Research Article

Thuan Duc Lao#, Hanh Van Trinh#, Loi Vuong, Luyen Tien Vu, Thuy Ai Huyen Le, Hiep Minh Dinh, Nguyen Binh Truong*

Molecular record for the first authentication of *Isaria cicadae* from Vietnam

https://doi.org/10.1515/biol-2021-0074
received February 18, 2020; accepted June 19, 2021

Abstract: The entomopathogenic fungus T011, parasitizing on nymph of Cicada, collected in the coffee garden in Dak Lak Province, Vietnam, was preliminarily morphologically identified as *Isaria cicadae*, belonged to order Hypocreales and family Clavicipitaceae. To ensure the authenticity of T011, phylogenetic analysis of the concatenated set of multiple genes including ITS, nrLSU, nrSSU, Rpb1, and Tef1 was applied to support the identification. Genomic DNA was isolated from dried sample T011. The PCR assay sequencing was applied to amplify *ITS*, *nrLSU*, *nrSSU*, *Rpb1*, and *Tef1* gene. For phylogenetic analysis, the concatenated data containing 62 sequences belonged to order Hypocreales, families Clavicipitaceae, and 2 outgroup sequences belonged to order Hypocreales, genus *Verticillium*. The phylogenetic analysis results indicated that T011 was accepted at subclade *Cordyceps* and significantly formed the monophyletic group with referent *Cordyceps cicadae* (Telemorph of *Isaria cicadae*) with high bootstrap value. The phylogenetically analyzed result was strongly supported by our morphological analysis described as the *Isaria cicadae*. In summary, phylogenetic analyses based on the concatenated dataset were successfully applied to strengthen the identification of T011 as *Isaria cicadae*.

Keywords: nuclear small ribosomal subunit, nuclear large ribosomal subunit, *Isaria cicadae*, phylogeny

1 Introduction

*Isaria cicadae* Miq., Bull. Sci. phys. nat. néerl.: 86 (1838) (Mycobank: MB#204858), also known *Cordyceps cicadae* (Miq.) Massee (1895) (Mycobank: MB#311793), is the entomopathogenic fungi capable of parasitizing on cicada nymph, belongs to the order Hypocreales, and the family Clavicipitaceae [4,5]. *C. cicadae* usually distribute in many regions of the world with temperatures ranging from 18 to 24°C, relative humidity of >80°C, and grows vertically on the sunny slopes at an altitude of 700–950 m [3]. The distribution of *C. cicadae* is recorded in China (Province of Yunnan, Sichuan, Guizhou, Jiangsu, Guangdong, Hunan, Hubei, etc.), Korea (Jeju Island), and Japan (South of Fukushima). Furthermore, *C. cicadae* is also seen in Thailand, North America, and Europe [3–24].

Due to their numerous bioactivities, *I. cicadae*, as well as *C. cicadae*, is considered the most valued traditional Chinese medicine. Its medicinal bioactive components, such as adenosine, cordycepin, ergosterol, etc., which have been used to relieve exhaustion remedy, treat numerous diseases, such as antitumor activities, and food source, have been recorded [3,19–22]. To obtain precious valued herbal medicine, the exploration and collection of local *I. cicadae* (*C. cicadae*) play an important role to apply for further medicinal applications. During our expedition to validate the fungal diversity in Ea Knop Town – Ea Kar District (Latitude: 13°34′26″N–13°02′09″N; Longitude: 108°22′08″E–108°43′2″E) located in Dak Lak Province, we collected the sample T011, parasitizing on the nymph of Cicada, which was classified and confirmed by the specialist on the entomologist, Faculty of Biotechnology, Ho Chi Minh City Open University, Ho Chi Minh City, Ho Chi Minh City, Vietnam.

# Equal contributors.

* Corresponding author: Nguyen Binh Truong, Faculty of Biology, Dalat University, Lam Dong, Vietnam, e-mail: nguyentb@dlu.edu.vn
Thuan Duc Lao, Luyen Tien Vu, Thuy Ai Huyen Le: Department of Pharmaceutical and Medical Biotechnology, Faculty of Biotechnology, Ho Chi Minh City Open University, Ho Chi Minh City, Vietnam
Hanh Van Trinh: University of Science, VNU-HCM, Ho Chi Minh City, Vietnam
Loi Vuong: University of Science, VNU-HCM, Ho Chi Minh City, Vietnam; Institute of Applied Technology, Thu Dau Mot University, Binh Duong, Vietnam
Hiep Minh Dinh: Department of Agriculture and Rural Development of Ho Chi Minh City, Ho Chi Minh City, Vietnam

Open Access. © 2021 Thuan Duc Lao et al., published by De Gruyter. This work is licensed under the Creative Commons Attribution 4.0 International License.
Minh City, Vietnam. In this paper, to ensure the origin and authenticity of T011 as *I. cicadae*, we conducted the morphology analysis and molecular phylogenetic analysis of the concatenated set genes including ITS, *nrLSU*, *nrSSU*, *Rpb1*, and *Tef1*.

2 Materials and methods

2.1 Sample collection

The specimen T011, parasitizing on the nymph of Cicada, was collected in the coffee garden in Ea Knop Town – Ea Kar District, Dak Lak Province on the morning of June 24, 2018. In the laboratory, the specimen was conditioned to be dried at 60°C and stored for further analysis.

2.2 Morphology analysis

Macroscopic characteristics of the fresh body were carefully observed in the many macroscopic characteristics. For the microscopic analysis, a bunch of conidiogenous cells was cut into small species, then, soaked in the water for about 3 min. A sample of the synemata containing the conidiogenous cells was immersed in distilled water for 3 min. Asexual spores were removed using a clean brush. The fertile part was then analyzed under a microscope. Conidia size was recorded. 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, 10 g/L Malt extract, 4 g/L Glucose, incubated at 20°C within a period of 20 days.

2.3 DNA extraction, PCR amplification, target gene sequencing

Genomic DNA was extracted from dried material by using the phenol/chloroform method (pH = 8). The dried material was added to a lysis buffer (2.0% SDS, Tris-HCl pH 8.0, 150 mM NaCl, 10 mM EDTA, 0.1 mg/mL Proteinase K). During the incubation at 65°C for overnight, it was mixed thoroughly by inverting the tube several times. Then, the supernatant was collected by centrifugation. About 700 μL of phenol/chloroform/isoamyl alcohol at a ratio of 25:24:1 was added and centrifuged. The upper solution was collected, precipitated with absolute ethanol, and washed with 70% ethanol. DNA concentration was identified by using OD260. Finally, isolated genomic DNA was stored in Tris-EDTA buffer at −20°C for further studies.

The primer pairs used to amplify ITS, *nrLSU*, *nrSSU*, *Rpb1*, and *Tef1* regions were shown in Table 1. The final volume for PCR was done in the total of 15 μL with the thermal program: 1 cycle for 95°C for 5 min; 40 cycles of 95°C for 30 s, X°C for 30 s, 72°C for 2 min; 1 cycle for 72°C for 5 min (Note: X°C is the annealing temperatures for each target gene, shown in Table 1). About 5 μL aliquots of amplification products were electrophoresed on a 2.0% agarose gel and visualized in a UV transilluminator. The amplified product was sequenced at Nam Khoa (Vietnam) company.

2.4 Taxa and ITS, *nrLSU*, *nrSSU*, *Rpb1*, and *Tef1* sequences collection, DNA proofreading, and phylogeny analysis

The data set of ITS, *nrLSU*, *nrSSU*, *Rpb1*, and *Tef1* sequences were established by sequences downloaded from Genbank.

---

**Table 1:** The primers’ sequences used in this study

| Target gene | Primer | Sequence (5’–3’) | \(T_a\) (°C) | Reference |
|-------------|--------|-----------------|-------------|-----------|
| *nrLSU*     | LR0R (F) | GTACCGCTGAACTTAAGC | 55 | [18] |
|             | LRS (R) | ATCCGAGGGAACCTTC | \(42.2\) | [20] |
| *nrSSU*     | NS1 (F) | GTAGTCATATGCTTCTC | \(55\) | [20] |
|             | NS4 (R) | CTTCCGTCAATCCCTTAAG | \(46.3\) | [13] |
| **ITS**     | ITS1F | CTTGGTCAATTAGGAAAGTA | \(55\) | [20] |
|             | ITS4  | TCCTCCGCTTATTGATATGC | \(55\) | [2] |
| *Rpb1*      | CRPB1 | CCWGGTATAGCAGAAGT | \(46.3\) | [13] |
|             | RPB1Cr | CCNGCDATCTCRTCTCATRA | \(55\) | [2] |
| *Tef1*      | 983F | GACYGGHGACGCTGGATYAT | \(55\) | [2] |
|             | 2218R | ATGACACCRARCGRACRGTYTG | \(55\) | [2] |

Note: F: forward primer; R: reverse primer; \(T_a\): annealing temperature.
and based on the previous data published by Sung et al. (2007) [16]. The ITS, nrLSU, nrSSU, Rpb1, and Tef1 were noted with accession number, name of taxon, and locality. The multiple gene data used in the current study were established based on the combination of ITS, nrLSU, nrSSU, Rpb1, and Tef1 data. 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 the neighbor-joining (NJ), maximum parsimony (MP), and maximum likelihood (ML) by using Molecular Evolutionary Genetics Analysis (MEGA) version X. Additionally, the best evolution model was predicted by using jModelTest.

### Table 2: BLAST results of T011 specimen’s ITS, nrLSU, nrSSU, Rpb1, and Tef1

| Target gene | BLAST description     | Total score | Per. Ident. | E-value | Accession   |
|-------------|-----------------------|-------------|-------------|---------|-------------|
| nrLSU-F     | Cordyceps cicadae     | 1,509       | 100.00      | 0.0     | MH879588    |
| nrLSU-R     | Cordyceps cicadae     | 1,509       | 99.64       | 0.0     | MH879588    |
| nrSSU-F     | Cordyceps cicadae     | 1,109       | 100.00      | 0.0     | MH879636    |
| nrSSU-R     | Cordyceps cicadae     | 1,048       | 99.65       | 0.0     | MH879636    |
| ITS-F       | Cordyceps cicadae     | 1,000       | 99.82       | 0.0     | MT555324    |
| ITS-R       | Cordyceps cicadae     | 1,444       | 99.82       | 0.0     | MN128643    |
| Tef1-F      | Cordyceps cicadae     | 1,729       | 99.17       | 0.0     | MH879662    |
| Tef1-R      | Cordyceps cicadae     | 1,676       | 98.33       | 0.0     | MN576985    |
| Rpb1-F      | Cordyceps cicadae     | 1,247       | 100.00      | 0.0     | MN913552    |
| Rpb1-F      | Cordyceps cicadae     | 1,280       | 99.57       | 0.0     | MN576876    |

Note: F: forward sequence; R: reverse sequence; Per. Ident.: percentage of identity.

(NCBI) and based on the previous data published by Sung et al. (2007) [16]. The ITS, nrLSU, nrSSU, Rpb1, and Tef1 were noted with accession number, name of taxon, and locality. The multiple gene data used in the current study were established based on the combination of ITS, nrLSU, nrSSU, Rpb1, and Tef1 data. 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 the neighbor-joining (NJ), maximum parsimony (MP), and maximum likelihood (ML) by using Molecular Evolutionary Genetics Analysis (MEGA) version X. Additionally, the best evolution model was predicted by using jModelTest.

### 3 Results

#### 3.1 Morphology analysis

The sample of T011 was collected in the soil of the coffee garden on the morning of June 24, 2018. The synnemata were emerging from the soil, while the host was in the soil. **Host**: unidentified cicada nymph. **Synnemata**: presence, branching, 15–60 mm in length × 1.0–2.5 mm in diameter. Synnemata originated from the head of cicada nymphs with the thick layer of mycelia (hiding under the ground). **Color**: white to cream. **Form**: simple, erect, and
| No. | Taxon | Genus | nrLSU Accession | nrSSU Accession | Rbp1 Accession | Teft Accession | ITS Accession |
|-----|-------|-------|-----------------|-----------------|----------------|---------------|---------------|
| 1   | Balansia pilulaeformis | Balansia | AF543788 | AF543764 | DQ522365 | DQ522319 | JN049816 |
| 2   | Beauveria caledonica | Beauveria | AF339520 | AF339570 | EF469086 | EF469057 | HQ880817 |
| 3   | Beauveria scarabaeicola | Beauveria | AF339524 | AF339574 | DQ522380 | DQ522335 | JN049827 |
| 4   | Beauveria staphylindicola | Beauveria | EF468836 | EF468981 | EF468881 | EF468776 | — |
| 5   | Claviceps fusiformis | Claviceps | UI7402 | DQ522539 | DQ522366 | DQ522320 | JN049817 |
| 6   | Claviceps paspali | Claviceps | U47826 | U32401 | DQ522367 | DQ522321 | JN049818 |
| 7   | Claviceps purpurea | Claviceps | AF469075 | EF469122 | EF469087 | EF469058 | KX777396 |
| 8   | Cordyceps unilateralis | Cordyceps | AF469859 | AF469765 | AF469848 | AF469801 | JN049859 |
| 9   | Cordyceps purpurea | Cordyceps | AF469850 | EF468995 | EF468906 | EF468801 | JN049860 |
| 10  | Cordyceps cicala | Cordyceps | MIH879588 | MIH879636 | MIH85438 | MIH879662 | MT803085 |
| 11  | Cordyceps ksyysuenensis | Cordyceps | EF468813 | EF468960 | EF468863 | EF468754 | EF368021 |
| 12  | Cordyceps militaris | Cordyceps | AV489466 | AV48977 | DQ522377 | DQ522332 | JN049825 |
| 13  | Cordyceps pruinosa | Cordyceps | AV489468 | AV48979 | DQ522397 | DQ522351 | JN049826 |
| 14  | Cordyceps sp. | Cordyceps | MT239107 | — | MT268242 | MT268246 | MT924848 |
| 15  | Drechmeria balanoides | Drechmeria | AF339539 | AF339588 | DQ522388 | DQ522342 | EF466660 |
| 16  | Drechmeria zeaspora | Drechmeria | AF339589 | — | EF469091 | EF469062 | — |
| 17  | Isaria sp. | Isaria | MT239106 | — | MT268241 | MT268245 | MT924847 |
| 18  | Lecanicillium antillanum | Lecanicillium | AF339536 | AF339585 | DQ522386 | DQ522350 | MH861888 |
| 19  | Lecanicillium fusisporum | Lecanicillium | AF339549 | AF339598 | EF46889 | EF468783 | MH859358 |
| 20  | Lecanicillium psalliota | Lecanicillium | AF339559 | AF339608 | EF468890 | EF468874 | NH848648 |
| 21  | Lecanicillium tenuipes | Lecanicillium | AF339526 | AF339576 | DQ522387 | DQ522341 | JN036566 |
| 22  | Metarhizium guizhouense | Metarhizium | AF543787 | AF543763 | DQ522383 | AF543775 | JN049829 |
| 23  | Pochonia chlamydosoria | Pochonia | DQ518758 | DQ522544 | DQ522372 | DQ522327 | JN049821 |
| 24  | Metachrospora builbrosa | Metachrospora | AF339542 | AF339591 | EF468902 | EF468796 | EU999595 |
| 25  | Metachrospora rubescens | Metachrospora | AF339566 | AF339615 | EF468903 | EF468797 | MH862138 |
| 26  | Metarhizium anisopliae | Metarhizium | AF339530 | AF339579 | DQ522399 | AF543774 | JN049834 |
| 27  | Metarhizium carneum | Metarhizium | EF468842 | EF468989 | EF468895 | EF468878 | AY624170 |
| 28  | Metarhizium flavoviride | Metarhizium | EF468843 | EF468988 | EF468894 | EF468879 | AY624171 |
| 29  | Ophiocordycps acicularis | Ophiocordycps | EF468805 | EF468950 | EF468852 | EF468744 | JN049820 |
| 30  | Ophiocordycps acicularis | Ophiocordycps | EF468804 | EF468951 | EF468853 | EF468745 | — |
| 31  | Ophiocordycps aphidii | Ophiocordycps | DQ518755 | DQ522541 | — | DQ522323 | — |
| 32  | Ophiocordycps entomorrhiza | Ophiocordycps | EF468809 | EF468954 | EF468857 | EF468749 | JN049850 |
| 33  | Ophiocordycps melolonthae | Ophiocordycps | DQ518762 | DQ522548 | DQ522376 | DQ522331 | KF873753 |
| 34  | Ophiocordycps stylophora | Ophiocordycps | EF468837 | EF468982 | EF468882 | EF468777 | — |
| 35  | Ophiocordycps stylophora | Ophiocordycps | DQ518766 | DQ522552 | DQ522382 | DQ522337 | JN049828 |
| 36  | Ophiocordycps unilateralis | Ophiocordycps | DQ518768 | DQ522554 | DQ522385 | DQ522339 | AY949596 |
| 37  | Ophiocordycps variabilis | Ophiocordycps | EF468839 | EF468985 | EF468885 | EF468779 | — |
| 38  | Ophiocordycps variabilis | Ophiocordycps | DQ518769 | DQ522555 | DQ522386 | — | — |
| 39  | Ophiocordycps gracilis | Ophiocordycps | EF468810 | EF468955 | EF468858 | EF468750 | HM119586 |
| 40  | Ophiocordycps gracilis | Ophiocordycps | EF468811 | EF468956 | EF468859 | EF468751 | JN049851 |
| 41  | Ophiocordycps heteropoda | Ophiocordycps | AY489722 | AY489690 | AY489651 | AY489617 | — |
| 42  | Ophiocordycps heteropoda | Ophiocordycps | EF468812 | EF468957 | EF468860 | EF468752 | JN049852 |
| 43  | Ophiocordycps nigrella | Ophiocordycps | EF468818 | EF468963 | EF46886 | EF468758 | JN049853 |
| 44  | Ophiocordycps rhizoidea | Ophiocordycps | EF468825 | EF468970 | EF468873 | EF467764 | JN049857 |
| 45  | Ophiocordycps rhizoidea | Ophiocordycps | EF468824 | EF468969 | EF468872 | EF467865 | GU723769 |
| 46  | Ophiocordycps robertsi | Ophiocordycps | EF468826 | — | — | EF468766 | AJ309393 |
| 47  | Ophiocordycps sobolifera | Ophiocordycps | KJ878898 | KJ878933 | KJ879013 | KJ878979 | KT281884 |

(continued)
3.2 Amplification of ITS, nrLSU, nrSSU, Rpb1, and Tef1 gene

Isolated genomic DNA was amplified with the described primers; then, electrophoresis on 2.0% agarose gel showed a significant and clear band of gene ITS: 700 bps, nrLSU: 950 bps, nrSSU: 1,102 bps, Rpb1: 803 bps, and Tef1: 1,020 bps. The PCR product was sequenced. Sequencing signals of both strands of both target genes were unique and good for reading (data not shown). According to BLAST results, the ITS, nrLSU, nrSSU, Rpb1, and Tef1 of T011 were similar to ITS, nrLSU, nrSSU, Rpb1, and Tef1 of C. cicadae (Telemorph of I. cicadae) (Table 2).

3.3 The systematic concatenated ITS, nrLSU, nrSSU, Rpb1, and Tef1 dataset and phylogeny analysis

Total of 62 sequences of ITS, nrLSU, nrSSU, Rpb1, and Tef1 belonged to order Hypocreales, families Clavicipitaceae (served as referent data), and 2 sequences belonged to order Hypocreales, genus Verticillium (served as outgroup) were collected from Genbank and listed in Table 3 and T011 sequence. According to 62 sequences, they were divided into three families (Cordycipitaceae, Clavicipitaceae, Ophiocordycipitaceae), and each family was also divided intro genus, including Cordycipitaceae (genus: Cordyceps, Beauveria, Simplicillium, Lecanicillium), Clavicipitaceae (genus: Claviceps, Balansia, Pochonia, Coniocephalella, Metapochonia, Metarhizium), and Ophiocordycipitaceae (genus: Drechmeria, Ophiocordyceps). The best-fit model of DNA evolution for the analyses was obtained using the jModelTest2. Results are shown from General Time Reversible and Gramma distributed with invariant sites (G + I) with the following parameters: parameters = 109, BIC = 42628.811, lnL = -20671.999, (-I) = 0.450, (+G) = 0.466, R = 2.186, f(A) = 0.246, f(T) = 0.221, f(G) = 0.257, f(C) = 0.276, r(AC) = 0.030, r(AG) = 0.040, r(TA) = 0.030, r(TC) = 0.260, r(TG) = 0.040, r(CA) = 0.040, r(CT) = 0.220, r(CG) = 0.050, r(GA) = 0.100, r(GT) = 0.330, and r(GC) = 0.040. This model was used to construct phylogenetic trees using maximum likelihood from concatenated data set. Phylogenetic analysis was presented in Figure 2. As the results, the Clavicipitaceae formed a strong monophyletic group and separated from the out group. All the species in our dataset formed threes clades that were previously reported. According to T011, the T011 multiple gene sequences (ITS, nrLSU, nrSSU, Rpb1, and Tef1) formed a group with referent sequences of C. cicadae, Cordyceps sp., and Isaria sp., belonged to the clade Clavicipitaceae, subclade Cordyceps, with the high supported bootstrap values: 100, 100, 100 for NJ, MP, ML method (Figure 2, blanket). Therefore, the molecular identification indicated that T011 was identified as I. cicadae (anamorph of C. cicadae).
Morphology analysis indicated that T011 is *Isaria cicadae*, belonged to the family of Cordycipitaceae. Based on the morphology analysis, our specimen T011 shared the common features of *Isaria cicadae* Miq. Bull. Sci. phys. nat. Néerl.: 85 (1838) [17], including: (1) specimen grew in the soil, (2) parasite on the nymph of cicada, (3) synnemata were simple and erect with branching, white to cream, (4) colonies were floccose, white and turned into powdery with age, and (5) conidia: hyaline to white, cylindrical, large (T011: 4.7–6.5 × 2.6–3.1 mm, and referent: 3.5–8.0 × 1.5–3.5 µm).

To confirm the authenticity of T011 as *Isaria cicadae*, the construction of ITS, nrLSU, nrSSU, Rpbi, and Tef1-
based phylogeny was performed. According to Mitchell et al. (1995), they suggested that molecular phylogenetic approaches to fungal evolution have proved valuable information toward the goals of understanding the relationship among the specific fungal groups [8]. Additionally, the use of fungal molecular data, including ITS, nrLSU, nrSSU, Rpb1, and Tef1, for the identification of fungi ushered in a new era of molecular phylogenetic sequence identification in kingdom Fungi [1,12]. In this study, the combination of ITS, nrLSU, nrSSU, Rpb1, and Tef1 genes were applied to strongly strengthen the identification of T011, which was classified as *I. cicadae*. According to phylogenetic analysis, phylogenetic analysis of ITS, nrLSU, nrSSU, Rpb1, and Tef1 yielded consistent topology in different taxa of Clavicipitaceae. The phylogenetic position of T011 was obtained and accepted at subclade level: Cordyceps. Notably, within this clade, the highly supported monophyletic group with referent *C. cicadae* was obtained with high bootstrap value (Bootstrap >95: NJ: 100; MP: 100; ML: 100) and separated this group from other referent taxonomy in subclade Cordyceps, such as *C. ninchukisora*, *C. pruinosa*, and *C. kyusyuensis*. Additionally, T011 formed the group with referent *C. cicadae*, Cordyceps sp., and *Isaria* sp. Among them, Cordyceps sp. and Isaria sp. were proposed using the ancient Chinese name “chanhua” (*Cordyceps chanhua*) [25]. Therefore, based on the phylogenetic analysis, the T011 was identified as the *Isaria cicadae* (anamorph of *C. cicadae*), which was strongly similar to *Cordyceps chanhua*. Therefore, we have successfully applied the phylogenetic analyses based on the concatenated dataset to strengthen the identification of T011, collected in the local coffee garden in Ea Knop Town – Ea Kar District, as *I. cicadae* (anamorph of *C. cicadae*).

5 Conclusion

We have successfully applied the phylogenetic analysis of multiple genes of ITS, nrLSU, nrSSU, Rpb1, and Tef1 to demmit sample T011, which was collected in Ea Knop Town – Ea Kar District, Dak Lak Province, was strongly supported as *Isaria cicadae* (anamorph of *C. cicadae*), which was similar to our preliminary identification. This is the first molecular record of *Isaria cicadae* in Vietnam.

Acknowledgments: We express our special thanks to National Foundation For Science and Technology Development (NAFOSTED), Vietnam, and Ho Chi Minh City Open University for the genuine support throughout this research work.

Funding information: The research was funded by National Foundation For Science and Technology Development (NAFOSTED): 106-NN.06.2015.44, Vietnam, and Ho Chi Minh City Open University under the grant number E2019.06.3.

Conflict of interest: The authors state no conflict of interest.

Data availability statement: The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

References

[1] Bruns TD, White TJ, Taylor JW. Fungal molecular systematics. Annu Rev Ecol Evol Syst. 1991;22:525–64.
[2] Castlebury LA, Rossman AY, Sung G-H, Hyten AS, Spatafora JW. Multigene phylogeny reveals new lineage for Stachybotrys chartarum, the indoor air fungus. Mycol Res. 2004;108:864–72.
[3] Hsu JH, Jhou BY, Yeh SH, Chen Yi, Chen CC. Healthcare functions of Cordyceps cicadae. J Nutr Food Sci. 2015;5:432. doi: 10.4172/2155-9600.1000432.
[4] Kobayasi Y, Shimizu D. Monographic studies of Cordyceps 2: group parasitic on Cicadae. Bull Natl Sci Mus. 1963;6:286–314.
[5] Li L, Zhang T, Li C, Xie L, Li N, Hou T, et al. Potential therapeutic effects of Cordyceps cicadae and Paecilomyces cicadae on adenosine-induced chronic renal failure in rats and their phytochemical analysis. Drug Design Dev Ther. 2018;13:103–17. doi: 10.2147/DDDT.S180543.
[6] Liu T, Liu Z, Yao X, Huang Y, Qu Q, Shi X, et al. Identification of cordycepin biosynthesis-related genes through de novo transcriptome assembly and analysis in Cordyceps cicadae. R Soc Open Sci. 2018;5(12):181247. doi: 10.1098/rsos.181247.
[7] Luangsa-ard J, Hywel-Jones NL, Manoch L, Samson RA. On the relationships of Paeoliaecomyces sect. Isoiridae species. Mycol Res. 2005;109(PT 5):581–9. doi: 10.1017/s0953756205002741.
[8] Mitchell JJ, Roberts PJ, Moss ST. Sequence or structure: a short review on the application of nucleic acid sequence information to fungal taxonomy. Mycologist. 1995;9:67–76. doi: 10.1016/S0269-915X(09)80212-7.
[9] Olatunji OJ, Feng Y, Olatunji OO, Tang J, Ouyang Z, Su Z. Neuroprotective effects of adenosine isolated from Isaria cicadae against oxidative and ER stress damages induced by glutamate in PC12 cells. Environ Toxicol Pharmacol. 2016;44:53–61. doi: 10.1016/j.etap.2016.02.009.
[10] Paterson RR. Cordyceps: a traditional Chinese medicine and another fungal therapeutic biofactory? Phytochemistry. 2008;69(7):1469–95. doi: 10.1016/j.phytochem.2008.01.027.
[11] Prayook S, Siripuk S, Panida L. First report of Cordyceps sp. isolated from Cicada in Northeastern Thailand and their characterizations. J Biol Sci. 2013;13:587–95. doi: 10.3923/jbs.2013.587.595.
[12] Raja HA, Miller AN, Pearce CJ, Oberlies NH. Fungal identification using molecular tools: a primer for the natural products.
research community. J Nat Products. 2017;80(3):756–70. doi: 10.1021/acs.jnatprod.6b01085.

[13] Rehner S. Primers for elongation factor 1-a (EF1-a). Beltsville, MD, USA: Insect Biocontrol Laboratory USDA, ARS, PSI; 2001. p. 4. 1p. Available online: http://ocid.NACSE.ORG/research/deepphyphae/EF1primer.pdf.

[14] Shi Z, Pan HJ, Fan LF, Advances in research of polysaccharides in Cordyceps species. Food Technol Biotechnol. 2009;47(3):304–12.

[15] Sun YF, Sun Y, Wang ZA, Han RL, Lu HF, Zhang JL, et al. Isaria Cicadae conidia possess antiproliferative and inducing apoptosis properties in gynaecological carcinoma cells. Mycology. 2017;8(4):327–34. doi: 10.1080/21501203.2017.1386243.

[16] Sung GH, Hywel-Jones NL, Sung JM, Luangsa-Ard JJ, Shrestha B, Spatafora JW. Phylogenetic classification of Cordyceps and the clavicipitaceous fungi. Stud Mycol. 2007;57:5–59. doi: 10.3114/sim.2007.57.01.

[17] Vilgalys R, Hester M. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several Cryptococcus species. J Bacteriol. 1990;172:4238–46. doi: 10.1128/JB.172.8.4238-4246.1990.

[18] Vilgalys R, Hester M. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several Cryptococcus species. J Bacteriol. 1990;172:4238–46. doi: 10.1128/JB.172.8.4238-4246.1990.

[19] Weng SC, Chou CJ, Lin LC, Tsai WJ, Kuo YC. Immunomodulatory functions of extracts from the Chinese medicinal fungus Isaria cicadae. J Ethnopharmacol. 2002;83:79–85. doi: 10.1016/s0378-8741(02)00212-x.

[20] White TJ, Bruns T, Lee S, Taylor J. PCR protocols. San Diego: Academic Press, Inc; 1990. p. 315–22. doi: 10.1016/8978-0-12-372180-8.50042-1.

[21] Xiao JH, Zhong JJ. Secondary metabolites from Cordyceps species and their antitumor activity studies. Recent Pat Biotechnol. 2007;1(2):123–37. doi: 10.2174/187220807780809454.

[22] Xie H, Li X, Chen Y, Lang M, Shen Z, Shi L. Ethanolic extract of Cordyceps cicadae exerts antitumor effect on human gastric cancer SGC-7901 cells by inducing apoptosis, cell cycle arrest and endoplasmic reticulum stress. J Ethnopharmacol. 2019;231:230–40. doi: 10.1016/j.jep.2018.11.028.

[23] Zeng WB, Yu H, Ge F, Yang JY, Chen ZH, Wang YB, et al. Distribution of nucleosides in populations of Cordyceps cicadae. Molecules. 2014;19(5):6123–41. doi: 10.3390/molecules19056123.

[24] Zhang CB, Wang YL, Yi M, Dong DC, Su XQ. Identification and phylogenetic analysis of the strain isolated from infected Platylomia pieli in Mopan Mountain, Tianwang Town, Jiangsu province. Guangdong Agric Sci. 2013;40:152–4.

[25] Li ZZ, Luan FG, Hywel-Jones Nigel L, Zhang SL, Chen MJ, Huang B, et al. Biodiversity of cordicipitoid fungi associated with Isaria cicadae Miquel II: teleomorph discovery and nomenclature of chanhua, an important medicinal fungus in China. Mycosystema. 2021;40(2):1–12.