MORPHOLOGICAL AND PHYLOGENETIC STUDY OF OPHIOCORDYCEPS SPHECOCEPHAL A AND OPHIOCORDYCEPS ASIANA FROM VIETNAM

MAI, T. N.1* – THUY, T. P. D.1 – HONG, V. N.2 – TAWAT, T.3 – SHRESTHA, B.4

1Department of Biology, Faculty of Science, Nong Lam University, Linh Trung Ward, Thu Duc District, Ho Chi Minh City, Vietnam

2Institute of Geography, Vietnam Academy of Science and Technology, A27-No 18, Hoang Quoc Viet street, Cau Giay district, Hanoi, Vietnam

3RSTDC, Maerim–Samerng Road, Moo 1, Tambol Maeram, Amphur Maerim, Chiang Mai, Thailand

4Central Department of Biotechnology, Tribhuvan University, Kirtipur, Kathmandu, Nepal

*Corresponding author
e-mail: ngtpmai@hcmuaf.edu.vn; phone: +84-287-220-262

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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 stroma 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.crg.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 | O. asiana | (Khoa-ngam et al., 2021) |
| BCC82789      | MW280203 | MW285710 | Thailand | O. asiana | (Khoa-ngam et al., 2021) |
| BCC84229      | MW280199 | MW285706 | Thailand | O. asiana | (Khoa-ngam et al., 2021) |
| BCC84230      | MW280200 | MW285707 | Thailand | O. asiana | (Khoa-ngam et al., 2021) |
| BCC84234      | MW280201 | MW285708 | Thailand | O. asiana | (Khoa-ngam et al., 2021) |
| BCC84235      | MW280202 | MW285709 | Thailand | O. asiana | (Khoa-ngam et al., 2021) |
| BCC86436      | MW280211 | MW285717 | Thailand | O. asiana | (Khoa-ngam et al., 2021) |
| BCC86440      | MW280212 | MW285718 | Thailand | O. asiana | (Khoa-ngam et al., 2021) |
| BCC86875      | MW280204 | MW285711 | Thailand | O. asiana | (Khoa-ngam et al., 2021) |
| BCC86876      | MW280205 | MW285712 | Thailand | O. asiana | (Khoa-ngam et al., 2021) |
| BCC86878      | MW280207 | MW285713 | Thailand | O. asiana | (Khoa-ngam et al., 2021) |
| BCC86879      | MW280208 | MW285714 | Thailand | O. asiana | (Khoa-ngam et al., 2021) |
| BCC86880      | MW280210 | MW285716 | Thailand | O. asiana | (Khoa-ngam et al., 2021) |
| NLU202011     | MT235757 | MW684019 | Vietnam | O. asiana | This study |
| NLU202012     | MT235758 | MW684020 | Vietnam | O. asiana | This study |
| NLU202013     | MT235759 | MW525516 | Vietnam | O. asiana | This study |
| NLU202014     | MT235760 | MW684021 | Vietnam | O. sphecocephala | This study |
| NLU202015     | MT235761 | MW525517 | Vietnam | O. asiana | This study |
| MY11785       | MW280209 | MW285715 | Thailand | O. asiana | (Khoa-ngam et al., 2021) |
| MY11878       | MW280213 | MW285719 | Thailand | O. asiana | (Khoa-ngam et al., 2021) |
| MY11884       | MW280216 | MW285720 | Thailand | O. asiana | (Khoa-ngam et al., 2021) |
| HUA186097     | KC610765 | Columbia | O. australis | (Sanjuan et al, 2015) |
| Ophau11780    | KP200888 | Columbia | O. australis | (Sanjuan et al, 2015) |
| MFLU17.1961   | NG064484 | Thailand | O. cylindrospora | GenBank |
| BCC82256      | MH028157 | Thailand | O. granospora | (Araújo et al, 2020) |
| BCC82793      | MH028141 | Thailand | O. irangiensis | (Khosanat et al, 2019) |
| NBRC101399    | JN941425 | JN943334 | Thailand | O. irangiensis | (Sanjuan et al, 2015; Schoch et al, 2012) |
| NBRC101400    | JN941426 | JN943335 | Thailand | O. irangiensis | (Sanjuan et al, 2015; Schoch et al, 2012) |
| NBRC101401    | JN941427 | JN943336 | Thailand | O. irangiensis | (Sanjuan et al, 2015; Schoch et al, 2012) |
| NHJ10945      | GU723767 | Thailand | O. irangiensis | (Luangs-a-Ard et al, 2011) |
| NHJ3          | AJ786566 | Thailand | O. irangiensis | (Stensrud et al, 2005) |
| OSC 128579    | EF469076 | Thailand | O. irangiensis | (Sanjuan et al, 2015) |
| BUO537        | MH879600 | China | O. myrmecophila | (Zihong et al, 2019) |
| MFLU16.2913   | MF372586 | Thailand | O. myrmecophila | (Xiao et al, 2017) |
| FMF88         | KX197242 | Brazil | O. neonutans | (Friedrich et al, 2018) |
| KEL110        | KX197240 | Brazil | O. neonutans | (Friedrich et al, 2018) |
| KEL113        | KX197239 | Brazil | O. neonutans | (Friedrich et al, 2018) |
| KEL114        | KX197241 | Brazil | O. neonutans | (Friedrich et al, 2018) |
| KEL138        | KX197243 | Brazil | O. neonutans | (Friedrich et al, 2018) |
| 03Y3          | AB544452 | Japan | O. nutans | (Sasaki et al, 2012) |
| 06Fuka3       | AB544463 | Japan | O. nutans | (Sasaki et al, 2012) |
| 06Fuka7       | AB544467 | Japan | O. nutans | (Sasaki et al, 2012) |
| Voucher   | Accession No. | ITS       | Country | Species              | Reference                  |
|-----------|---------------|-----------|---------|----------------------|----------------------------|
| 06Tank1   | AB544473      | Japan     | O. nutans | (Sasaki et al, 2012) |
| 06Tank11  | AB544478      | Japan     | O. nutans | (Sasaki et al, 2012) |
| 06Tank21  | AB544485      | Japan     | O. nutans | (Sasaki et al, 2012) |
| 06Tank22  | AB544486      | Japan     | O. nutans | (Sasaki et al, 2012) |
| 06Yak2    | AB544489      | Japan     | O. nutans | (Sasaki et al, 2012) |
| 06Yak3    | AB544490      | Japan     | O. nutans | (Sasaki et al, 2012) |
| 06Yaka1   | AB544491      | Japan     | O. nutans | (Sasaki et al, 2012) |
|AUoO113.78 | AJ786583      | Thailand  | O. nutans | (Stensrud et al, 2005) |
| G97035    | AJ309367      | China     | O. nutans | (Sasaki et al, 2012) |
| GDGM20887 | JX177484      | China     | O. nutans | GenBank                |
| Iso1      | AJ536560      | China     | O. nutans | (Sasaki et al, 2012) |
| KA12.1247 | KR673498      | Korea     | O. nutans | (Kim et al, 2015)     |
| KA12.1340 | KR673559      | Korea     | O. nutans | (Kim et al, 2015)     |
| NBRC100944| JN941428      | Japan     | O. nutans | (Ban et al, 2015)     |
| NBRC101749| AB968408      | Japan     | O. nutans | (Sasaki et al, 2012) |
| Oph994    | KJ917567      | Columbia  | O. nutans | (Sanjuan et al, 2015) |
| OSC110994 | DQ518763      | n/a       | O. nutans | (Sanjuan et al, 2015) |
| T37       | AB366634      | Japan     | O. nutans | (Sasaki et al, 2012) |
| T62       | AB366626      | Japan     | O. nutans | (Sasaki et al, 2012) |
| T70       | AB366623      | Japan     | O. nutans | (Sasaki et al, 2012) |
| MRCIF53   | EU573348      | Thailand  | O. oxycephala | (Qu et al, 2018) |
| Iso6578   | AJ536548      | China     | O. polyarthra | (JiaJun et al, 2021) |
| 20877     | AJ536550      | China     | O. sphecocephala | (Tian et al, 2010) |
| MRCIF54   | EU573347      | Thailand  | O. sphecocephala | GenBank |
| NBRC101416| JN941443      | Thailand  | O. sphecocephala | (Sanjuan et al, 2015) |
| NBRC101752| JN941445      | Japan     | O. sphecocephala | (Ban et al, 2015) |
| NBRC101414| JN943443      | Thailand  | O. sphecocephala | (Sanjuan et al, 2015; Schoch et al, 2012) |
| NBRC101415| JN941442      | Thailand  | O. sphecocephala | (Sanjuan et al, 2015) |
| NBRC101752| JN943351      | Japan     | O. sphecocephala | (Ban et al, 2015; Schoch et al, 2012) |
| NBRC101753| JN943350      | Japan     | O. sphecocephala | (Ban et al., 2015; Schoch et al., 2012) |
| NHJ4224   | GU723778      | Thailand  | O. sphecocephala | (Luangsa-Ard et al., 2011) |
| OSC 110998| DQ518765      | Thailand  | O. sphecocephala | (Sanjuan et al., 2015) |
| BCC79226  | MW280219      | Thailand  | O. tesseratominorarum | (Kha-ngam et al., 2021) |
| MY10827   | MW280217      | Thailand  | O. tesseratominorarum | (Kha-ngam et al., 2021) |
| MY10830   | MW280218      | Thailand  | O. tesseratominorarum | (Kha-ngam et al., 2021) |
| MFLU16.2908| MF362990     | Thailand  | O. thanathonensis | (Xiao et al., 2017) |
| NBRC106968| AB968423      | Japan     | O. tricinti | (Ban et al., 2015) |
| BCC49498  | KF016996      | Outgroup  | Aschersonia narathiwatensis | GenBank |
| JM0807    | HM135162      | Outgroup  | Cordyceps militarius | (Zhong et al., 2010) |
| BCC55524  | KF016995      | Outgroup  | Hypocreella sianensis | GenBank |
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.
Host insect. The specimens of *O. sphecocephala* were only found on German wasps (*Vespula germanica*, Vespidae). Similar host insect family is reported in Thai *O. sphecocephala* (Hywel-Jones, 1995a).

Locality. Bidadu 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 altitude 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 part spores 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) |
|----------|--------------|-------------|-------------------|-------------------|-----------|-----------------|
| *O. sphecocephala* (This study) | On *Vespula germanica*, 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 |
| *O. sphecocephala* Thailand (Hywel-Jones, 1995a) | to 45 × 0.15–0.8 | 2.2–11 × 1.2–1.9 | 880–1000 × 200–260 | 700– up x to 7 | 10–14 × 1.5–2.5 |
| on *Halyomorpha halys* | Pentatomidae (NLU202011) | | | | | |
| on *Acanthosoma labiduroides* | Acanthosomatidae (NLU202012) | | | | | |
| on *Clavigralla scutellaris* | Coreidae (NLU202013) | | | | | |
| on *Proxys punctulatus* | Pentatomidae (NLU202015) | | | | | |
| *O. asiana* 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 |
| *O. nutans* Japan (Type I) (Sasaki et al, 2008) | n/a | n/a | 950–970 × 250–260 | n/a | n/a |
| *O. nutans* 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 |
| *O. nutans* 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 |
| *O. neonutans* 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 asc 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 asiaca**

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

Mycobank MB838742.

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

The specimens of *O. asiaca* from Vietnam shared morphological characteristics with recently described characteristics of *O. asiaca* 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: Ancanthosomatide, Pentatomidae and also in Coreidiae (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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