 1949); Colombia (Sanjuan et al. 2001, 2015); Ghana (Samson et al. 1982); Guyana, Uganda, Venezuela (Kobayasi 1941).

Specimens examined: ARGENTINA: MISIONES Province, General Manuel Belgrano, Reserva Natural Estricta, San Antonio, 29 April 2015, leg. Ramirez (CTES 0524834; CTES 0524835).

Remarks: the species was originally described as a variety, *Cordyceps unilateralis* (Tul.) var. a*ustralis* Speg. from Brazil on *Pachycondyla striata* (Formicidae, Ponerinae) (Spegazzini 1881), and later raised to specific rank (Saccardo 1883). Mains (1949) studied and described several species of *Cordyceps* on ants having been described with bicolored clavae from South America, among which are the specimens described by Spegazzini (1881, 1889), as the type specimen of *C. australis* (Speg.) Sacc. Sung et al. (2007) re-classified species that formerly were included in *Cordyceps* sensu Kobayasi & Mains, in the genus *Ophiocordyceps* as *O. australis*.

Ophiocordyceps australis has been suggested to be a host specialist parasitizing exclusively *Paltothyreus tarsatus* (Samson et al. 1982). Our material fits with the description of *C. australis* given by them Samson et al (1982) from Ghana. However,

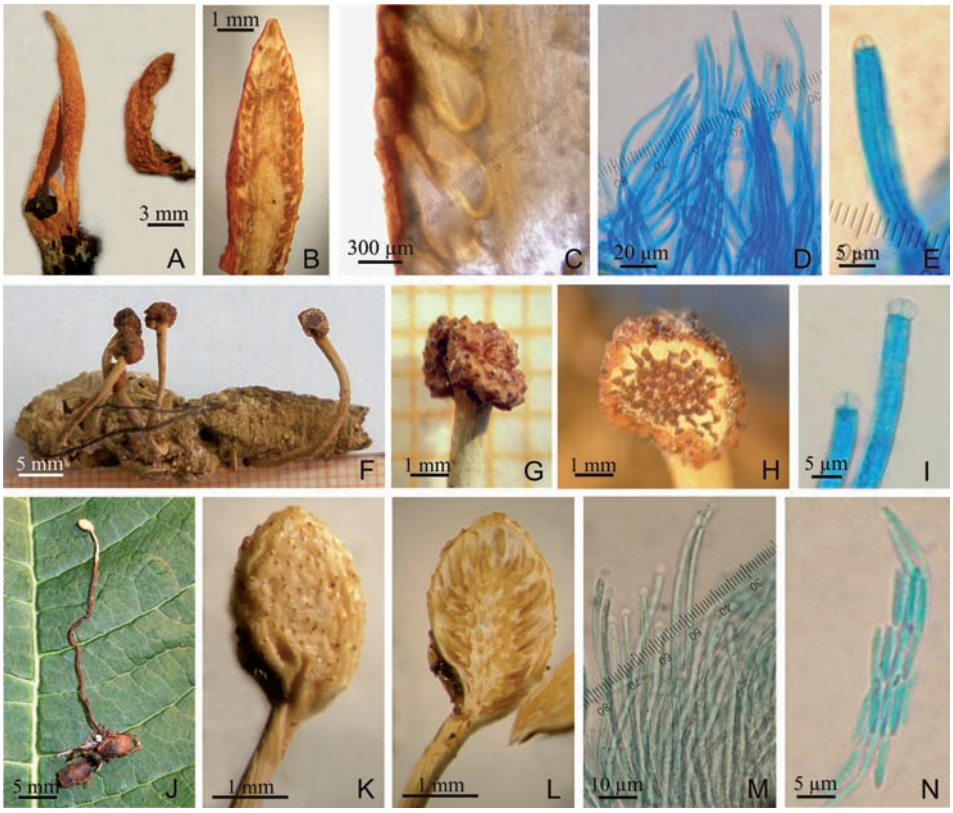

Fig. 2. A–E: *Metarhizium martiale*. A. Stromata on larvae of Coleoptera. B. Section through stroma. C. Section of perithecia in stroma, in an oblique position. D. Asci. E. Ascus and ascus apex. F–I: *Ophiocordyceps amazonica*. F. Capitate stromata on grasshopper, Orthoptera. G. Detail of fertile part. H. Section of immersed perithecia. I. Asci, apical apparatus. J–N: *Ophiocordyceps australis*. J. Stroma on ant *Pachycondyla striata*. K. Detail of fertile part. L. Section through stromata. M. Asci. N. Ascospores.

there are differences in stromata [34–42 µm vs 15–35(–55) µm long], bigger perithecia (420–750 × 170–250 µm vs 480–960 × 90–380 µm), thicker asci (5.5–6.0 µm vs 4–5 µm) that indicate the need of molecular data for assessing the significance of these deviations.

According to the description given by Mains in 1949, our material differs in having smaller perithecia (420–750 × 170–250 µm vs 720–960 × 230–336 µm) and asci (280–450 µm vs 500–720 µm long). Mains (1959) studied a collection on ants from Argentina, Misiones Province, and identified it as *Cordyceps australis*. Hence, this is the second record from Argentina.

Ophiocordyceps melolonthae (Tul. & C.Tul.) G.H.Sung, J.M. Sung, Hywel-Jones & Spatafora, Stud. Mycol. 57: 44 (2007). Fig. 3 A–F

≡ *Torrubia melolonthae* Tul. & C.Tul., Sel. Fung. Carpol. 3: 12 (1865).

≡ *Cordyceps melolonthae* (Tul. & C.Tul.) Sacc., Michelia 1 (3): 320 (1878).

Stromata gregarious, subcylindric to clavate, up to 60 mm long., yellow to yellowish brownish. Fertile head clavate to subcylindrical, slightly rough through the protruding perithecial ostioles, yellowish brownish, 16–20 × 2.5–5 mm. Stipe pale yellowish, 2–3 mm wide. Perithecia embedded in ordinal orientation, ovoid, 400–490 × 180–220 µm. Asci cylindric, (150–)200–230 × 5.5–7.5(–8.0) µm, apical apparatus 3.5–4.0 µm thick. Ascospores filiform, hyaline, multiseptate, partspores cylindrical, 4.5–7.5 × 1.0–1.5(–2.0) µm. No conidial state observed.

Host: larvae of Melolonthidae (Coleoptera).

Known distribution: Argentina (**new record**), Belize, Brazil, Costa Rica, West Indies, USA (Kobayasi 1941); Brazil, Guyana, Nicaragua, Trinidad, USA (Mains 1958); Ecuador (Evans et al. 1999); Mexico (Pérez Silva 1977; Guzmán et al. 2001); Bolivia, Colombia (http://mushroaming.com/Rainforest_Cordyceps).

Specimen examined: ARGENTINA: MISIONES Province, no date and data collector (BAFC 28579).

Remarks: *Cordyceps melolonthae* was first described by Tulasne & Tulasne (1865) as *Torrubia melolonthae* on *Melolonthae* larvae in Pennsylvania. Subsequently, Sung et al. (2007) reassigned the species to the new genus *Ophiocordyceps* as *O. melolonthae*. This fungus is considered one of the most pathogenic to Scarabaeidae, and there have been many attempts to use it as an agent for biological control of melolonthid pests (Salgado-Neto et al. 2015). The studied material matches the description of Mains (1958), who reported this species as parasitic on larvae of Coleoptera. This author designated two varieties: var. *melolonthae*, a pathogen of the June bug or May beetle (*Phyllophaga* spp.) in the USA and var. *rickii* (Lloyd) Mains, restricted to tropical North and South American lamellicorns. Guzmán et al. (2001) recorded and described the species *C. melolonthae* var. *rickii* on larvae of *Enema endymion* (Coleoptera, Melolonthidae) in different states of Mexico, in subtropical rain forests and coffee plantations. Salgado-Neto et al. (2015) reported it for the first time in Brazil on the larvae of the white grub *Diloboderus abderus*.

Ophiocordyceps melolonthae is reported for the first time from Argentina, in Misiones Province, where the vegetation is subtropical forest, which represents the greatest animal and plant biodiversity in the country.

Ophiocordyceps nutans (Pat.) G.H.Sung, J.M.Sung, Hywel-Jones & Spatafora, Stud. Mycol. 57: 45 (2007) Fig. 3 G–M

≡ *Cordyceps nutans* Pat., Bull. Soc. Mycol. France 3: 127 (1887).

≡ *Cordyceps bicephala* subsp. *nutans* (Pat.) Moureau, Mém. Inst. Roy. Colon. Belge 7: 47 (1949).

Stromata one to two, simple, elongated, up to 50 mm long, with an acute apex. Fertile parts oblong, with apex acute, smooth, yellowish orange, to yellowish brown toward the base, 8–10 × 0.35–1 mm. Stipe blackish brown to black, long straight or curved,

longitudinally furrowed, 48 × 0.8–1.4 mm. Perithecia immersed, ovoid to conical, obliquely orientated with a curved neck, light yellow to yellowish brown, 350–750 × 125–200 µm. Asci cylindrical, up to 450 × 5–6 µm, apical apparatus 5–7 × 6–7 µm. Ascospores filiform, hyaline, multiseptate, partspores subfusiform, 7–9 × 0.9–1.5 µm. Anamorph not observed.

Host: on adult stinkbug (Hemiptera, Pentatomidae).

Known distribution: Argentina: Misiones (Mains 1959); Belgium, Brazil, China, Colombia, Costa Rica, Ecuador, Ghana, Guyana, Indonesia, Japan, Korea, Madagascar, Malaysia, Panama, Papua New Guinea, Peru, Philippines, Taiwan, Thailand, Vietnam (Chen & Sung 2015); Ghana (Samson & Evans 1975); Siberia, Colombia (Kobayasi 1981b, Sanjuan et al. 2015); Japan, Taiwan, China and Papua New Guinea (Sasaki et al. 2004).

Specimen examined: ARGENTINA: MISIONES Province, San Pedro, Tobuna, 4 November 2009, Robledo 1954 (CORD).

Remarks: *Ophiocordyceps nutans* was originally described as *Cordyceps nutans* by Patouillard (1887) from a specimen on adult stinkbug (Hemiptera) from Japan. *Ophiocordyceps nutans* shows host specificity, being recorded only on hemipteran bugs (Hywel-Jones 1995, Sasaki et al. 2004) and is one of the most common species of *Cordyceps* s.l. (Sung 1996). Sasaki et al. (2012) investigated the host specificity of *O. nutans* against hemipteran insects in Japan. Their results suggest that *O. nutans* is highly host specific demonstrating its value as a biological control agent.

Mains (1959) studied material from Misiones Province, and identified it as *C. nutans* on *Edessa* sp. (Pentatomidae). Our collection is from the same place, and is the second report for Argentina. Our material differs from that described by Mains (1959) in having shorter stromata (up to 50 mm vs up to 75 mm long), perithecia (up to 750 x 200 µm vs up to 1100 x 300 µm) and asci (up to 450 x 5–6 µm vs 500–650 x 6–7 µm). This is due possibly to differences in the age of the stromata. Furthermore, in our material, the stipe is smooth, but longitudinally furrowed, as described by Shrestha & Sung (2005).

Samson & Evans (1975), in their study on entomogenous fungi from Ghana, collected *C. nutans* and its anamorph *Hymenostilbe nutans*. They described morphological features of *H. nutans* but were unable to isolate it from the conidia. Hywel-Jones (1995) reported *Hymenostilbe nutans* for the first time from Thailand, and re-described it from fresh material and its association with the teleomorph. Recently, Wang et al. (2015) identified and described a synnematous fungus, *Polycephalomyces yunnanensis*, as a new species that parasitizes the stromata of *Ophiocordyceps nutans* and stink bugs (hemipteran adults).

Purpureocillium atypicola (Yasuda) Spatafora, Hywel-Jones & Luangsa-Ard, IMA Fungus 6 (2): 361 (2015) Fig. 3 N–P

≡ *Isaria atypicola* Yasuda, Bot. Mag., Tokyo 29: 117 (1915).

= *Nomuraea atypicola* (Yasuda) Samson, Stud. Mycol. 6: 84 (1974).

= *Spicaria atypicola* (Yasuda) Petch, Trans. Brit. Mycol. Soc. 23: 140 (1939).

= *Cordyceps cylindrica* Petch, Trans. Brit. Mycol. Soc. 21: 46 (1937).

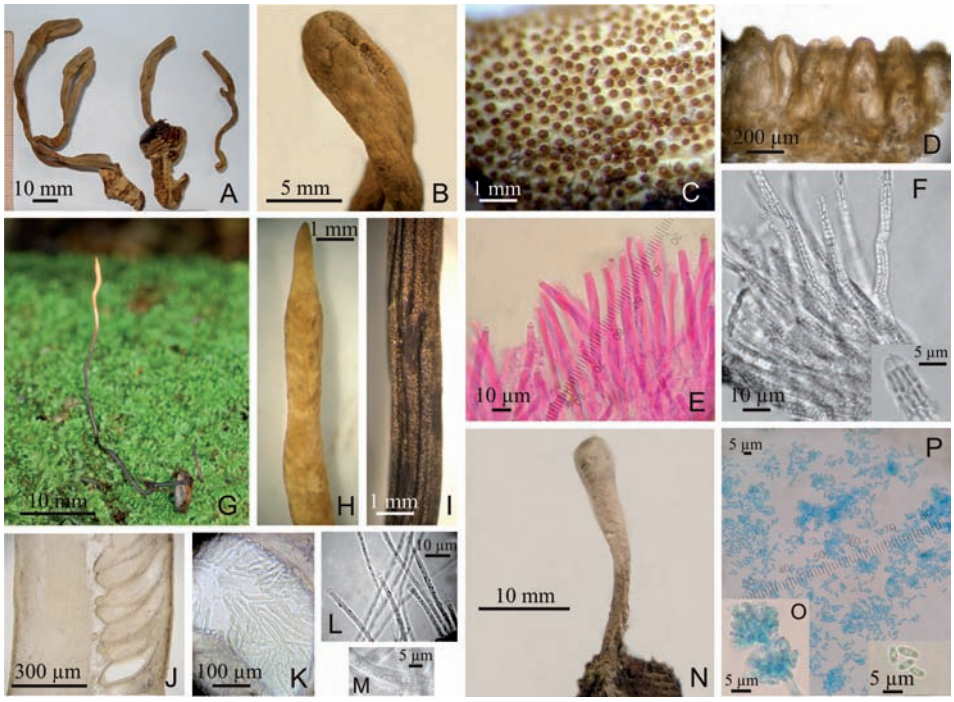

Fig. 3. A–F: *Ophiocordyceps melolonthae*. A. Stromata on larvae of Coleoptera. B. Clavate fertile part. C. Detail of apices of perithecia. D. Section showing perithecia embedded, except the apices. E. Asci. F. Asci and partspores. G–M: *Ophiocordyceps nutans*. G. Stroma on adult stinkbug. H. Fertile part, apex acute. I. Stipe, longitudinally furrowed. J. Section of stroma, perithecia obliquely orientated with curved necks. K. Perithecium and asci, detail. L. Asci, detail apical apparatus. M. Multiseptate ascospores. N–P: *Purpureocillium atypicolum*. N. Synnema on spider. O. Conidiophore with dense clusters of branches and phialides. P. Conidia.

Synnemata erect, cylindric clavate, 25 mm long, 1–2 mm thick, pale grey lavender. Fertile part roughened, velvety, with round apex, 8–9 × 2–2.5 mm, with conidiogenous structures. Conidiophores densely grouped, up to 155 µm long, 3.5–4.5 µm wide, forming dense clusters of branches and phialides. Branches 5–6 × 3–4.5 µm. Phialides cylindrical, 3–4.5 × 2.5–3.5 µm. Conidia cylindrical, smooth, hyaline, 4–5 × 1.5–2 µm. Teleomorph not observed.

Host: spider, Nemesiidae, Mygalomorphae (Opistothelae, Araneae).

Purpureocillium atypicola occurs on a wide range of soil dwelling mygalomorphs, and it has also been found on araneomorph spiders (Coyle et al. 1990).

Known distribution: Argentina: Salta, Tucumán (Coyle et al. 1990); Ghana, USA, Japan (Samson 1974); Brazil (Greenstone et al. 1987); Panama (Nentwig 1985); Thailand (Hywel-Jones & Sivichai 1995).

Specimens examined: ARGENTINA: MISIONES Province, Parque Nacional Iguazú, Cataratas, 3 March 1980, Capelli M-3286 (BAFC 27025).

Remarks: *Nomuraea atypicola* was first described from Japan, one of the most common pathogenic fungi attacking spiders (Samson 1974). It is the asexual morph of *Cordyceps cylindrica* Petch (Kobayasi & Shimizu 1978, Hywel-Jones & Sivichai 1995) and its phylogenetic relationships remains unknown (Sung et al. 2007). Detailed phylogenetic analysis showed that *N. atypicola*, *Paecilomyces lilacinus* and *Isaria takamizusanense* are closely related (Sung et al. 2007, Luangsa-Ard et al. 2011). Luangsa-Ard et al. (2011) proposed the new genus *Purpureocillium* Luangsa-Ard, Hywel-Jones, Houbraken & Samson in the family Ophiocordycipitaceae to accommodate them. Quandt et al. (2014) recognized six genera within Ophiocordycipitaceae (*Drechmeria*, *Harposporium*, *Ophiocordyceps*, *Polycephalomyces*, *Purpureocillium* and *Tolypocladium*), based on an exhaustive phylogenetic reconstruction and taxonomically detailed revision of the family. Spatafora et al. (2015) introduced new species combinations for the family Ophiocordycipitaceae, based on the taxonomic and nomenclatural recommendations of Quandt et al. (2014) and previous phylogenetic analyses (e.g., Sung et al. 2007, Luangsa-Ard et al. 2011). *Nomuraea atypicola* was re-classified in the genus *Purpureocillium* as *P. atypicolum*.

The host of *P. atypicola* studied by Coyle et al. (1990) in north-west Argentina (Salta and Tucumán Provinces) was identified as *Actinopus* trapdoor spiders. The specimen examined here was collected in Misiones Province and agrees with Coyle's description of the host. It is the first report for this province.

Discussion

This study is the first survey carried out in protected areas of Argentina and revealed three new records: *Cordyceps pseudomilitaris*, *Ophiocordyceps amazonica*, and *Ophiocordyceps melolonthae* (Table 1). Most of the material studied (herbarium specimens and own collections) were found in subtropical humid ecosystems such as Yungas forests (Northwest) and Atlantic rain forests (Northeast) (Fig. 4). The occurrence of *O. amazonica* in this latitude suggests that it could be the southernmost limit of its distribution. This pattern has been observed in wood-decay fungi also (Robledo et al. 2014).

Until now, the southernmost record of *Cordyceps* s.l. was from the temperate *Nothofagus* forests of Patagonia. It was identified as *Cordyceps militaris* (Mueller & Rajchenberg 1991) (Fig. 4).

Hitherto, our knowledge of *Cordyceps* has been concentrated in the temperate and tropical region of Asia and in the Amazon region in South America. This preliminary study demonstrates the paucity of knowledge of this group in the southern hemisphere and it does not yet represent the real diversity in Argentina. Methodical sampling efforts throughout Argentina and Chile will be necessary in order to know what could be the real southernmost distribution of *Cordyceps* s.l.

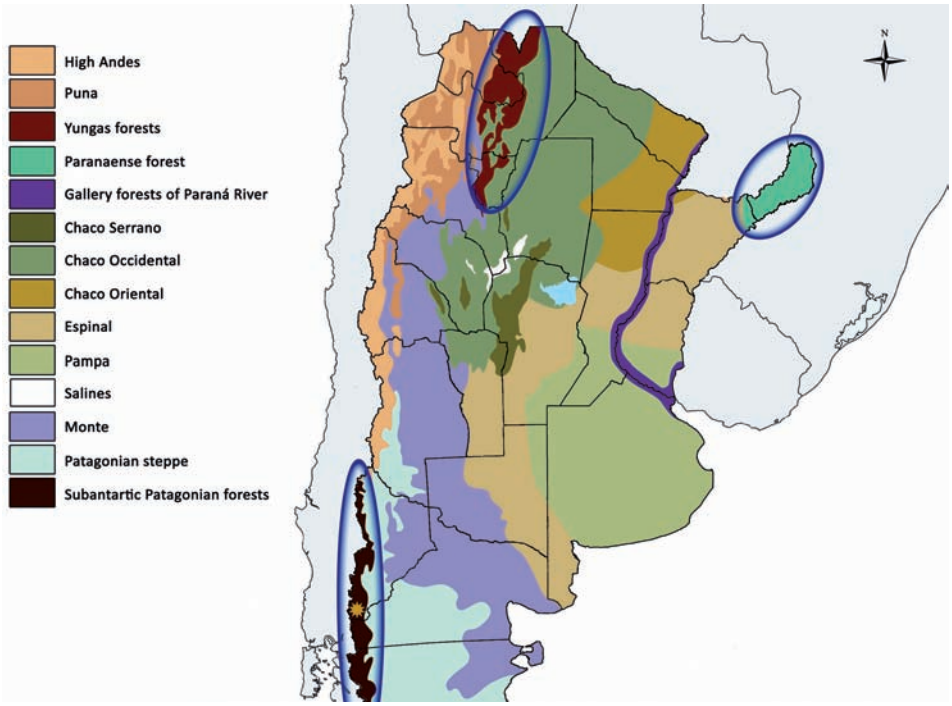

Fig. 4. Phytogeographic regions of Argentina (modified from Rajchenberg & Robledo 2013). Circled areas in the North represent areas where most of the records of *Cordyceps* s.l. occur. *Cordyceps militaris*, yellow star, is the only species recorded in Patagonian Forests.

Key to *Cordyceps* s.l. species found in Argentina

1 Associated with spiders 2
1' Associated with insects 4

2 Teleomorphic stromata absent, synnemata erect, cylindrical claviform, grey-pale lavender; conidiophores densely grouped, forming clusters of branches and phialides; conidia cylindrical, smooth, hyaline, 4–5 × 1.5–2 µm ..... ***Purpureocillium atypicola***
2' Teleomophic stromata present 3

3 Stromata simple, cylindricic to clavate, up to 20 mm long, light brown to red orange ..... ***Cordyceps singeri***
3' Stromata simple or branched, slender, up to 60 mm long, acuminate at the apices, purplish brown ..... ***Cordyceps ignota***

4 Parasitizing immature stage of insects 5
4' Parasitizing adult stage of insects 13

5 Teleomorph absent. Synnemata gregarious, simple or irregularly branched, yellow to yellow-greenish, terminating in fertile head, white or pale-yellow. On pupa of Lepidoptera ..... ***Cordyceps takaomontana***
5' Teleomorph present 6

6 Fertile head with perithecia sub-immersed or pseudo-immersed 7
6' Fertile head with perithecia immersed in a pseudo-parenchyma tissue 9

7 Stromata cespitose, narrowly clavate, long slender cylindric, 30–44 mm long. Synnemata present or absent ..... ***Cordyceps polyarthra***
7' Stromata not cespitose. Synnemata absent 8

8 On pupa of Lepidoptera. Rhizomorphs absent, ascospores breaking into numerous smaller part-spores, stromata 8–70 mm long, asci 300–510 × 3.5–5 µm ..... ***Cordyceps militaris***
8' On larva of Lepidoptera. Rhizomorphs present, ascospores not breaking into part-spores, stromata 12–25 mm long. Asci 210–395 × 5–6 µm..... ***Cordyceps pseudomilitaris***

9 Stromata ovoid capitate, red brownish with red dark ostioles 10
9' Stromata in other arrangement 11

10 On larva of butterflies (Lepidoptera: Papilioidae) ..... ***Ophiocordyceps gracilis***
10' On imago of grasshopper (Orthoptera: Acridoidea) ..... ***Ophiocordyceps amazonica***

11 Stromata acuminate, rough surface, with dark ostioles, perithecia oblique, on larva of beetle (Coleoptera: Elateridae)..... ***Metarhizium martiale***
11' Stromata clavate with other characters 12

12 On larva of beetle (Coleoptera: Scarabeidae). Stromata with fertile head slightly differentiated, yellow to yellowish brownish ..... ***Ophiocordyceps melolonthae***
12' On nymph of cicada (Hemiptera: Cicadidae). Stromata with fertile head cylindrical, purple brown ..... ***Ophiocordyceps sobolifera***

13 Stromata bicolored..... 14
13' Stromata not distinctly bicolored..... 16

14 On beetles (Coleoptera: Curculionidae) ..... ***Ophiocordyceps curculionum***
Ophiocordyceps curculionum exclusively infects Curculionidae
14' On ants (Hymenoptera) and stinkbugs (Hemiptera)..... 15

15 Fertile head narrowly fusoid with an acute apex, stipe longitudinally furrowed, 0.8–1.4 mm thick, on adult stinkbugs (Hemiptera, Pentatomidae)..... ***Ophiocordyceps nutans***
15' Fertile head ovoid-subglobose, stipe not longitudinally furrowed, 0.4–0.7 mm thick, on bullet ants (Hymenoptera, Ponerinae) ..... ***Ophiocordyceps australis***

16 Stromata with lateral fertile head..... ***Ophiocordyceps unilateralis* complex**
Ophiocordyceps unilateralis species complex of specific on *Camponotus* ants, Hymenoptera: Formicidae, Formicinae
16' Stromata with terminal fertile head 17

17 On flies (Diptera: Tabanidae, Tachinidae). Stromata usually paired, short and light yellow. Perithecia in parallel orientation ..... ***Ophiocordyceps dipterigena***
17' On wasps (Hymenoptera: Vespidae). Stromata usually single, long, light cream to brownish yellow, perithecia in oblique orientation..... ***Ophiocordyceps sphaecocephala***

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