Morphological and genetic characteristics of the novel entomopathogenic fungus *Ophiocordyceps langbianensis* (Ophiocordycipitaceae, Hypocreales) from Lang Biang Biosphere Reserve, Vietnam

Thuan Duc Lao¹, Thuy Ai Huyen Le¹ & Nguyen Binh Truong²*

An entomopathogenic fungus newly named *Ophiocordyceps langbianensis* was collected from Lang Biang Biosphere Reserve, located in Lam Dong Province, Vietnam. It is characterized as a species of *Ophiocordyceps* (Ophiocordycipitaceae, Hypocreales) having the unique characteristics of a cylindrical fertile part and several branched apical appendices. Each ascospore develops as two swollen, constricted part-spores. A phylogenetic analysis of multiple genes, including *nrLSU*, *nrSSU*, *Rpb1*, *ITS* and *Tef*, supported its systematic position in the genus of *Ophiocordyceps*; it is related to *O. brunneipunctata*. Based on morphological and phylogenetic analyses, *O. langbianensis* was confirmed as a new species from Vietnam.

The genus *Ophiocordyceps*, first established by Petch in 1931, belongs to the family Ophiocordycipitaceae, order Hypocreales, comprising approximately 250 species¹.². Originally, *Ophiocordyceps* was classified as a subgenus of *Cordyceps* by Kobayasi (1941, 1982) and Mains (1958)³–⁵. In 2007, Sung et al. established a new called family Ophiocordycipitaceae, comprising *Ophiocordyceps*, based on morphological and phylogenetic analyses⁶–⁷. The distinction of the genus *Ophiocordyceps* from *Cordyceps* was done due to the darkly pigmented stromata of *Ophiocordyceps*, which are pliant, wiry or fibrous and tough in texture, compared to the brightly pigmented stromata of *Cordyceps*⁷. Species of *Ophiocordyceps* are entomopathogenic on a wide range of insects. The hosts of species of *Ophiocordyceps* are the larvae of Coleoptera and Lepidoptera as well as the adults of Araneae, Diptera, Hemiptera, Hymenoptera, Odonata and Orthoptera⁶–⁷. Although *Ophiocordyceps* has worldwide distribution, the tropics and subtropics are where the highest numbers of the species are recorded. Moreover, it is considered that there is an underestimation of the number of *Ophiocordyceps* species.

Vietnam is located in a tropical region with terrestrial ecosystems. The forests feature a rich biodiversity of both flora and fauna due to the tropical monsoon climate with high temperature and rainfall. This is a favorable environment for the development of entomopathogenic fungi. Lang Biang Biosphere Reserve is located in Lam Dong Province and comprises a vast primitive jungle with the Lang Bian Mountain at its core, one of Vietnam’s four biodiversity centers. During our expedition to discover the diversity of entomopathogenic fungi, we collected the sample DL0017. In this study, we introduce this specimen as a new species of *Ophiocordyceps* that parasitizes the larva of *Coleoptera*. We present a morphological description and phylogenetic analysis based on the phylogenetic construction of nuclear large ribosomal subunit (nrLSU), nuclear small ribosomal subunit (nrSSU) and RNA Polymerase II Subunit B1(rpb1) of species of *Ophiocordyceps*, including this new species.

¹Faculty of Biotechnology, Ho Chi Minh City Open University, Ho Chi Minh City, Vietnam. ²Faculty of Biology, Dalat University, Dalat, Lam Dong, Vietnam. ³email: nguyentb@dlu.edu.vn
Materials and methods

Fungal specimen collection. The specimen, DL0017, used for this study was collected from Lang Biang Biosphere Reserve (N 12°2′19.0″, E108°26′04.7″, elevation 1680 m) in 9th August, 2016. The specimen, including the host, was extracted carefully, noted, and photographed in the field using a digital camera. The specimen was immediately wrapped in wax paper, placed in a collection bag, and taken to the laboratory.

Cultivation techniques. According to the identification of conidia, phialides and colony coloration, the isolate cultures were grown on YMG media, composed of 4 g/l yeast extract (Sigma-Aldrich, Germany), 10 g/l malt extract (Sigma-Aldrich, Germany), 4 g/l glucose (Sigma-Aldrich, Germany), and incubated at 20 °C for a period of 20 days with PDA media (potato extract 4 g/l, dextrose 20 g/l, agar 15 g/l; Merck, Germany).

For fruit body induction, cultures were grown on millet substrate (millet/silkworm pupae powder = 20:1 (w/w)) and brown rice substrate (brown rice/silkworm pupae powder = 20:1 (w/w)) at 20 °C under 12 h light and 12 h darkness with relative humidity of over 90%.

Morphological study: macro- and micro-morphological analysis. Morphological observations were carried out and recorded according to the guidelines of Kobayasi and Sung et al.3,4,7. The macroscopic characteristics of the fresh fruit body were carefully observed, including the stipe, stroma, etc. Moreover, the color was noted according to Kornerup and Wanscher8. Additionally, the host insect was identified based on morphological characteristics, such as mandibulate mouthparts, antennae, shape of head and thorax. For the micro-morphological analysis, one or two perithecia were removed from the stroma and placed on a microscope slide in lactophenol-cotton blue to measure the sizes and shapes of the perithecia, asci and ascospores. Finally, the nomenclatural novelty and descriptions were deposited in MycoBank.

DNA extraction, PCR amplification, target gene sequencing. Genomic DNA was isolated by using the phenol/chloroform method (pH = 8)11. The fruiting body was incubated in a lysis buffer (2.0% SDS, Tris–HCl pH 8.0, 150 mM NaCl, 10 mM EDTA, 0.1 mg/ml Proteinase K) at 65 °C overnight. The supernatant was collected by centrifugation, and a volume of 700 μL of phenol/chloroform/isoamyl alcohol (25:24:1) was supplemented and centrifuged. The supernatant was collected and precipitated with absolute isopropanol. Finally, the isolated genomic DNA was stored in Tris–EDTA buffer at − 20 °C for further studies.

The primer pairs used to amplify nrLSU, nrSSU, rpbl, ITS and Tef regions are shown in Table 1. The final volume of PCR was done in a total of 15 μL with the thermal program: 1 cycle at 95 °C for 5 min, 40 cycles at 95 °C for 30 s, X °C for 30 s, 72 °C for 2 min, 1 cycle at 72 °C for 5 min (Note: X °C is the annealing temperatures for each target gene shown in Table 1); 5 μL aliquots of amplification product were electrophoresed on a 2.0% agarose gel and visualized in a UV transilluminator. The amplified product was sequenced at Nam Khoa (Vietnam) company.

Taxa and nrLSU, nrSSU, rpbl, ITS and tef sequences collection, DNA proofreading and phylogeny analysis. The data set of nrLSU, nrSSU, rpbl, ITS and tef sequences were established by sequences downloaded from Genbank (NCBI) and based on the previous data published by Sung et al.7. The nrLSU, nrSSU, rpbl, ITS and tef were noted with accession number, name of taxon and locality. The amplified DNA sequences were proofread to remove ambiguous signals at both ends by different software, including Seaview 4.2.12 and Chromas Lite 2.1.1. The phylogenetic tree was constructed based on neighbor-joining (NJ), maximum parsimony (MP), and maximum likelihood (ML), using Molecular Evolutionary Genetics Analysis (MEGA) version 5. Additionally, the best evolution model was predicted using jModelTest.

Results

Taxonomy. Ophiocordyceps langbianensis T. D. Lao, T. A. H. Le & N. B. Truong, sp. nov. Mycobank MB836716 Figs. 1, 2, 3.
**Figure 1.** Overview of *Ophiocordyceps langbianensis*. (A–D) Ecology of collected plots; (E) Stroma developing from the head of hosts; (F) Immature stromata of fungus emerging from the larva of Coleoptera; (G) Stromata in moist soil surrounded by dried leaves.

**Figure 2.** *Ophiocordyceps langbianensis*. (A) Stroma on host; (B–D) Fertile part and apical appendix, surface of fertile part with perithecium ostioles, cortex; (E) Host; (F) Mycelium on the host; (G) Perithecia; (H, I) Asci with thick cap; (J, K) Ascospores.
**Typification.** VIETNAM. Lam Dong Province, Lang Bian Biosphere Reserve, Lang Bian mountain: N12°02′19.0″, E108°26′04.7″; elevation 1680 m; humidity: over 85%; temperature: day 20 °C–22 °C, night: 14 °C–16 °C; collected between 9h00–15h00 of the day on 9 August, 2016, from the larva of a beetle of Coleoptera in moist soil surrounded by dried leaves. Truong B.N. DL0017 (Holotype DLU; Iso VNMN, DLU).

**Distribution.** Vietnam, only known from Lang Bian Mountain.

**Etymology.** “Langbianensis” refers to Lang Bian Mountain, Lam Dong province, Vietnam.

**Host.** On the larva of a beetle of Coleoptera. Larva: 28–32 mm long, hard-body, shiny, smooth, dark brownish yellow; body composed of 13 segments with black edges; larva with three pairs of jointed legs attached to thorax.

**Habitat.** Individuals of associated species appeared at the type locality, including pioneer species such as Acer laurinum (Aceraceae), Baccaurea harmandii (Euphorbiaceae), Castanopsis chinensis (Fagaceae), Eriobotrya poilanei (Rosaceae), Jasminum longisepalum (Oleaceae), Phoebe petelotii (Lauraceae) and Tetrastigma lanceolarium (Vitaceae).

**Sexual morph.** Stroma arising from the head of the host larva, solitary, rarely branched, 40–100 mm long; host covered with thin, tough layer of mycelium. Stipe filiform, cylindrical, 30–67 mm × 0.7–1.0 mm, pale yellow. Fertile portion, cylindrical, 7.0–14.0 mm × 1.5–2.0 mm, brownish yellow with dark brown ostiolar dots of perithecia. Apical appendices, pale yellow, 2–10 primary or secondary branches, 4.0–10.0 × 0.5 mm. Perithecia immersed, ovate or pyriform, 260–400 μm × 100–190 μm. Asci, cylindrical, 200–250 μm × 5.0–6.0 μm, with thickened cap. Ascospores filiform, multiseptate, articulated in long-chain after discharging, sometimes breaking into 1-celled part spores, cylindrical, swollen, two waist-like constrictions, 5–7.5 μm × 1.3–2 μm.

**Asexual morph.** Germination of ascospores after 48 h on PDA; white colony, slow growing on YMG and PDA media, 25.00 mm and 24.58 mm after 40 days (respectively); septate hyphae, branched, chlamydospores developing in intercalary or terminal cells. Aerial hyphae with divergent phialides; elliptical conidia in chains after release from the phialide. Stromata without fertile part forming on cereal substrates. Minor differences in morphological characteristics of stromata developing from different substrates. Stromata, white, branched when developing on millet substrate; brownish yellow, solitary, rarely branched, when developing on brown rice substrate.

**Amplification of nrLSU, nrSSU, rpb1, ITS and tef genes.** Target genes, including nrLSU, nrSSU, rpb1, ITS and tef, were successfully amplified with corresponding primers (Table 1). The bands of 950-bp, 1102-bp, 803-bps, 700-bps, and 1030-bps corresponding to the amplified nrLSU, nrSSU, rpb1, ITS and tef were observed.
in the electrophoresis on 2.0% agarose gel. The PCR products were sequenced with the signal of the peaks in both strands of target genes; the sequence was significant, unique and good for reading.

**The systematic concatenated **nrLSU, **nrSSU, **rpb1, **ITS and **tef **gene dataset.** To construct a phylogeny of major lineages, representative taxa were chosen based on previous study. The data set of **nrLSU, **nrSSU, **rpb1, **ITS and **tef consisted of 50, 50, 46, 39 and 42 taxa representing the morphological and ecological diversity of genera in Ophiocordycipitaceae, Clavicipitaceae, and Cordycipitaceae, including the outgroup taxon *Glomerella cingulata* (Glomerellaceae, Glomerellales) (Table 2). A combined concatenated dataset consisting of 30 representative taxa was constructed based on the list of individual target genes.

**Molecular phylogeny analysis.** The sequences of **nrLSU, **nrSSU, **rpb1, **ITS and **tef of DL0017 were similar to the representative sequence of *Cordyceps brunnipeunctata* (similarity > 90%), with accession numbers of DQ518756, DQ522542, DQ522369, GU723777 and DQ522324. Sequences were aligned and edited using the MEGA 5.2. Gaps were excluded from the phylogenetic analysis. The dataset of representative taxa and DL0017 target gene sequence consisted of 451 bp for **nrLSU, 674 bp for **nrSSU, 392 bp for **rpb1, 158 bp for **ITS and 790 bp for **tef. The evolution model that was most fixed with **nrLSU, **nrSSU, **rpb1, **ITS and **tef was TN93 + G, K2 + G + I, T92 + G + I, K2 + G, and TN93 + G + I respectively. The phylogenetic trees were generated with Neighbor Joining (NJ), Maximum Parsimony (MP), and Maximum Likelihood (ML) methods with replication of 1000. Based on the NJ, MP, and ML phylogenetic trees, individual **nrLSU, **nrSSU, **rpb1, **ITS, and **tef of DL0017 clustered together with *Ophiocordyces brunnipeunctata* within separate branches with credible bootstrap (> 50%), suggesting that these species are related (Table 3).

Information from molecular phylogenetic analysis based on separate genes is not enough to reconstruct trees for higher classification compared to multigene analysis. Therefore, a combined data set, including 2,319 bp of five target genes, **nrLSU-nrSSU-Rpb1-ITS-tef, was analyzed. The evolution model that was most fixed with the combined dataset was TN93 + G + I, as determined by MEGA 5.2. The phylogenetic trees, based on analysis of the combined data, could be broadly separated into three groups, which corresponded to the families of Clavicipitaceae, Ophiocordycipitaceae and Cordycipitaceae. In the phylogenetic tree, DL0017 clustered with *Ophiocordyces brunnipeunctata* with bootstrap of 100/100/100 (NJ/MP/ML phylogenetic tree) and formed a separate, monophyletic branch. Within this monophyletic branch, DL0017 and *O. brunnipeunctata* clustered together closely, suggesting that these species were truly associated (Fig. 4). The molecular phylogenetic analysis confirmed that there were differences between DL0017 and other related species.

To confirm the authenticity of DL0017 as the most closely associated with *Ophiocordyces brunnipeunctata*, the reconstruction of Neighbor-Net network of DL0017 and its allies was performed. The Neighbor-Net analysis supported the results from the phylogenetic analysis (Fig. 5). The network presented three complex groups, corresponding to three families: Clavicipitaceae, Ophiocordycipitaceae and Cordycipitaceae. The DL0017 closely clustered with Ophiocordycipitaceae complex. Additionally, speciation was observed between the cluster of DL0017 and *O. brunnipeunctata*.

**Comparison of Ophiocordyces langbianensis with close species.** In the phylogenetic analysis, the *Ophiocordyces langbianensis* clustered with *Ophiocordyces brunnipeunctata* with high bootstrap support, suggesting a close relationship. To confirm the authenticity of DL0017 as a new species, we compared DL0017 and its close species, *O. brunnipeunctata*. It differed from *O. brunnipeunctata* by the morphological characteristics described in Table 4. Therefore, DL0017 was confirmed as a new species, namely *O. langbianensis*.

**Discussion**

Lang Biang Biosphere Reserve, located in Lam Dong Province, is classified as Vietnam’s biodiversity center and considered a hotspot of fungal biodiversity, including entomopathogenic fungi. During our expedition to validate the diversity of entomopathogenic fungi in Lang Biang Biosphere Reserve, the sample DL0017 was collected.

Morphological analysis indicated that DL0017, named *Ophiocordyces langbianensis*, is a new taxon. Species belonging to the family Ophiocordycipitaceae have stromata that are darkly pigmented or rarely brightly colored), tough, fibrous, pliant, and rarely fleshy. Additionally, ascii are usually cylindrical with thickened ascus apex. Ascospores are usually cylindrical, multisepitate, and disarticulate into part-spores or non-disarticulating. Our specimen shares these common characteristics.

Based on the phylogenetic analysis, the specimen DL0017 clustered with *Ophiocordyces brunnipeunctata* in Ophiocordycipitaceae. However, the morphologies of these two species are different in many characteristics, including color, size of stroma, stipe, and dots in the fertile portion. The apical appendix of *O. brunnipeunctata* lacks branching, while *O. langbianensis* has 2–10 branches. Additionally, the ascospores of *O. brunnipeunctata* break into part-spores, while the ascospores of *O. langbianensis* stick together to form a multisepitate chain, which could only be ruptured into unicellular part-spores by a strong force, while ascospores of *Cordyceps furcicaodata* often break into unicellular part-spores.
| Taxon                  | Genus     | nrLSU         | nrSSU         | rpb1       | ITS        | Tef       |
|----------------------|-----------|---------------|---------------|------------|------------|-----------|
| Claviceps fusiformis | Claviceps | U17402        | U32401        | -          | JN049817   | DQ522320  |
| Claviceps paspali    | Claviceps | U47826        |               | -          | JN049818   | DQ522321  |
| Claviceps purpurea   | Claviceps | AF543789      | AF543765      | AY489648   | KI529004   | AF543778  |
| Claviceps purpurea   | Claviceps | EF469075      | EF469122      | EFE469087  | KX977396   | EFE469058 |
| Metacordyceps chlamydosporia | Metacordyceps | DQ518758 | DQ522544 | DQ522372 | - | EFE469069 |
| Metacordyceps taitai | Metacordyceps | DQ543787 | AF543763 | DQ522383 | - | AF543775 |
| Metacordyceps langshanensis | Metacordyceps | EF468815 | EF468962 | - | EF468756 |
| Metacordyceps langshanensis | Metacordyceps | EF468814 | EF468961 | - | EF468755 |
| Conioideocrella luteostratata | Conioideocrella | EF468830 | EF468995 | EF489060 | JN049859 | EFE468801 |
| Conioideocrella luteostratata | Conioideocrella | EF468849 | EF489094 | EF488905 | JN049860 | EFE468800 |
| Ophiocordyceps acicularis | Ophiocordyceps | EF468805 | EF468950 | EF488852 | JN049820 | EFE468744 |
| Ophiocordyceps acicularis | Ophiocordyceps | EF468804 | EF488951 | EF488853 | GU723772 | EFE468745 |
| Ophiocordyceps aphyllus | Ophiocordyceps | DQ518755 | DQ522541 | - | - | - |
| Ophiocordyceps brauneipunctata | Ophiocordyceps | DQ518756 | DQ522542 | DQ522369 | GU723777 | DQ522324 |
| Ophiocordyceps sinensis | Ophiocordyceps | EF468827 | MF403011 | EF488874 | JN049854 | EFE468767 |
| Ophiocordyceps stylophora | Ophiocordyceps | EF468837 | EF488982 | EF488882 | - | EFE468777 |
| Ophiocordyceps stylophora | Ophiocordyceps | DQ521766 | DQ522552 | DQ522382 | JN049828 | DQ522337 |
| Ophiocordyceps australis | Ophiocordyceps | DQ518768 | DQ222554 | DQ522385 | - | - |
| Ophiocordyceps variabilis | Ophiocordyceps | EF468839 | EF488985 | EF488885 | - | EFE468779 |
| Ophiocordyceps entomohirra | Ophiocordyceps | EF468809 | EF488954 | EF488875 | JN049850 | EFE468749 |
| Ophiocordyceps gracilis | Ophiocordyceps | EF468810 | EF488955 | EF488858 | AJ786563 | EFE468750 |
| Ophiocordyceps gracilis | Ophiocordyceps | EF468811 | EF488956 | EF488959 | AJ786564 | EFE468751 |
| Ophiocordyceps heteropoda | Ophiocordyceps | AYA89722 | AYA89690 | AYA89651 | FJ76028 | AYA89617 |
| Ophiocordyceps heteropoda | Ophiocordyceps | EF468812 | EF488957 | EF488860 | JN049852 | EFE468752 |
| Ophiocordyceps nigrella | Ophiocordyceps | EF468818 | EF488963 | EF488866 | JN049853 | EFE468758 |
| Ophiocordyceps rhizoida | Ophiocordyceps | EF468825 | EF488970 | EF488873 | JN049857 | EFE468764 |
| Ophiocordyceps rhizoida | Ophiocordyceps | EF468824 | EF488969 | EF488872 | MH175420 | EFE468765 |
| Beauveria caledonica | Beauveria | AF339520 | AF339570 | EF490064 | HQ880017 | EFE469057 |
| Cordyceps cf. pruinosa | Cordyceps | EF468820 | EF488965 | EF488868 | - | DQ522351 |
| Cordyceps cf. pruinosa | Cordyceps | EF468821 | EF488966 | EF488869 | - | - |
| Cordyceps cf. pruinosa | Cordyceps | EF468823 | EF488968 | EF488871 | - | EFE468761 |
| Cordyceps cicadae | Cordyceps | MH879588 | MH879636 | MH885438 | MH93774 | - |
| Cordyceps cicadae | Cordyceps | MK761212 | MK761207 | MF416553 | MH937742 | - |
| Cordyceps kyusyuensis | Cordyceps | EF468813 | EF488960 | EF488863 | - | - |
| Cordyceps militaris | Cordyceps | AYA184966 | AYA184977 | DQ522377 | - | DQ522332 |
| Cordyceps pruinosa | Cordyceps | AYA184968 | AYA184979 | DQ522397 | - | EFE468763 |
| Cordyceps scarabaeicola | Cordyceps | AF339524 | AF339574 | DQ522380 | JN049827 | DQ522335 |
| Cordyceps staphylinidicola | Beauveria | EF468836 | EF488981 | EF488881 | - | EFE468776 |
| Lecanicillium antillanum | Lecanicillium | AF339536 | AF339585 | DQ522396 | MH861888 | DQ522350 |
| Lecanicillium fusciporum | Lecanicillium | AF339549 | AF339598 | EF488889 | - | EFE468776 |
| Lecanicillium psalliota | Lecanicillium | AF339599 | AF339608 | EF488909 | - | - |
| Lecanicillium tenuipes | Lecanicillium | AF339526 | AF339576 | DQ522387 | JN036556 | DQ522341 |
| Cordyceps ninchikispora | Cordyceps | EF468846 | EF488991 | EF489000 | - | EFE468795 |
| Cordyceps ninchikispora | Cordyceps | EF468847 | EF488992 | EF489001 | - | EFE468794 |
| Simplicillium lamellicola | Simplicillium | AF339552 | AF339601 | DQ522404 | MH854806 | DQ522356 |
| Simplicillium lazosonivum | Simplicillium | AF339554 | AF339603 | DQ522405 | - | - |
| Simplicillium lazosonivum | Simplicillium | AF339553 | AF339602 | DQ522406 | - | DQ522357 |
| Simplicillium obclavatum | Simplicillium | AF339517 | AF339567 | - | MH860859 | DQ522358 |
| Glomerella cingulate | Colletotrichum | AF543786 | AF543762 | AY489659 | FJ904831 | AF543773 |
| Glomerella cingulata | Colletotrichum | U48428 | U48427 | DQ858454 | EU520087 | AF543772 |

Table 2. Representative taxon information and GenBank accession numbers for sequences used in current study. -: no accession number recorded. *Outgroup.
The asexual morph of *O. langbianensis* consists of long and divergent phialides, elliptical conidia usually in chains considered paecilomyces-like or purpureocillium-like. Conversely, *O. bruneipunctata* produced a mononematous hirsutella-like asexual morph from colonies after 3–4 weeks.

**Conclusion**

We successfully applied morphological characterization in combination with phylogenetic analysis of multiple genes, including *nrLSU*, *nrSSU*, *Rpb*, *ITS*, and *Tef*, to delimit sample DL0017, collected from Lang Biang Biosphere Reserve located in Lam Dong Province, Vietnam, as a new species named *Ophiocordyceps langbianensis*, belonging to the genus of *Ophiocordyceps* (Ophiocordycipitaceae, Hypocreales).

| Gene     | Bootstrap value (NJ/MP/ML) | DL0017_nrlSU            | Ophiocordyceps_brunneipunctata_nrlSU_DQ518756 |
|----------|---------------------------|-------------------------|-----------------------------------------------|
| nrLSU    | 90/87/91                 | Ophiocordyceps_brunneipunctata_nrlSU_DQ518756 |
| nrSSU    | 75/74/72                 | DL0017_nrSSU            | Ophiocordyceps_brunneipunctata_nrSSU_DQ522542 |
| Rpb1     | 100/99/98                | DL0017_RPB              | Ophiocordyceps_brunneipunctata_RPB_DQ522369  |
| ITS      | 50/50/79                 | DL0017_ITS              | Ophiocordyceps_brunneipunctata_ITS_DQ723777  |
| Tef      | 90/90/85                 | DL0017_TEF              | Ophiocordyceps_brunneipunctata_tef_DQ522324  |

Table 3. DL0017 clustered together with *Ophiocordyceps brunneipunctata* with bootstrap support.

Figure 4. Phylogenetic relationship between *O. langbianensis* and its allies based on five regions, *nrLSU*- *nrSSU*- *Rpb*- *ITS*- *Tef* data. Bootstrap values (1,000 replicates) are indicated above the nodes.
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Figure 5. Reconstruction of Neighbor-Net network of DL0017 and its allies.

Table 4. Comparison between Ophiocordyceps langbianensis và Ophiocordyceps brunneipunctata. *Reference from Ophiocordyceps brunneipunctata (Hywel-Jones) G.H. Sung, J.M. Sung, Hywel-Jones & Spatafora.

|                      | Ophiocordyceps langbianensis | Ophiocordyceps brunneipunctata* |
|----------------------|-----------------------------|---------------------------------
| Stromata             | Arising from the head of host larva | Arising from one end of the insect larva |
|                      | Solitary, rarely branch, 40–100 mm long | Solitary, rarely up to 3, simple, 25–90 mm long |
| Stipe               | Fibrous, cylindrical 30–67 mm × 0.7–1.0 mm, light yellow | Simple, cylindrical, 5–15 mm × 1–1.8 mm, base reddish-brown |
| Fertile portion      | Cylindrical 7.0–14.0 mm × 1.5–2.0 mm, brownish yellow with dark brown dots, that present in the ostiole of the perithecia | Subterminal, cinnamon in color, with brown ostioles apparent, 5–15 × 1–1.8 mm |
| Perithecia           | Embedded, ovate or pyriform, 260–400 µm × 100–190 µm | Immersed, ovate to pyriform, brown, 270–335 µm × 110–160 µm |
| Asci                | Cylindrical, 200–250 µm × 5.0–6.0 µm, with thick cap | Hyaline, cylindrical, 280–295 µm × 6–7 µm, with prominent apical cap |
| Ascospores           | Filiform, multiseptate, disarticulating into unicellular partspores | Filiform, filiform, flexuous, breaking into partspores |
|                      | Partspores: cylindrical, swollen, two waist-like constriction, 5.0–7.5 µm × 1.25–2.0 µm | Partspores truncate, 4–6 µm × 1–1.5 µm |
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Author contributions
N.B.T. collected the sample DL0017. T.D.L., T.A.H.L. conceived, planned and carried out the experiments and contributed to the interpretation of the results; T.D.L. took the lead in writing the manuscript. All authors provided critical feedback and revised the manuscript.

Competing interests
The authors declare no competing interests.

Additional information
Correspondence and requests for materials should be addressed to N.B.T.

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