

## MORPHOLOGICAL AND PHYLOGENETIC STUDY OF *OPHIOCORDYCEPS SPHECOCEPHALA* AND *OPHIOCORDYCEPS* *ASIANA* FROM VIETNAM

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**Abstract.** *Ophiocordyceps* is a megagenus of entomopathogenic fungi belonging to Ophiocordycipitaceae of Hypocreales, Ascomycota. We report here the morphological and phylogenetic analyses of two *Ophiocordyceps* species from Bidoup Nui Ba National Park, Lam Dong Province, southern Vietnam. Our data showed that one of our studied *Ophiocordyceps* is a new intraspecies of *O. sphecocephala* and another is a new record of *O. asiana* from Vietnam.

**Keywords:** *Bidoup Nui Ba*, *D1–D2*, *insect fungi*, *ITS*, *species*

### Introduction

The genus *Ophiocordyceps* (Hypocreales, Ascomycota) comprises fungal species that exclusively parasitize members of arthropods, kill them and ultimately grow on their cadavers. *Ophiocordyceps sinensis*, growing on moth larvae in the alpine grasslands of Himalayan range and the Tibetan Plateau, is used in traditional oriental medicine to treat kidney diseases, asthma and lung infection (Paterson, 2008). Recent publications have also reported roles of *Ophiocordyceps* species in immunomodulation; cellular apoptosis; anticancer, lung, hepatic and renal support (Paterson, 2008; Zhou et al., 2009; Tuli et al., 2013; Wu et al., 2016). Such properties therefore generate interest in the usage of these fungi as potential sources of bioactive compounds (Shrestha and Sung, 2005; Wang and Yao, 2011; Sasaki et al., 2012; Shrestha et al., 2017; Xiao et al., 2019).

*Ophiocordyceps* have a worldwide distribution in ecosystem, ranging from sea level up to 5000 m above sea level (Shrestha and Sung, 2005; Li et al., 2011; Araújo et al., 2015; Xiao et al., 2019). The biodiversity of *Ophiocordyceps* is highly endangered due to intensive collection, deforestation and climate change (Hopping et al., 2018; Wei et al., 2021). Hence, study on *Ophiocordyceps* species is essential to provide valuable information for biodiversity monitoring and conservation of these fungi.

Bidoup Nui Ba National Park is located in the northern part of Lam Dong Province, which lies in the Central Highlands of southern Vietnam. In 2005, UNESCO recognized Bidoup Nui Ba as the core zone of Langbiang Biosphere Reserve due to its rich

biodiversity. We describe here two species of *Ophiocordyceps* collected in Bidoup Nui Ba National Park, using morphological characteristics and phylogenetic analyses of ribosomal sequences (D1–D2 and ITS).

## Materials and methods

### *Field collection*

Specimens of *Ophiocordyceps* species were collected in August 2019 and August 2020 in Bidoup Nui Ba National Park (12°00'00" to 12°52'00" N, 108°17'00" to 108°42'00" E) (Fig. 1). The light intensity and relative humidity at the sampling areas were measured using an environmental meter (Extech 45170, Taiwan). All the collected specimens were primarily grouped based on the host insects, one group growing on wasps and the others on bugs. These specimens were either kept in sterile sampling boxes, at 4°C for further analysis or air dried and deposited in the Herbarium of Faculty of Science, Nong Lam University, Ho Chi Minh City, Vietnam (<http://sweetgum.nybg.org/>, NLU).

### *Morphological observations*

Thirty stromata of each group were observed for morphological measurements. For the microscopic measurements, cross sections of the fertile heads were mounted in sterile distilled water and observed under Olympus CX22 microscope (Olympus, Tokyo, Japan).

### *DNA extraction and sequencing*

DNA was extracted from the specimens using CTAB method (Wu et al., 2001). The D1–D2 region of the 28S rRNA subunit was amplified using NL1/NL4 primer pairs (O'Donnell, 1993). Similarly, the ITS sequence was amplified using ITS1/ITS4 primer pairs (White et al., 1990).

DNA amplification was performed in 35 cycles with a ProFlex PCR System (Thermo Fisher Scientific, MA, USA), each cycle consisting of 3 min at 95°C, 30 sec at 55°C and 2 min at 72°C. High fidelity DNA polymerase (BioFact™ H–Star, Korea) was used for the amplification. The PCR reaction mixture was prepared according to the manufacturer's instruction and the PCR products were kept at 4°C until used further.

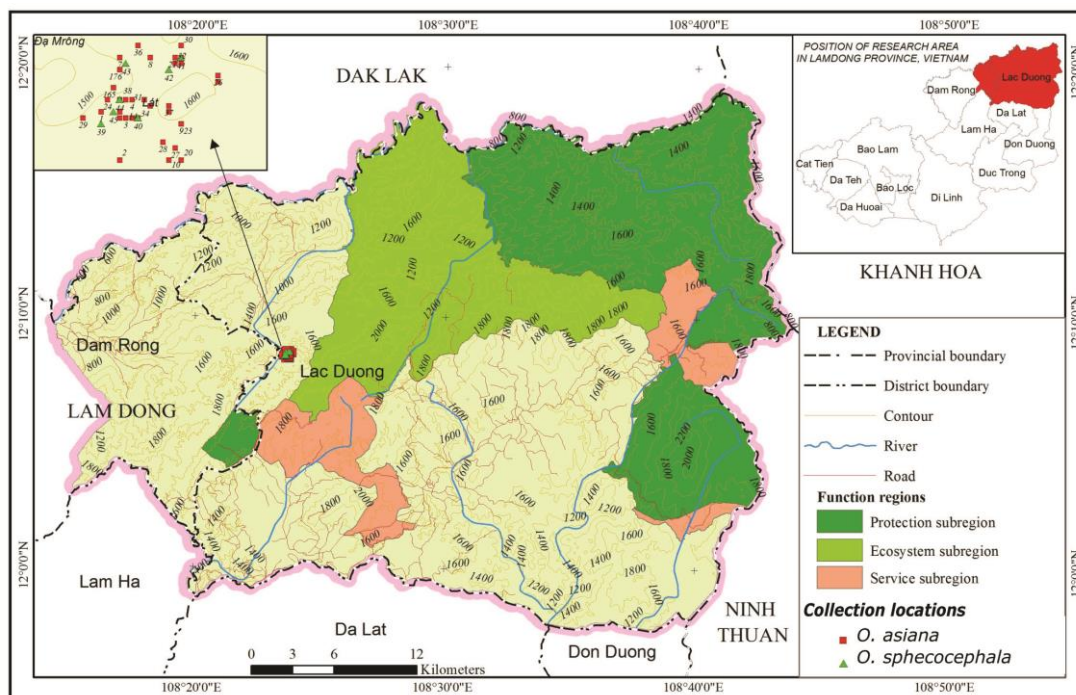
The DNA fragments were purified using a PCR purification kit (MEGAquick–spin™ Plus Total Fragment DNA Purification Kit, Intron, MA, USA). The resulted purified fragments were subsequently sequenced using an ABI 3500 genetic analyzer (Thermo Fisher Scientific, MA, USA) with a BigDye® Terminator v3.1 Cycle Sequencing Kit. The sequenced data were deposited in GenBank with accession numbers.

Preliminary species identification was performed using nBLAST against the GenBank nucleotide database (NCBI, Bethesda MD, USA). To evaluate phylogenetic relationships of Vietnamese specimens with closely related *Ophiocordyceps* species (Table 1), we conducted multiple sequence alignments using TCoffee (<http://tcoffee.org.cat>) with manual corrections using BioEdit (Hall, 1999; Notredame et al., 2000). The alignments were deposited in TreeBASE under accession number ID 28946. Phylogenetic analyses were conducted using RAxML–HPC2 on XSEDE (<https://www.phylo.org>) (Stamatakis, 2014) with 1000 bootstrap replicates. Default parameters were used under a GTR + G + I model. The tree with the highest likelihood was obtained. The Bayesian inference was performed using MrBayes v.3.2.7a (Ronquist et al., 2012) on XSEDE using default parameters. The outputs were then imported into FigTree v1.4.3 for viewing the phylogenetic trees.

**Table 1.** List of D1–D2 and ITS sequences used in this phylogenetic analysis. Vietnamese *Ophiocordyceps* sequences are indicated in bold

Accession No.					
Voucher	D1–D2	ITS	Country	Species	Reference
BCC86880	MW280210	MW285716	Thailand	<i>O. asiana</i>	(Khao-ngam et al., 2021)
BCC82789	MW280203	MW285710	Thailand	<i>O. asiana</i>	(Khao-ngam et al., 2021)
BCC84229	MW280199	MW285706	Thailand	<i>O. asiana</i>	(Khao-ngam et al., 2021)
BCC84230	MW280200	MW285707	Thailand	<i>O. asiana</i>	(Khao-ngam et al., 2021)
BCC84234	MW280201	MW285708	Thailand	<i>O. asiana</i>	(Khao-ngam et al., 2021)
BCC84235	MW280202	MW285709	Thailand	<i>O. asiana</i>	(Khao-ngam et al., 2021)
BCC86436	MW280211	MW285717	Thailand	<i>O. asiana</i>	(Khao-ngam et al., 2021)
BCC86440	MW280212	MW285718	Thailand	<i>O. asiana</i>	(Khao-ngam et al., 2021)
BCC86875	MW280204	MW285711	Thailand	<i>O. asiana</i>	(Khao-ngam et al., 2021)
BCC86876	MW280205	MW285712	Thailand	<i>O. asiana</i>	(Khao-ngam et al., 2021)
BCC86878	MW280207	MW285713	Thailand	<i>O. asiana</i>	(Khao-ngam et al., 2021)
BCC86879	MW280208	MW285714	Thailand	<i>O. asiana</i>	(Khao-ngam et al., 2021)
BCC86880	MW280210	MW285716	Thailand	<i>O. asiana</i>	(Khao-ngam et al., 2021)
<b>NLU202011</b>	<b>MT235757</b>	<b>MW684019</b>	<b>Vietnam</b>	<b><i>O. asiana</i></b>	<b>This study</b>
<b>NLU202012</b>	<b>MT235758</b>	<b>MW684020</b>	<b>Vietnam</b>	<b><i>O. asiana</i></b>	<b>This study</b>
<b>NLU202013</b>	<b>MT235759</b>	<b>MZ255516</b>	<b>Vietnam</b>	<b><i>O. asiana</i></b>	<b>This study</b>
<b>NLU202014</b>	<b>MT235760</b>	<b>MW684021</b>	<b>Vietnam</b>	<b><i>O. sphecocephala</i></b>	<b>This study</b>
<b>NLU202015</b>	<b>MT235761</b>	<b>MZ255517</b>	<b>Vietnam</b>	<b><i>O. asiana</i></b>	<b>This study</b>
MY11785	MW280209	MW285715	Thailand	<i>O. asiana</i>	(Khao-ngam et al., 2021)
MY11878	MW280213	MW285719	Thailand	<i>O. asiana</i>	(Khao-ngam et al., 2021)
MY11884	MW280216	MW285720	Thailand	<i>O. asiana</i>	(Khao-ngam et al., 2021)
HUA186097	KC610765		Columbia	<i>O. australis</i>	(Sanjuan et al., 2015)
Ophaus1780		KP200888	Columbia	<i>O. australis</i>	(Sanjuan et al., 2015)
MFLU17.1961	NG064484		Thailand	<i>O. cylindrospora</i>	GenBank
BCC82256	MH028157		Thailand	<i>O. granospora</i>	(Araújo et al., 2020)
BCC82793		MH028141	Thailand	<i>O. irangiensis</i>	(Khonsanit et al., 2019)
NBRC101399	JN941425	JN943334	Thailand	<i>O. irangiensis</i>	(Sanjuan et al., 2015; Schoch et al., 2012)
NBRC101400	JN941426	JN943335	Thailand	<i>O. irangiensis</i>	(Sanjuan et al., 2015; Schoch et al., 2012)
NBRC101401	JN941427	JN943336	Thailand	<i>O. irangiensis</i>	(Sanjuan et al., 2015; Schoch et al., 2012)
NHJ10945		GU723767	Thailand	<i>O. irangiensis</i>	(Luangsa-Ard et al., 2011)
NHJ3		AJ786566	Thailand	<i>O. irangiensis</i>	(Stensrud et al., 2005)
OSC 128579	EF469076		Thailand	<i>O. irangiensis</i>	(Sanjuan et al., 2015)
BUO537	MH879600		China	<i>O. myrmecophila</i>	(Zihong et al., 2019)
MFLU16.2913	MF372586		Thailand	<i>O. myrmecophila</i>	(Xiao et al., 2017)
FMF88		KX197242	Brazil	<i>O. neonutans</i>	(Friedrich et al., 2018)
KEL110		KX197240	Brazil	<i>O. neonutans</i>	(Friedrich et al., 2018)
KEL113		KX197239	Brazil	<i>O. neonutans</i>	(Friedrich et al., 2018)
KEL114		KX197241	Brazil	<i>O. neonutans</i>	(Friedrich et al., 2018)
KEL138		KX197243	Brazil	<i>O. neonutans</i>	(Friedrich et al., 2018)
03Y3		AB544452	Japan	<i>O. nutans</i>	(Sasaki et al., 2012)
06Fuka3		AB544463	Japan	<i>O. nutans</i>	(Sasaki et al., 2012)
06Fuka7		AB544467	Japan	<i>O. nutans</i>	(Sasaki et al., 2012)

Accession No.					
Voucher	D1–D2	ITS	Country	Species	Reference
06Tank1		AB544473	Japan	<i>O. nutans</i>	(Sasaki et al, 2012)
06Tank11		AB544478	Japan	<i>O. nutans</i>	(Sasaki et al, 2012)
06Tank21		AB544485	Japan	<i>O. nutans</i>	(Sasaki et al, 2012)
06Tank22		AB544486	Japan	<i>O. nutans</i>	(Sasaki et al, 2012)
06Yak2		AB544489	Japan	<i>O. nutans</i>	(Sasaki et al, 2012)
06Yak3		AB544490	Japan	<i>O. nutans</i>	(Sasaki et al, 2012)
06Yaka1		AB544491	Japan	<i>O. nutans</i>	(Sasaki et al, 2012)
AUoO113.78		AJ786583	Thailand	<i>O. nutans</i>	(Stensrud et al, 2005)
G97035		AJ309367	China	<i>O. nutans</i>	(Sasaki et al, 2012)
GDGM20887		JX177484	China	<i>O. nutans</i>	GenBank
Iso1		AJ536560	China	<i>O. nutans</i>	(Sasaki et al, 2012)
KA12.1247		KR673498	Korea	<i>O. nutans</i>	(Kim et al, 2015)
KA12.1340		KR673559	Korea	<i>O. nutans</i>	(Kim et al, 2015)
NBRC100944	JN941428		Japan	<i>O. nutans</i>	(Ban et al, 2015)
NBRC101749		AB968408	Japan	<i>O. nutans</i>	(Sasaki et al, 2012)
Oph994	KJ917567		Columbia	<i>O. nutans</i>	(Sanjuan et al, 2015)
OSC110994	DQ518763		n/a	<i>O. nutans</i>	(Sanjuan et al, 2015)
T37		AB366634	Japan	<i>O. nutans</i>	(Sasaki et al, 2012)
T62		AB366626	Japan	<i>O. nutans</i>	(Sasaki et al, 2012)
T70		AB366623	Japan	<i>O. nutans</i>	(Sasaki et al, 2012)
MRCIF53		EU573348	Thailand	<i>O. oxycephala</i>	(Qu et al, 2018)
Iso6578		AJ536548	China	<i>O. polyarthra</i>	(JiaJun et al, 2021)
20877		AJ536550	China	<i>O. sphecocephala</i>	(Tian et al, 2010)
MRCIF54		EU573347	Thailand	<i>O. sphecocephala</i>	GenBank
NBRC 101416	JN941443		Thailand	<i>O. sphecocephala</i>	(Sanjuan et al, 2015)
NBRC 101752	JN941445		Japan	<i>O. sphecocephala</i>	(Ban et al, 2015)
NBRC101414	JN941441	JN943443	Thailand	<i>O. sphecocephala</i>	(Sanjuan et al, 2015; Schoch et al, 2012)
NBRC101415	JN941442		Thailand	<i>O. sphecocephala</i>	(Sanjuan et al, 2015)
NBRC101752	JN941445	JN943351	Japan	<i>O. sphecocephala</i>	(Ban et al, 2015; Schoch et al, 2012)
NBRC101753	JN941446	JN943350	Japan	<i>O. sphecocephala</i>	(Ban et al., 2015; Schoch et al., 2012)
NHJ4224		GU723778	Thailand	<i>O. sphecocephala</i>	(Luangsa-Ard et al., 2011)
OSC 110998	DQ518765		Thailand	<i>O. sphecocephala</i>	(Sanjuan et al., 2015)
BCC79226	MW280219	MW285723	Thailand	<i>O. tessaratomidarum</i>	(Khao-ngam et al., 2021)
MY10827	MW280217	MW285721	Thailand	<i>O. tessaratomidarum</i>	(Khao-ngam et al., 2021)
MY10830	MW280218	MW285722	Thailand	<i>O. tessaratomidarum</i>	(Khao-ngam et al., 2021)
MFLU16.2908	MF362990		Thailand	<i>O. thanathonensis</i>	(Xiao et al., 2017)
NBRC106968	AB968423		Japan	<i>O. tricentri</i>	(Ban et al., 2015)
BCC49498		KF016996	Outgroup	<i>Aschersonia narathiwatensis</i>	GenBank
JM0807	HM135162		Outgroup	<i>Cordyceps militaris</i>	(Zhong et al., 2010)
BCC55524		KF016995	Outgroup	<i>Hypocrella sianmensis</i>	GenBank



**Figure 1.** Collection locations of *O. sphecocephala* and *O. asiana* in Bidoup Nui Ba National Park, Lam Dong, Vietnam

Molecular analyses used the dataset of 101 taxa (including 10 new sequenced data) (Table 1). Analysis using the D1–D2 sequences included 45 taxa with a total length of 2200 characters in the final dataset, while the analysis using the ITS sequences included 66 taxa with a total length of 920 characters in the final dataset.

## Results and discussion

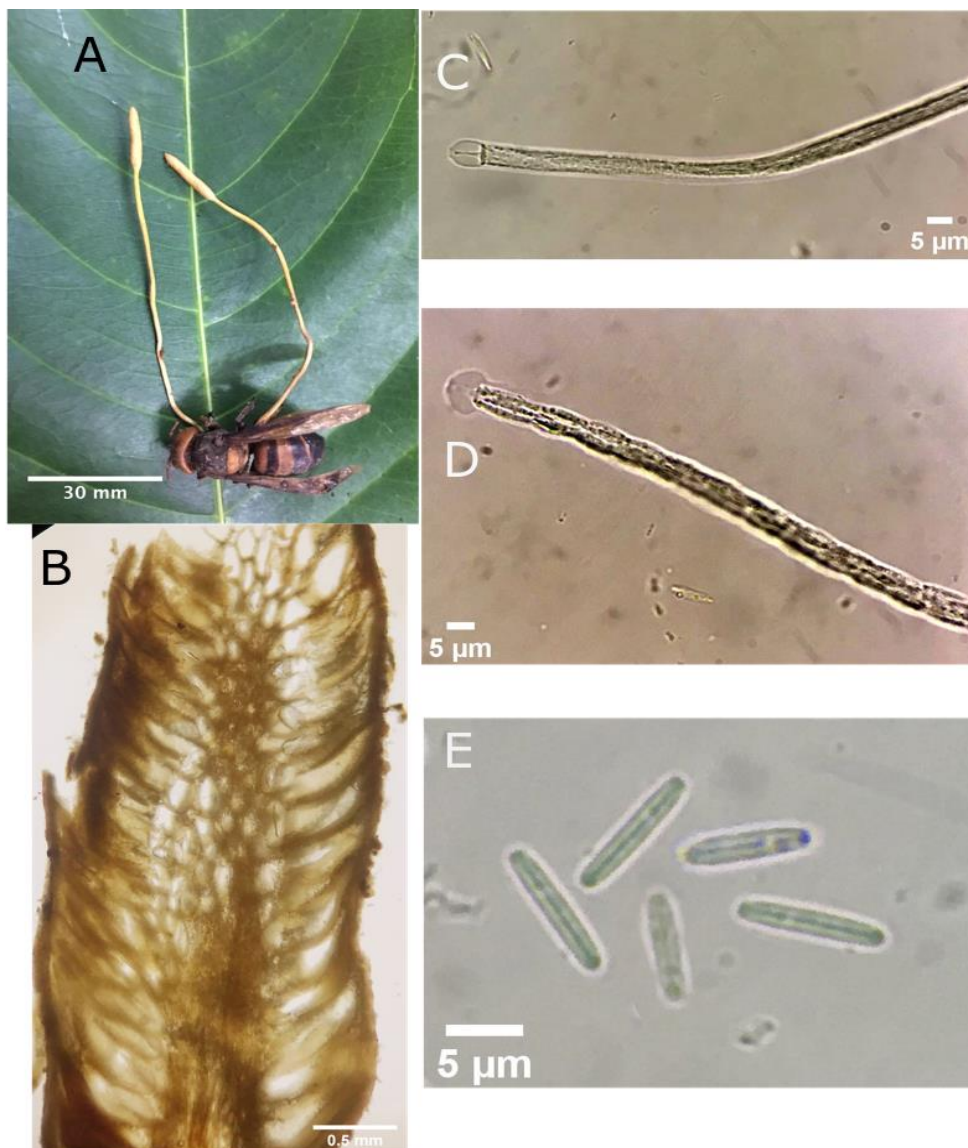
### *The analyses of Ophiocordyceps sphecocephala*

*Ophiocordyceps sphecocephala* (Klotzsch ex Berk.) G.H. Sung, J.M. Sung, Hywel-Jones & Spatafora, Stud. Mycol. 2007: 5-59.  
Mycobank MB504343.

### *Taxonomy (Fig. 2, Table 2)*

The specimens from Vietnam shared the morphological characteristics with the described morphology of *O. sphecocephala* (Sung et al, 2007).

**Diagnosis.** Stromata solitary or two, thin, creamy yellow, 72–106 mm long, arising from the region between the head and thorax of the host insect. Stipe stout, 0.7–1.0 mm in diam. Fertile head soft when fresh, 10–13 × 1.5–2.0 mm. Perithecia long, oblique in position, thick-walled and immersed in the fertile head, 610–730 × 130–220 μm. Ascospores thread-like and fragmented, 120–260 × 4–6 μm. Part spores fusoid, 7.5-8.5 × 1.5–2.0 μm.



**Figure 2.** *O. sphecocephala* on German wasp (*Vespa germanica*, Vespidae). A. Stromata and host insect, B. Perithecia, C. immature ascus, D. mature ascus with part spores. E. part spores

**Host insect.** The specimens of *O. sphecocephala* were only found on German wasps (*Vespa germanica*, Vespidae). Similar host insect family is reported in Thai *O. sphecocephala* (Hywel-Jones, 1995a).

**Locality.** Bidoup Nui Ba National Park, Lam Dong province, Vietnam (12°00'00" to 12°52'00" N, 108°17'00" to 108°42'00" E), between 1200 m to 1600 m attitude above sea level, specimens arising from thick layer of decaying leaves on the floor of mixed forest, near the streams at the relative humidity of 62%–68% and less than 200 lx of scattering light.

**Remarks.** Even though Vietnamese *Ophiocordyceps sphecocephala* shared many characteristics with previous description of *O. sphecocephala*, we observed that Vietnamese *O. sphecocephala* has longer stromata and fertile head while the asci and partspores were smaller (Table 2).

**Table 2.** Morphological characteristics of the collected *Ophiocordyceps* and the references from Thailand, Japan and Brazil

Specimen	Host/Voucher	Stroma (mm)	Fertile head (mm)	Peritheticum (mm)	Asci (µm)	Part spore (µm)
<i>O. sphecocephala</i> (This study)	On <i>Vespula germanica</i> , Vespidae (NLU202014)	72–106 × 0.7–1	10–13 × 1.5–2.0	610–730 × 130–220	120–260 × 4–6	7.5–8.5 × 1.5–2
<i>O. sphecocephala</i> Thailand (Hywel-Jones, 1995a)		to 45 × 0.15–0.8	2.2–11 × 1.2–1.9	880–1000 × 200–260	700– up × to 7	10–14 × 1.5–2.5
	on <i>Halyomorpha halys</i>					
	Pentatomidae (NLU202011)					
	on <i>Acanthosoma labiduroides</i>					
	Acanthosomatidae (NLU202012)					
	on <i>Clavigralla scutellaris</i>					
	Coreidae (NLU202013)					
	on <i>Proxys punctulatus</i>					
	Pentatomidae (NLU202015)					
<i>O. asiana</i> Thailand (Khao-ngam et al, 2021)		30–130 × 1–2	5–20 × 2–3	750–1200 × 200–300	200–600 × 5–6	6–14 × 1.5–2
<i>O. nutans</i> Japan (Type I) (Sasaki et al, 2008)		n/a	n/a	950–970 × 250–260	n/a	n/a
<i>O. nutans</i> Japan (Type II) (Sasaki et al, 2008)		32–112	2.5–14 × 1.5–3.7	610–1170 × 190–560	200–285 × 5–9	3.5–20 × 1–2
<i>O. nutans</i> Thailand (Hywel-Jones, 1995b)		50–90 × 0.4–0.8	6–17 × 3–5	550–800 × 130–300	780 × 7–8	9.3–15 × 1.5–2
<i>O. neonutans</i> Brazil (Friedrich et al, 2018)		23–170 × 1–2	6–19 × 0.9–2.0	550–1200 × 130–360	220–900 × 3–8	6–15 × 1.2–3

The BLAST analysis using the D1–D2 sequence of *O. sphecocephala* from Vietnam showed 96.87% identity with *O. sphecocephala* sequence (NBRC 101414) and 96.48% with *O. irangiensis* sequence (NBRC 101399). The phylogenetic analysis using the D1–D2 dataset showed that Vietnamese *O. sphecocephala* formed a monophyletic cluster with high support (95% RAxML, BPP 1.00 and 0.02 pairwise distance) to the group of *O. sphecocephala* (NBRC 101414) and *O. irangiensis* (NBRC101399) from Thailand (Fig. 3). It is known that D1–D2 sequences are slowly evolved and the nucleotide substitution values within a species is not higher than 0.01, whereas greater value of nucleotide substitution is recorded in separate biological species (Kurtzman and Robnett, 1997; Raja et al., 2017). In the analysis using the ITS dataset, *O. sphecocephala* again showed its closest relationship to Thai *O. sphecocephala* (NBRC 101414) and *O. irangiensis* (NBRC101399). Even though the support was moderate (79% RAxML, BPP 0.82), the pairwise distances between Vietnamese *O. sphecocephala* and Thai *O. sphecocephala* (NBRC 101414) was 0.09 and the pairwise distance to *O. irangiensis* (NBRC101399) was 0.06 (Fig. 4). Chen et al (2004) reported that the ITS sequence distance within a species should be from 0.00 to 0.05. Our results using ITS sequences therefore indicated a genetic variable between Vietnamese *O. sphecocephala* and Thai *O. sphecocephala* (NBRC101414).

It has been known that *O. irangiensis* infects only ants while *O. sphecocephala* grows on wasps only (Hywel-Jones, 1995a; 1996; Araújo et al., 2020). All specimens of *O. sphecocephala* were found on wasps only. Mains (1958) pointed out the presence of longitudinal hyphae at the core of the fertile head as a key character to distinguish *O. sphecocephala* from similar species. Similar descriptions on *O. sphecocephala* were also reported later (Hywel-Jones, 1995a; Sung et al., 2007). Here, we observed the presence of this diagnostic character in Vietnamese *O. sphecocephala* specimens (Fig. 2).

So far, data on *O. sphecocephala* were either reported as genetic data or morphological data (Hywel-Jones, 1995a; Sung et al., 2007). There is no morphological description for *O. sphecocephala* (NBRC101414) and many other reported *O. sphecocephala*. Only morphological data of *O. sphecocephala* specimens collected in Thailand is available (Hywel-Jones, 1995a) (Table 2), however these specimens are not analyzed phylogenetically. In comparison to the data by Hywel-Jones (1995a), Vietnamese *O. sphecocephala* had longer stromata and fertile heads, while the length of the asci and part spores were smaller (Table 2). Our study therefore the first report providing both morphological and genetic data on *O. sphecocephala*.

Our phylogenetic and morphological data consistently showed the differences of Vietnamese *O. sphecocephala* and other reported *O. sphecocephala*. We therefore propose Vietnamese *O. sphecocephala* as a new intraspecies of *O. sphecocephala*.

### ***The analyses of Ophiocordyceps asiana***

*Ophiocordyceps asiana* Mongkolsamrit, Khao-ngam, Himaman, Rungjindamai & Luangsa-Ard, 2021: 341–353.

Mycobank MB838742.

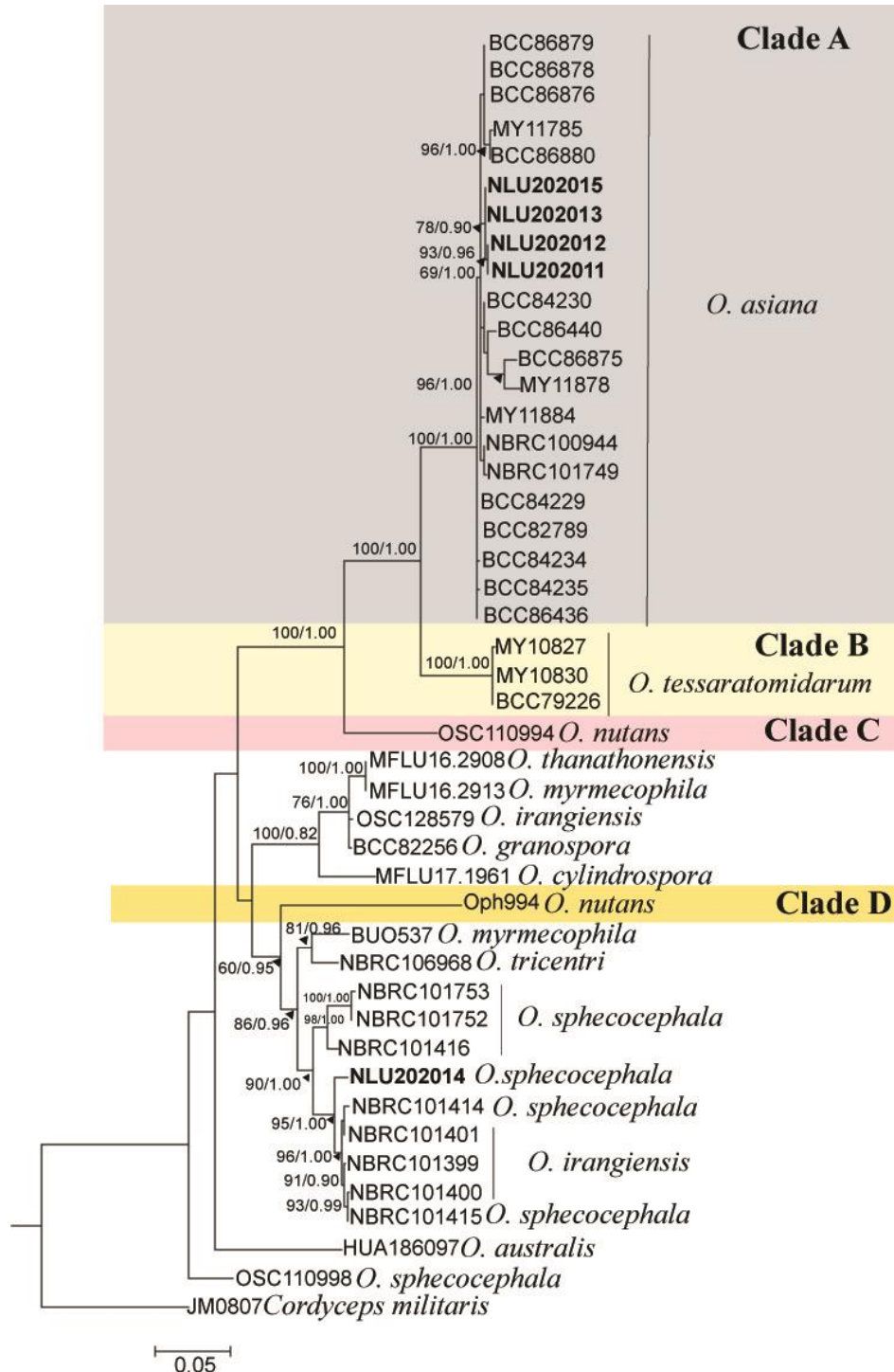
### ***Taxonomy (Table 2, Fig. 5)***

The specimens of *O. asiana* from Vietnam shared morphological characteristics with recently described characteristics of *O. asiana* from Thailand (Khao-ngam et al., 2021).

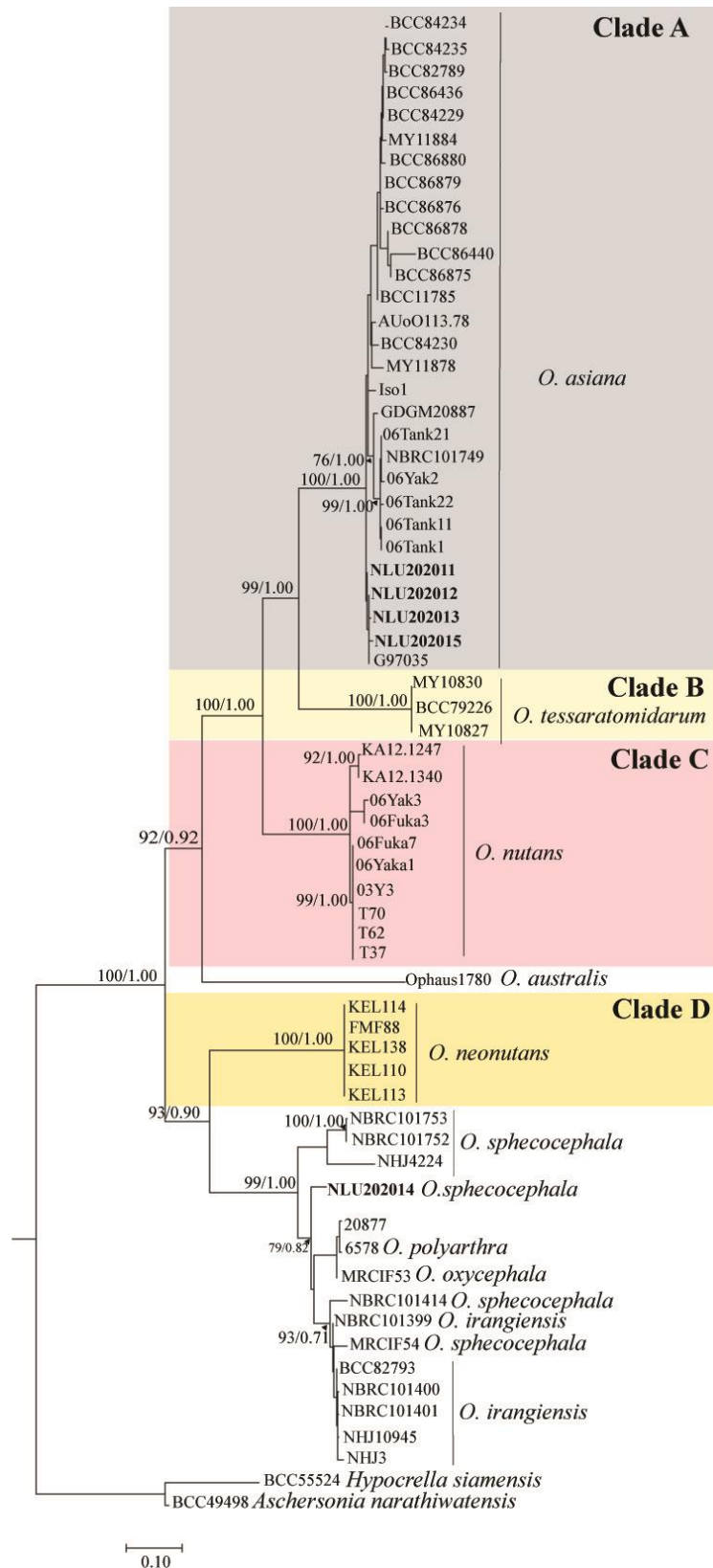
**Diagnosis.** Stromata solitary or up to four, cylindrical, 72–189 mm long, arising from the thorax of adult bugs. Stipe stout, black and wiry, 0.5 to 1.0 mm in diam. Fertile head



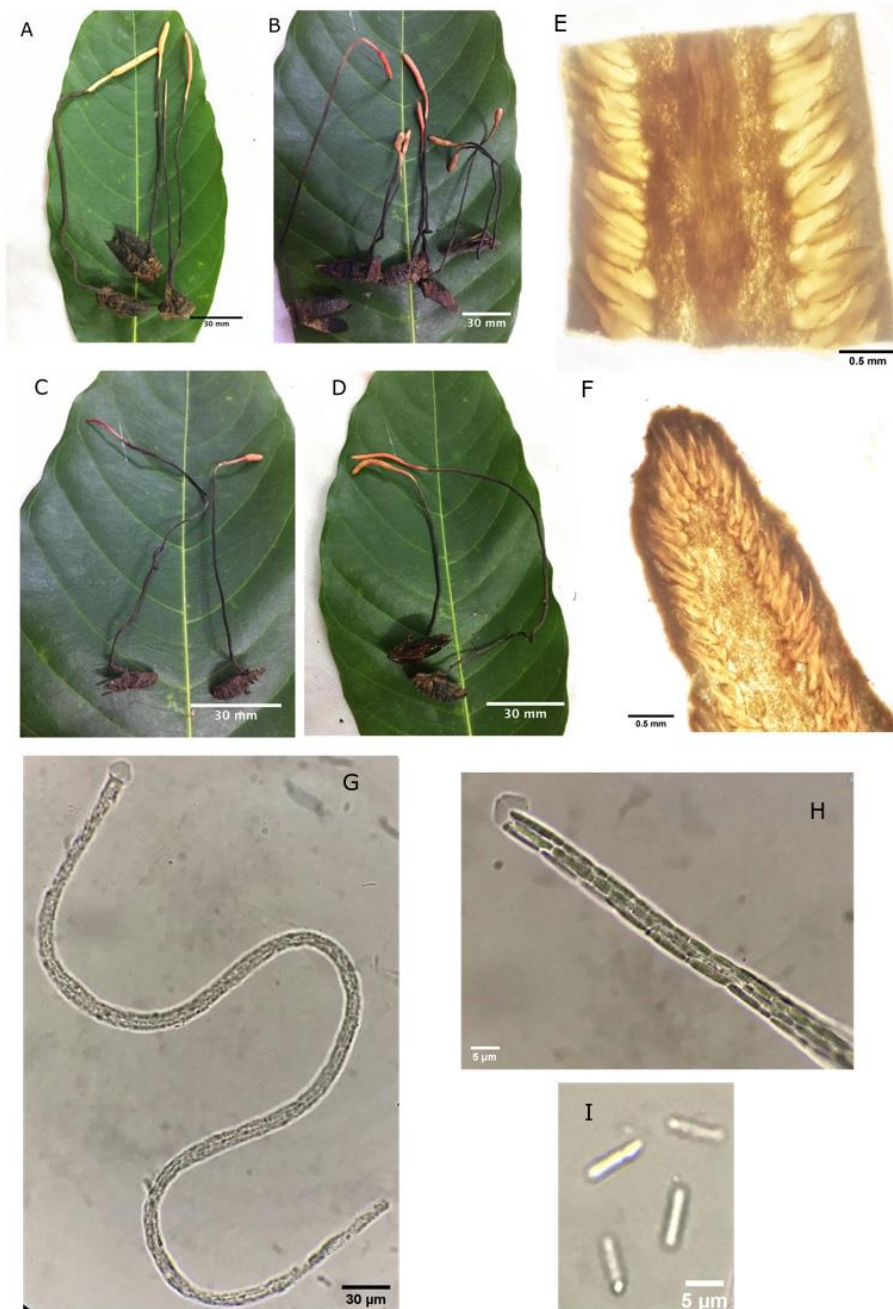
cylindrical, yellow to reddish orange and soft when fresh, 4.5–31.5 × 0.5–2.5 mm. Perithecia elongated pyriform, thick-walled and immersed in the fertile head, 140–810 × 4–7 μm. Ascospores are thread-like and fragmented. Partspores 7.5–14 × 1.5–3 μm, cylindrical with truncate ends.



**Figure 3.** Phylogenetic tree of the studied *O. sphecocephala* and *O. asiana* and the related taxa generated from RAxML analysis using D1–D2 sequences. The RAxML and Bayesian posterior probability values were indicated above the nodes as RAxML/BPP. Vietnamese *Ophiocordyceps* sequences are indicated in bold



**Figure 4.** Phylogenetic tree of the studied *O. sphecocephala*, *O. asiana* and the related taxa generated from RAxML analysis using ITS sequences. The RAxML and Bayesian posterior probability values were indicated above the nodes as RAxML/BPP. Vietnamese *Ophiocordyceps* sequences are indicated in bold



**Figure 5.** A–D. Stromata of *O. asiana* on *Halyomorpha halys* (Pentatomidae), *Acanthosoma labiduroides* (Acanthosomatidae), *Clavigralla scutellaris* (Coreidae), *Proxys punctulatus* (Pentatomidae), respectively. E, F. Perithecia, G. immature ascus, H. mature ascus with partspores, I. Part spores

**Host insects.** The collected specimens were found on a broad range of host insect families. They infected black stinkbug (*Proxys punctulatus*, Pentatomidae), brown marmorated stinkbug (*Halyomorpha halys*, Pentatomidae), scissors turtle bug (*Acanthosoma labiduroides*, Acanthosomatidae) and legume bug (*Clavigralla scutellaris*, Coreidae). Similar results are also reported in *O. asiana* from Thai Lan (Khao-ngam et

al., 2021) and *O. nutans* from Japan (Sasaki et al., 2012) while *O. neonutans* is only found in Pentatomidae (Friedrich et al., 2018).

*Locality.* Bidoup Nui Ba National Park, Lam Dong province, Vietnam (12°00'00" to 12°52'00" N, 108°17'00" to 108°42'00" E) from 1200 m to 1600 m above sea level, near the stream in mixed forest, specimens arising from thick layers of decaying leaves on the forest floor under 62%–68% relative humidity and less than 200 lx of scattering light.

*Remarks.* Although the specimens from Vietnam of *O. asiana* shared many characteristics with *O. asiana* and *O. nutans* reported from Thailand, Japan and *O. neonutans* reported from Brazil, we observed that the stroma and the fertile head of Vietnamese specimens are longer than those of Thailand, Japan and Brazil (Table 2), while the perithecia, asci and partspores are shorter (Table 2).

We recorded a broad variation in the morphology of *Ophiocordyceps asiana* infecting different bug species. For example, longer stromata, fertile heads, perithecia and part spores were observed in the specimens infecting *Halyomorpha halys* (Pentatomidae) and *Acanthosoma labiduroides* (Acanthosomatidae) (Table 2). Besides, the differences in the stroma color, the sizes of the stromata, fertile head and perithecia were also recorded (Table 2).

Four groups of *Ophiocordyceps asiana* from Vietnam had identical D1–D2 and ITS sequences regardless of having different families of host insects (Acanthosomatidae, Coreidae and Pentatomidae).

The nucleotide BLAST analyses using D1–D2 sequences of Vietnamese *O. asiana* specimens revealed more than 99.3% of homology with the sequences of *O. nutans* from Japan (NBRC 101749), Thailand (NBRC 100944) and *O. asiana* from Thailand. The phylogenetic analysis using the D1–D2 sequences showed that Vietnamese *O. asiana* sequences formed a monophyletic group with high support (100% RAxML, BPP 1.00) to the group of *O. asiana* in Clade A reported by Khao-ngam et al. (2021) (Fig. 2). This clade includes Thai *O. asiana* (Khao-ngam et al., 2021) and Japanese *O. nutans* type I (Sasaki et al., 2012). In our analysis, the pairwise distance between Vietnamese *O. asiana* and others in clade A was lower than 0.01 (Fig. 3).

It is known that D1–D2 sequences are slowly evolved and the nucleotide substitution values of intraspecies is not higher than 0.01, whereas greater value of nucleotide substitution is recorded in separate biological species (Kurtzman and Robnett, 1997; Raja et al., 2017). The results therefore indicated that *O. asiana* from Vietnam belonged to the Clade A of *O. asiana* of Khao-ngam et al. (2021) and *O. nutans* Type I of Sasaki et al. (2012). Since D1–D2 regions are more conserved than ITS regions, we analyzed *O. asiana* at the ITS region to further investigate if there is any genetic variation between Vietnamese *O. asiana* and other *O. asiana* in clade A. Consistent to the analysis results using D1–D2 sequences, the analysis using ITS sequences also showed that Vietnamese *O. asiana* was in Clade A with high support (100% RAxML, BPP 1.00) and low pairwise distance (0.01) (Fig. 4). It is therefore confirmed the genetic similarity between Vietnamese *O. asiana*, Thai *O. asiana* and *O. nutans* Type I from Japan.

However, we still noticed that Vietnamese *O. asiana* had longer stroma and fertile heads but shorter perithecia, asci and partspores than those of *O. asiana* from Thailand, of *O. nutans* from Japan and also of *O. neonutans* from Brazil (Table 2).

Sasaki et al. (2012) had found that the ITS sequences of *O. nutans* infecting different species of Acanthosomatidae and Pentatomidae are similar. However, the ITS sequences of *O. nutans* infecting Coreidae are different from those on other insect families (Sasaki et al., 2012). Differently, Vietnamese *O. asiana* possessed identical D1–D2 and ITS

sequences in all the recorded bug families: Ancanthosomatidae, Pentatomidae and also in Coreidae (Figs. 3,4).

In the study of *O. nutans* collected in Japan, Sasaki et al. (2008) did not record any significant differences in the morphology of *O. nutans* among the host insect species. In contrast, we noticed a strong impact of the host insect on the morphological diversity of Vietnamese *O. asiana*, which could be observed in the size of the stroma, fertile heads, asci and part spores (Table 2, Fig. 5).

## Conclusions

The collected specimens of *Ophiocordyceps* on wasps were an intraspecies of *O. sphecocephala* and the specimens on bugs were *O. asiana*.

*O. asiana* could infect a wide range of host insects and the influence of the host insects on *O. asiana* morphology was also observed, while the host of *O. sphecocephala* was more specific and found only on wasps (Vespidae).

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