

PAPER • OPEN ACCESS

Observations of the host habitat of *Ophiocordyceps nutans* in the Bidoup Nui Ba National Park, Lam Dong, Vietnam.

To cite this article: T M Nguyen *et al* 2023 *IOP Conf. Ser.: Earth Environ. Sci.* **1155** 012010

View the [article online](#) for updates and enhancements.

You may also like

- [Antioxidant and cytotoxic effects of isolated polysaccharides from cultured *Ophiocordyceps sinensis*](#)
Thu Huynh, Minh-Trang Tran and Minh-Hiep Dinh
- [Thermophysical properties of *Cordyceps militaris* change during the freezing process](#)
Van – Phuc Nguyen, Nguyen-An Nguyen and Duc – Vu Luong
- [Diversity evaluation of *Cordyceps* spp. in Bidoup Nui Ba, Lam Dong province, Vietnam](#)
M H Tran, T M Nguyen and V B Huynh



ECS
The
Electrochemical
Society
Advancing solid state &
electrochemical science & technology

DISCOVER
how sustainability
intersects with
electrochemistry & solid
state science research

Observations of the host habitat of *Ophiocordyceps nutans* in the Bidoup Nui Ba National Park, Lam Dong, Vietnam.

T M Nguyen¹, P T T Doan¹ and V H Nguyen²

¹ Department of Biology, Faculty of Science, Nong Lam University, Ho Chi Minh City, Vietnam

² Institute of Geography, Vietnam Academy of Science and Technology, Cau Giay district, Hanoi, Vietnam

E-mail: ngtpmai@hcmuaf.edu.vn

Abstract. Endoparasitic Cordyceps species infect pest insects and other arthropods all throughout the world. *Ophiocordyceps nutans* like specimens were found in the Lat and Dung K'no of the Lac Duong district during entomogenous fungi forays in Bidoup Nui Ba National Park. However, observations made over the last two years (2021 - 2022) in various forest locations across different altitudinal ranges revealed that *O. nutans* only occurs in two high-altitude forests, with elevations between 1409 and 1589 m asl with the temperature fluctuating from 20,3 and 25,7°C. Morphological comparisons and molecular phylogenetic analyses using D1 - D2 led us to conclude that the neotropical specimens represent *Ophiocordyceps nutans* (Pat.). In this study, the parasitic host of *O. nutans* of Hemiptera and host habitas is the bark and leaves of *Lasianthus bidoupensis* (Rubiaceae). Because *Ophiocordyceps nutans* parasitize and kill different species of stinkbugs that pose a hazard to silviculture and agriculture, making it a viable biocontrol agent.

Keywords: Bugs, Cordyceps, Hemiptera, *Lasianthus*, species.

1. Introduction

Entomopathogenic fungi from a specific fungal group have been studied and applied in the development of traditional Chinese remedies, pharmaceuticals, and bioactive compounds. Many varieties of this fungus have been widely employed as biological control agents for insect pests.

Ophiocordyceps contains fungal species that only parasitize bugs, slay them, and then develop on their dead body. *Ophiocordyceps* is a fungus genus that belongs to the phylum Ascomycota. More than 680 species of *Ophiocordyceps* are reported worldwide, distributed across six continents in very diverse addresses, climates, and hosts. Most of them were found in areas with the high altitude of Nepal, Bhutan, India, China, Korea, Thailand, and Brazil [1, 10, 16, 25]. In nature, *Ophiocordyceps* is considered an entomo-pathogens since the fungus parasitizes its host insects, exploits nutrients of the hosts for their proliferation and reproduction, and ultimately tells the fungus takes over the insects' bodies. *Ophiocordyceps* species are found on a wide variety of insect hosts and are virtually entirely entomopathogenic. Coleoptera, Diptera, Hemiptera, Hymenoptera, Isoptera, Lepidoptera, Neuroptera, and Odonata are the insect orders they prey on. They may infect all phases of the insect life cycle, from larva to pupa to nymph to adult [6, 11, 18]. It has been known that *Ophiocordyceps* have specificities toward a broad range of host insects. Therefore, such property has aroused interest in the possible application of these fungi as biological characteristics [16, 18, 23, 25].

Ophiocordyceps nutans is an entomogenous fungus that thrives on actual bugs and is thought to be found globally. *Ophiocordyceps nutans* is known for its yellow to orange or red, apical, cylindrical broadly ovoid head, obliquely submerged perithecia, ascospores that easily fracture into part spores, and stink bugs as hosts [17].



The Langbian mountain Lam Dong, Vietnam have a diversity of *Cordyceps* fungus. Le Thuy Lien et al discovered the *Cordyceps neovolkiana* at Langbian mountain Dalat in 2013 [9]. In 2017, the *Cordyceps takaomontana* identified by morphology and ITS was used in research of Dinh Minh Hiep [4]. Results of Vu Tien Luyen et al shows that, using the primer pair LR0R and LR5 to analyze the nrLSU gene to support identification of fungus belonging to *Cordyceps* genus and Clavicipitaceae family. The final analysis confirms the DL0004 specimen as *Cordyceps neovolkiana* [22].

This report aims to provide morphological descriptions of the *Ophiocordyceps nutans* in Bidoup Nui Ba National Parks, placed in the northern section of Lam Dong Province in Vietnam. Studied of the ecological niche, the phylogeny relationships of ribosomal sequences D1-D2 and analyze its host habitat of *Ophiocordyceps nutans*.

2. Materials and methods

2.1. Materials

All the specimens were from Bidoup Nui Ba National Park in Lam Dong Province, Vietnam. The specimens were collected, placed in sterile sample boxes, transported to the laboratory within 24 hours, and then stored at 4°C until analyzed.

Morphological analyzing of 65 fungal specimens collected in the research sites, we recorded *Ophiocordyceps nutans* that only parasitize bugs with 4 species belonging to genera Hemiptera, to select 4 representing specimens to DNA sequencing.

2.2. Methods

2.2.1. Field survey and examination, sample collection

Field examinations were conducted from May 22nd, 2021, to October 2nd, 2022, to study the ecological characteristics of *Ophiocordyceps nutans* niches in Bidoup Nui Ba National Park, Lam Dong province. In this examination, the locations of sampling areas were located using a GPS device, and the environmental factors such as altitude, light, and relative humidity at the sampling sites were recorded and evaluated using the Extech 45170 tool in the location where *Ophiocordyceps nutans* were discovered and analyzed (Figure 1).

The sequence of fungal occurrence was calculated using the equation as follow:

$TS (\%) = (t_i/T) \times 100$ with t_i being the number of specimens of the studied species.

T is the total number of specimens collected in each field examination

These specimens were either maintained in sterile sample boxes at 4°C for future study in the Herbarium of Nong Lam University's Faculty of Science in Ho Chi Minh City, Vietnam. (<http://sweetgum.nybg.org/>, NLU).

2.2.2. Morphological analysis

Morphological analysis method based on some characteristics: color, dimension and shape of stroma. The stromata of each specimen were used to obtain the morphological measurements, the measurements were made according to the method of Sasaki et al [14, 15]. The microscopic analyses were observed using an Olympus CX22 microscope from Japan. The observation and measurement of morphological was based on reference to the cordyceps document [19, 20] and for the host [3].

2.2.3. DNA sequencing

DNA of the collected samples was extracted using the CTAB method [24]. The extracted DNA was then used to amplify the D1-D2 regions of the 28S rRNA subunits using the primer sequences [8] as follows:

NL1: 5'-GCATATCAATAAGCGGAGGAAAAG-3'

NL4: 5'-GGTCCGTGTTTCAAGACGG -3'

A ProFlex PCR System was used to accomplish 35 cycles of DNA amplification. (Thermo Fisher Scientific, MA, USA), each cycle lasted 3 minutes at 95 °C, 52 °C at 30 sec, and 72 °C at 2 min. For the amplification, high-fidelity DNA polymerase (BioFact™ H-Star, Korea) was employed, and the PCR products were stored at 4°C until further usage. PCR products were detected using agarose gel electrophoresis stained with ethidium bromide.



Figure 1. Collection of *Ophiocordyceps* in Bidoup Nui Ba National Park, Lam Dong province.

A PCR purification kit (MEGAquick-spin™ Plus Total Fragment DNA Purification Kit, Intron, MA, USA) was used to purify the DNA fragments. Following purification, the fragments were sequenced on an ABI 3500 genetic analyzer (Thermo Fisher Scientific, MA, USA) with a BigDye® Terminator v3.1 Cycle Sequencing Kit. Our sequencing data were entered into Genbank and assigned numbers (Table 2). To identify the species of the collected specimens, we performed nucleotide BLAST analysis (NCBI, Bethesda MD, USA) against the Genbank nucleotide database.

To evaluate the phylogenetic relationships among our specimens to the most common *Ophiocordyceps* species (listed in Table 2), we conducted the sequence alignment using ClustalX v2.0 with manual corrections. Molecular analysis using the D1–D2 sequences included 21 taxa (Table 2) with a total length of 2200 characters in the final dataset. The alignment output was then used in MEGA7's phylogenetic tree analysis, which employed the Maximum Likelihood technique based on the TamuraNei model [7]. The tree with the lowest log probability (-2017.87) was built. The starting tree for the heuristic search was created by applying the UPGMA, Neighbor, and BioNJ algorithms to a matrix of pairwise distances measured using the Maximum Composite Likelihood (MCL) method, and the topology with the highest log likelihood value was chosen.

3. Results

3.1. Distribution and ecological niche

Our collected *Ophiocordyceps nutans* were found in National Park Bidoup Nui Ba, Lam Dong Province, Vietnam (12°00'00" to 12°52'00" North latitude and 108°17'00" to 108°42'00" East longitude). The area belongs to a tropical rainforest road-leaf trees and woody bamboo.

In our field examination, we found 65 specimens, and most specimens were collected in the areas belonging to Lat and Đưng K'Nó communes. All specimens were collected in rugged terrains of various locations along the forest streams at the altitude of 1409 m to 1589 m. *Ophiocordyceps* specimens were discovered on the forest floor amid thick layers of decomposing leaves. The relative humidity ranged from 75% to 96% in the sample sites. The air temperature went from 20,3°C to 25,7°C in the research regions, the soil temperature went from 17,5°C to 18,5°C, and there was poor scattered light (less than 200 lux) in the sample sites. It was also noted that *Ophiocordyceps nutans* were only founded in decaying vegetation layers of the forest floor that were close to water, such as streamlines or in damp, shady areas of the mixed forests broad with leave saved wooden trees, bamboos, and vines. Among 65 collected specimens, all *O. nutans* specimens have stinkbugs as the host insects, thertherespecimens were *Halyomorpha halys* corresponding to the frequencies of occurrence at 43,1%;

19 specimens were *Acanthosoma labiduroides* (29,2%); 10 specimens were (15,4%), and 8 specimens were *Proxys punctulatus* corresponding to the frequencies of occurrence at 12,3%. The environmental factors of the collection areas, stroma, and host insects are shown in Table 1. The distribution map of *Ophiocordyceps* in National Park Bidoup Nui Ba, Lam Dong Province, is shown in Figure 2.

Table 1. The environmental factors at 65 areas where *Ophiocordyceps nutans* were collected

Location	North longitude	East longitude	Altitudes (m)	Temperature (°C)	Humidity (%)	Host insects	Stroma (cm)
1	12°8'28"	108°23'35"	1545.660	23,6	87	11	16,7
2	12°8'20"	108°23'38"	1545.202	23,3	88	11	16,9
3	12°8'27"	108°23'39"	1537.864	23,8	80	12	15,1
4	12°8'30"	108°23'40"	1546.317	23,4	90	12	12,7
5*	12°8'32"	108°23'37"	1537.261	23,1	92	11	15,7
6	12°8'35"	108°23'38"	1531.693	22,4	94	13	9,6
7	12°8'37"	108°23'38"	1525.457	22,6	92	15	11,2
8	12°8'37"	108°23'43"	1547.625	22,3	96	11	16,2
9	12°8'26"	108°23'48"	1542.959	25,0	83	13	10,1
10	12°8'20"	108°23'46"	1510.834	25,6	82	15	11,6
11	12°8'27"	108°23'40"	1542.337	23,1	75	11	14,3
12	12°8'28"	108°23'38"	1547.717	23,8	87	11	14,7
13	12°8'30"	108°23'38"	1545.573	23,2	88	12	12,8
14	12°8'27"	108°23'39"	1537.864	23,3	80	12	12,1
15*	12°8'30"	108°23'39"	1546.010	23,4	90	11	15,8
16*	12°8'32"	108°23'37"	1537.261	23,1	92	11	16,4
17	12°8'35"	108°23'38"	1531.693	22,4	94	11	18,5
18	12°8'27"	108°23'38"	1536.135	22,6	92	11	16,5
19	12°8'37"	108°23'48"	1575.612	25,3	82	11	13,2
20*	12°8'20"	108°23'48"	1505.873	23,1	75	11	9,7
21	12°8'37"	108°23'47"	1568.848	23,4	84	13	9,8
22	12°8'36"	108°23'48"	1589.729	23,7	80	13	10,1
23	12°8'26"	108°23'48"	1542.959	22,9	80	15	12,1
24	12°8'30"	108°23'36"	1545.545	23,0	82	12	11,5
25	12°8'33"	108°23'54"	1571.542	21,0	85	12	13,2
26*	12°8'34"	108°23'54"	1586.203	20,9	88	11	14,5
27*	12°8'22"	108°23'47"	1526.962	23,6	87	11	13,8
28	12°8'23"	108°23'45"	1531.952	25,2	86	12	12,9
29	12°8'27"	108°23'32"	1544.378	23,3	90	13	11,2
30	12°8'39"	108°23'48"	1548.566	21,5	79	13	9,8
31	12°8'38"	108°23'42"	1531.919	22,1	81	15	10,4
32	12°8'36"	108°23'47"	1582.661	25,7	76	15	7,8
33	12°8'28"	108°23'46"	1555.148	24,9	80	11	12,8
34*	12°8'27"	108°23'41"	1546.384	23,2	76	11	10,5
35	12°8'29"	108°23'43"	1554.329	24,0	79	15	11,4
36	12°8'39"	108°23'41"	1528.409	25,3	92	12	18,4
37	12°8'29"	108°23'46"	1561.419	22,4	86	13	12,0
38	12°11'33"	108°26'57"	1568.580	22,8	87	12	13,2
39	12°11'34"	108°26'59"	1552.139	22,7	83	11	14,5
40	12°11'25"	108°26'89"	1509.695	24,1	84	13	11,8
41	12°10'35"	108°27'45"	1458.038	24,3	80	12	12,8

42	12°10'24"	108°27'22"	1499.157	24,8	79	12	13,6
43*	12°9'29"	108°31'18"	1794.135	21,5	95	11	13,8
44*	12°9'31"	108°31'42"	1757.507	22,1	94	11	15,4
45*	12°9'36"	108°31'57"	1688.801	20,7	93	11	15,8
46	12°13'32"	108°34'47"	1565.915	21,1	88	11	14,8
Location	North longitude	East longitude	Altitudes (m)	Temperature (°C)	Humidity (%)	Host insects	Stroma (cm)
47	12°13'34"	108°34'35"	1476.045	20,3	91	13	10,5
48	12°13'49"	108°35'47"	1470.148	20,0	93	12	11,4
49	12°12'29"	108°33'25"	1446.451	20,3	93	12	16,4
50*	12°12'54"	108°33'41"	1476.882	20,5	91	11	15,0
51*	12°14'35"	108°26'89"	1474.265	20,7	92	11	15,8
52	12°14'45"	108°27'45"	1503.798	25,0	82	12	16,9
53	12°14'84"	108°27'22"	1615.709	21,0	92	11	15,2
54	12°14'42"	108°27'44"	1509.872	20,0	87	12	16,0
55	12°13'29"	108°31'18"	1490.964	25,5	91	15	9,8
56	12°13'31"	108°31'42"	1447.982	20,3	96	11	12,4
57	12°13'26"	108°31'57"	1482.628	21,4	94	12	16,9
58*	12°12'22"	108°34'47"	1429.307	20,0	96	11	14,7
59	12°12'54"	108°34'35"	1508.011	20,2	95	12	13,5
60	12°14'41"	108°35'47"	1409.490	20,5	92	13	12,4
61	12°9'28"	108°37'49"	1513.236	23,3	94	12	16,9
62	12°9'67"	108°37'53"	1621.466	23,0	92	12	17,2
63*	12°9'49"	108°37'39"	1523.640	22,9	91	11	15,7
64*	12°10'24"	108°42'75"	1508.205	23,4	89	11	17,5
65	12°10'32"	108°42'83"	1548.407	23,6	88	15	13,5

(11) *Halyomorpha halys*; (12) *Acanthosoma labiduroides*; (13) *Clavigralla scutellaris*; (15) *Proxys punctulatus*

* in places *Lasianthus bidoupensis* growing

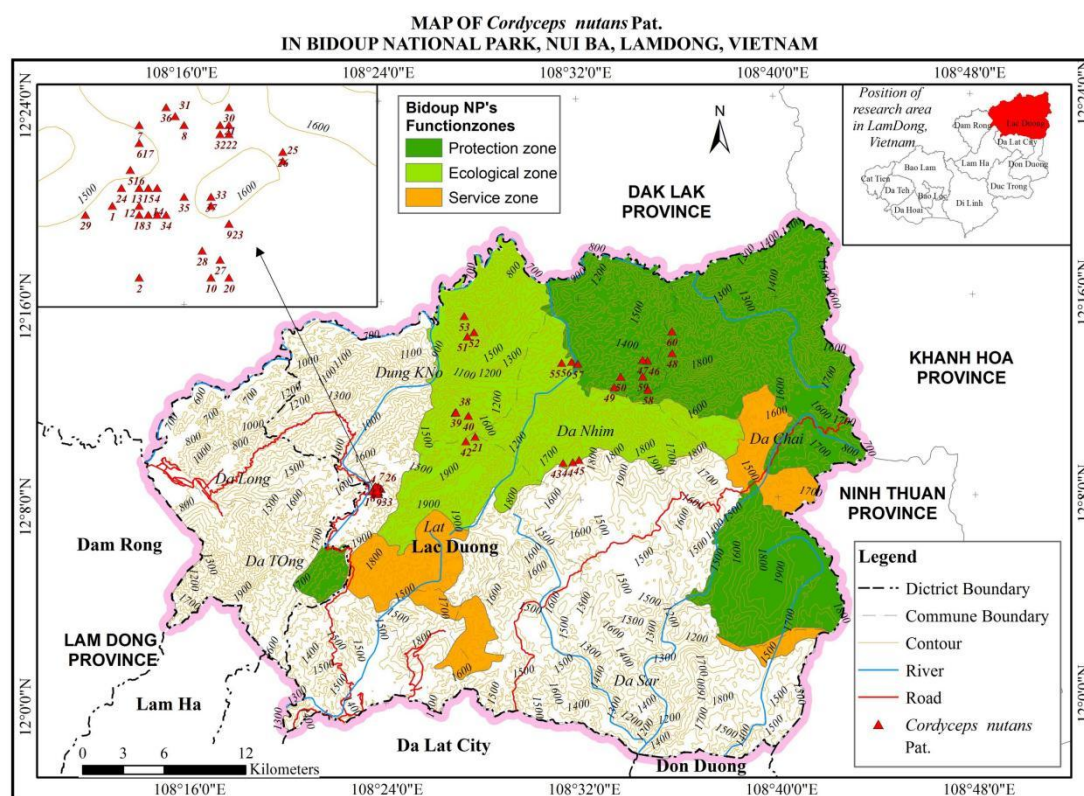


Figure 2. The distribution map of *Ophiocordyceps nutans* in Bidoup Nui Ba National Park, Lam Dong province.

3.2. Host

The collected *Ophiocordyceps nutans* were discovered on a wide variety of host insect groups. They infected the *Proxys punctulatus*; the *Halyomorpha halys*, the *Acanthosoma labiduroides*, the *Acanthosoma labiduroides* and the *Clavigralla scutellaris*. The similarity in findings have been observed in *O. nutans* from Japan [15], although *O. neonutans* is exclusively found in the Pentatomidae family [2] (Figure 3).

Among 65 collected specimens, *O. nutans* specimens have stinkbugs as the host insects, with 28 specimens being *Halyomorpha halys*, and there were 15 specimens were *Lasianthus bidoupensis*, corresponding to the frequencies of occurrence at 64,3%.

Lasianthus bidoupensis (Rubiaceae), a new evergreen tree species, has been described and illustrated in Bidoup Nui Ba National Park, Lam Dong Province, southern Vietnam. Larger leaves and bracts, longer petioles, stipules, calyx and corolla tubes, and deep-red mature fruits distinguish this species. The *Lasianthus bidoupensis* is an economically valuable and naturally distributed tree species that serve in Bidoup Nui Ba, with altitudes of 1436 m [21] (Figure 4).



Figure 3. Accumulated dead stink bugs *Halyomorpha halys* (11), *Acanthosoma labiduroides* (12), *Clavigralla scutellaris* (13), *Proxys punctulatus* (15) on the floor of *Lasianthus bidoupensis* grove; fruit bodies of *Ophiocordyceps nutans* (a), dorsal view of dead stink bugs (b), ventral view of dead stink bugs (c).

3.3. Morphology

Our specimens shared the morphological parameters of the published *O. nutans* morphology [16, 18, 19].

Stromata solitary, cylindrical, with the length is 9.6 -17.5 cm (Table 1), emerging from the adult insects' head and thorax, with up to five stromata on each host. Below fertile head stout, similar color to fertile the ad. Productive head cylindrical, yellow-orange, soft and fleshy when fresh, 0.3 x 3.6 cm, occupying 2.5 to 20.4 % of the stromata length. Ascospores are thread-like and fragmented. Part spores 7.31 to 14.72 μm , cylindrical with blunt ends. The *Ophiocordyceps nutans* parasitize 4 different stink bugs species, with distinguish morphology: size and color of stromata, and fertile head. Furthermore, changes in stroma color, size, productive head, and perithecia structures were also recorded (Figure 5).



Figure 4. Grove of *Lasianthus bidoupensis* in Bidoup Nui Ba National Park (a), leaves (b), inflorescence (c, d), developing fruit (d).

3.4. Phylogenetic analysis

D1-D2 sequences are known to develop slowly, with interspecies nucleotide replacement values less than 0.01. On the other hand, the increased weight of nucleotide replacement is observed in distinct biological species [7, 12]. The D1-D2 sequence of 11, 12, 13, and 15BidoupNLU has the pairwise distances at 0.01 with that of Japanese *O. nutans* (NBRC 10749), thus indicating these specimens are genetically *O. nutans*. These molecular results were consistent with the morphological characteristics of 11, 12, 13, and 15BidoupNLU specimens in that the morphology of our specimens resembled the described morphology of *O. nutans* [16, 19, 18].

The nucleotide BLAST analyses using our four sequences showed that 11, 12, 13, and 15 BidoupNLU sequences shared the identity of 99.47%, 99.40%, 99.48%, 99.44%, respectively, with the 28S ribosomal DNA sequence of *O. nutans* (Genbank accession no. NBRC 100944). The phylogenetic analysis revealed four sequences (11, 12, 13, and 15BidouNLU) formed a monophyletic cluster of 100% bootstrap with *O. nutans*. The phylogenetic analysis indicated that four specimens 11, 12, 13, and 15BidoupNLU were *O. nutans*. These molecular results were consistent with our morphological results (Figure 5 and Table 2) and the taxonomic descriptions by Shrestha and Sung on *O. nutans* [16, 18, 19]. Our results confirmed that all four specimens 11, 12, 13, and 15BidouNLU) were *Ophyocordyceps nutans* (Figure 6).

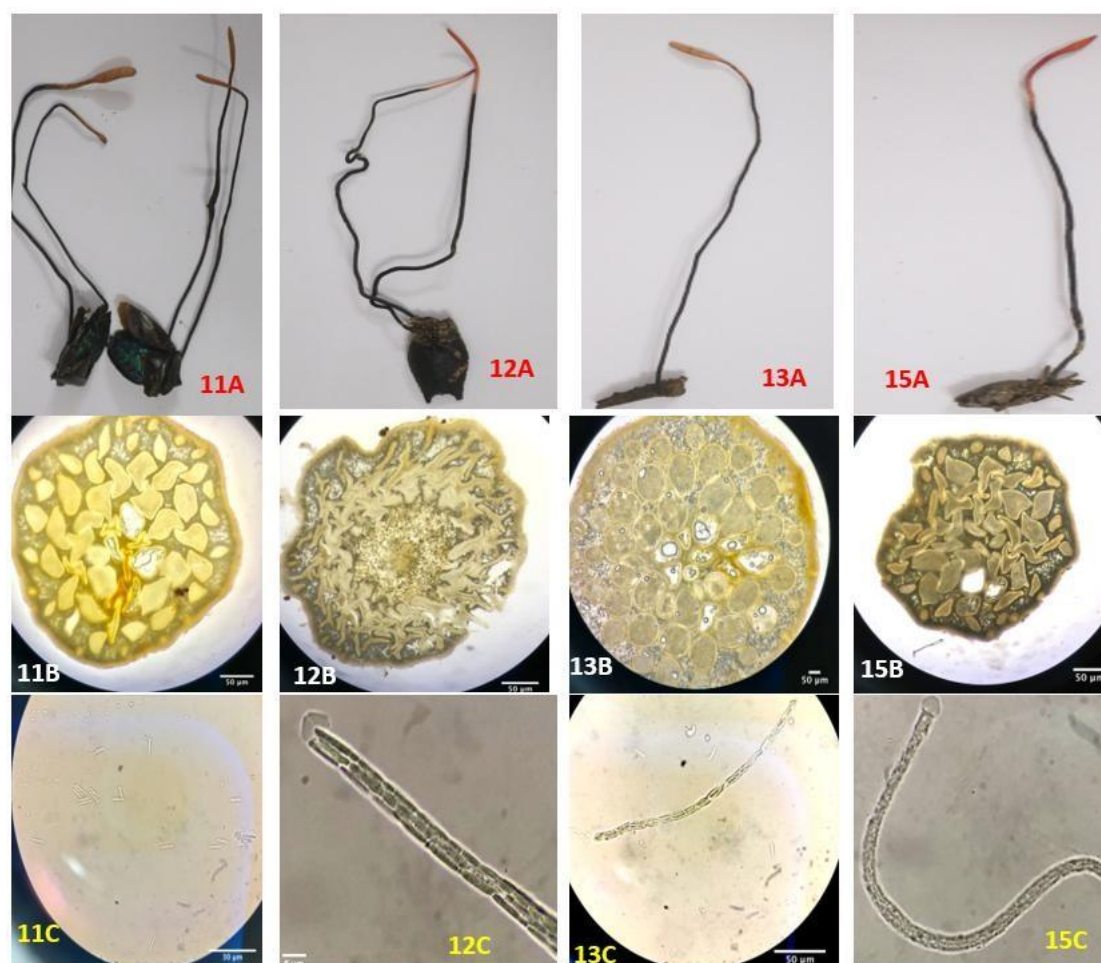


Figure 5. (11A) *O. nutans* on brown marmorated stink bug (*Halyomorpha halys*, Pentatomidae). (12A) *O. nutans* on scissors turtle bug (*Acanthosoma labiduroides*, Acanthosomatidae). (13A) *O. nutans* on legume bug (*Clavigralla scutellaris*, Coreidae). (15A) *O. nutans* on black stink bug (*Proxys punctulatus*, Pentatomidae). (B) fertile head with immersed perithecia; (C) Thread-like ascospore with cylindrical, blunt end part spores

Table 2: Specimens and GenBank sequences used in the phylogenetic analysis

Species	Voucher	Country	D1-D2
<i>Ophyocordyceps nutans</i>	Oph994	Columbia	KJ917567.1
<i>Ophyocordyceps nutans</i>	NBRC 101749	Japan	JN941429.1
<i>Ophyocordyceps nutans</i>	NBRC 100944	Thailand	JN941428.1
<i>Ophyocordyceps nutans</i>	11BidoupNLU	This study	MT235757
<i>Ophyocordyceps nutans</i>	12BidoupNLU	This study	MT235758
<i>Ophyocordyceps nutans</i>	13BidoupNLU	This study	MT235759
<i>Ophyocordyceps nutans</i>	15BidoupNLU	This study	MT235761
<i>O. sphecocephala</i>	NBRC101415	Thailan	JN941442.1

<i>O. sphecocephala</i>	NBRC101414	Thailand	JN941441.1
<i>O. sphecocephala</i>	NBRC 101416	Thailand	JN941446.1
<i>O. irangiensis</i>	NBRC101399	Thailand	JN941425.1
<i>O. irangiensis</i>	NBRC101400	Thailand	JN941426.1
<i>O. irangiensis</i>	NBRC101401	Thailand	JN941427.1
<i>O. myrmecophila</i>	BUO537	China	MH879600.1
<i>O. myrmecophila</i>	BCC82256	Thailand	MH028157.1
<i>O. myrmecophila</i>	MFLU16.2913	Thailand	MF372586.1
<i>O. cylindrospora</i>	MFLU17.1961	Thailand	NG_064484.1
<i>O. thanathonensis</i>	MFLU16.2908	Thailand	MF362990.1
<i>O. forquignonii</i>	Ophaus1780	Columbia	KJ878889.1
<i>O. australis</i>	HUA186097	Columbia	KC610765.1
<i>O. tricentri</i>	NBRC106968	Japan	AB968423.1

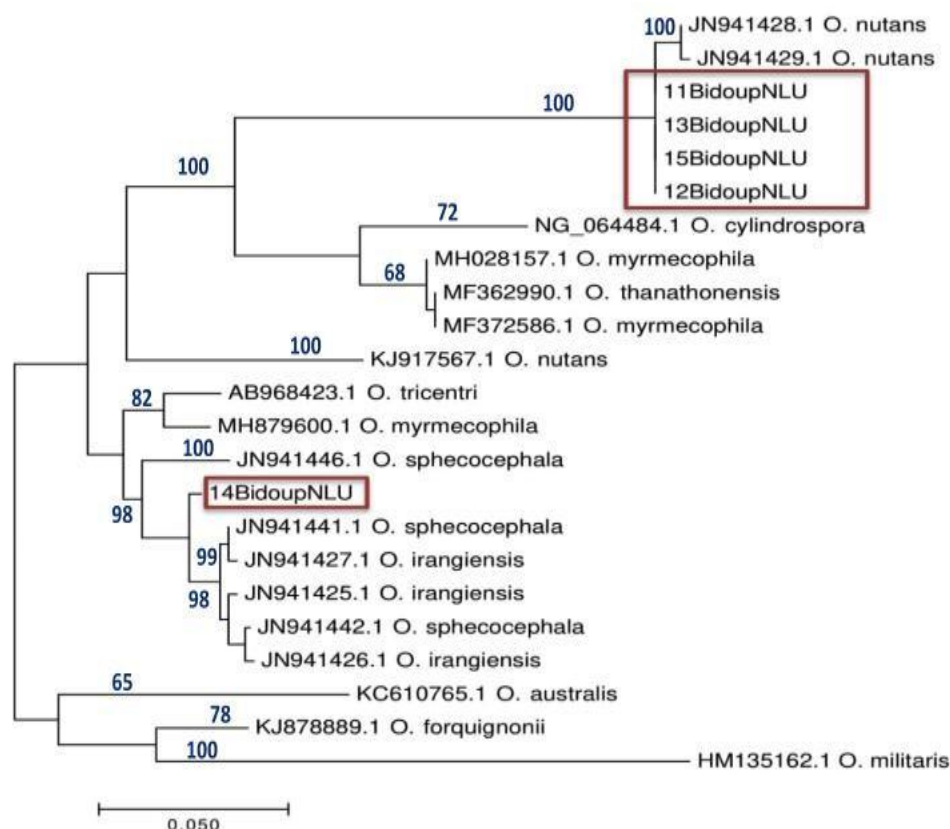


Figure 6: Molecular Phylogenetic analysis using five collected Ophiocordyceps.

4. Discussion

O. nutans parasitize approximately 22 kinds of stinkbugs that endanger agriculture and silviculture, making it a viable biocontrol agent [14]. Sasaki et al., (2008) discovered minimal diversity in the morphological and molecular variations in the stromata of *O. nutans* taken from nine hemipteran host

species. They looked examined *O. nutans* from three hemipteran host families: Acanthosomatidae, Pentatomidae, Pentotom [14].

In this report, *Ophiocordyceps nutans* were found to parasite adult stinkbugs; the fruiting bodies of *O. nutans* were red-orange and arose from the decaying vegetation layers and growing in rough, moist, shady terrains, although our studied *O. nutans* shared many characteristics to the reported *O. nutans*, we discovered that our specimens' stroma and fertile head were longer than those from Japan. Morphological differences between fruit bodies of *O. nutans* from four stink bugs include host, stroma head, and color. However, Sasaki et al. (2008) found no significant changes in the morphology of *O. nutans* across host bug types in their research of *O. nutans* collected in Japan. In contrast, we found that the host insect had a considerable influence on the morphological variety of our collected *O. nutans* and can be seen the stroma's size, fertile head, and part spore, as well as the form of the perithecia. Despite this, molecular data revealed that four sets of insect specimens were *Ophiocordyceps nutans* with highly similar molecular architectures. Molecular techniques aid in the identification of *Ophiocordyceps* and its host within an ecological niche [13].

At 65 areas where *Ophiocordyceps nutans* was discovered were the place with wet surface of decaying leaves at Bidoup Nui Ba National Park; it was also discovered that *O. nutans* could affect 4 types of host insects. In 28 areas, the *O. nutans* parasitize *Halyomorpha halys*. Moreover, in 15 of 28 areas, there are *Lasianthus bidoupensis*, which is a specie of Rubiaceae. According to Sasaki et al., (2008), cultivating *Cassine glauca* as a shade tree has numerous benefits in terms of reducing the assault of stinkbugs *Halyomorpha halys* on surrounding rice and horticulture crops. Despite containing the anamorphic antagonistic endophyte *Hymenostilbe nutans*, the level of harm produced to *C. glauca* demonstrates stinkbugs' tremendous capabilities. Inrush of *H. nutans* through bark tissue, on the other hand, is fatal to stinkbugs, resulting in death and the appearance of teleomorphic *O. nutans* [14].

The presence of *Lasianthus bidoupensis* has various benefits, including a decrease in the assault of stinkbugs *Halyomorpha halys* on surrounding crops. Because *Halyomorpha halys* consumption of *Hymenostilbe nutans* through the bark and leaf tissue is fatal to the stinkbugs, the appearance of teleomorphic *O. nutans* follows. Its dependency distinguishes *Ophiocordyceps* on both single and many hosts. Such *Ophiocordyceps* clades are extremely useful in the biocontrol of phytophagous insects.

5. Conclusion

Our molecular and morphological data consistently showed that 65 of our collected specimens belonged to *Ophiocordyceps nutans*, and this species could infect a broader range of insect species. Observations of the host habitat of *Ophiocordyceps nutans* in the Bidoup Nui Ba National Park in two years showed the regular presence of *O. nutans* at a certain altitude of 1409 - 1589 m; The temperature varies throughout this period from 20,3 to 25,7°C. *Ophiocordyceps nutans* mostly develop in the type ecosystems of locality coffee agroforest and the mixed forests with broad-leaved wooden trees. The 'host habitat' of *Ophiocordyceps nutans* to the settling of unrelated hosts in a niche, the relationship between *Halyomorpha halys* and *Lasianthus bidoupensis* has been established.'

Conflict of interest

The authors declare that there is no conflict of interest.

Acknowledgment

The authors acknowledge the financial support from the Nong Lam University Ho Chi Minh City, Ministry of Education and Training, Vietnam.

References

- [1] Araújo J P M *et al* 2015 Unravelling the diversity behind the *Ophiocordyceps unilateralis* (Ophiocordycipitaceae) complex Three new species of zombie-ant fungi from the Brazilian Amazon *Phytotaxa* **220** 15.
- [2] Friedrich R C S 2018 *Ophiocordyceps neonutans* sp. nov. a new neotropical species from *O. nutans* complex (Ophiocordycipitaceae, Ascomycota) *Phytotaxa* **344** 3 215-227.
- [3] Dang D K 2000 Fauna of Vietnam Coreidae *Science and Technics Publishing House Ha Noi Vietnam* **7** 170-323.
- [4] Dinh M H 2017 Discovery of entomopathogenic fungi Cordyceps takaomontana at Langbian mountain Lam Dong Vietnam *Journal of Science and Technology* **55** 1A 19-26.
- [5] Holliday J and Cleaver M 2008 Medicinal value of the caterpillar fungi species of the genus Cordyceps (Fr.) Link (Ascomycetes) A review *Int J Med Mush* **10** 219-234.
- [6] Kobayasi Y 1941 The genus Cordyceps and its allies *Science reports of the Tokyo Bunrika Daigaku* **5** 53-260.
- [7] Kumar S, Stecher G, and Tamura K 2016 MEGA7 Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets *Mol Biol Evol* **33** 7 1870-1874.
- [8] Leaw S N *et al* 2006 Identification of Medically Important Yeast Species by Sequence Analysis of the Internal Transcribed Spacer Regions *J Clin Microbiol* **44** 3 693-699.
- [9] Le T L *et al* 2013 Discovery of entomopathogenic fungi Cordyceps neovolkiana at Langbian mountain Dalat Vietnam *Vietnam Journal of Biotechnology* **8** 3A 1007-1013.
- [10] Li Y *et al* 2011 A survey of the geographic distribution of *Ophiocordyceps sinensis* *Journal of microbiology (Seoul Korea)* **49** 913-919.
- [11] Mains E B 1958 North American Entomogenous Species of Cordyceps *Mycologia* **50** 169-222.
- [12] Raja H A, Miller A N, Pearce C J, and Oberlies N H 2017 Fungal identification using molecular tools A primer for the natural products research community *Journal of Natural Products* **80** 3 756-770.
- [13] Sanjuan T I *et al* 2015 Five new species of entomopathogenic fungi from the Amazon and evolution of Neotropical Ophiocordyceps *Fungal Biol* **119** 901-916.
- [14] Sasaki F *et al* 2008 Morphological and genetic characteristics of the entomopathogenic fungus *Ophiocordyceps nutans* and its host insects *Mycol Res* **112** 1241-1244.
- [15] Sasaki F *et al* 2012 Relationship between intraspecific variations and host insects of *Ophiocordyceps nutans* collected in Japan *Mycoscience* **53** 85-91.
- [16] Shrestha B, and Sung J-M 2005 Notes on Cordyceps species Collected from the Central Region of Nepal *Mycobiology* **33** 4 235-239.
- [17] Shrestha B 2011 Diversity of Cordyceps fungi in Nepal *Nepal Journal of Science and Technology* **12** 103-110.
- [18] Shrestha B *et al* 2017 Mycosphere Essay 19 Cordyceps species parasitizing hymenopteran and hemipteran insects *Mycosphere* **8** 1424-1442.
- [19] Sung G-H *et al* 2007 Phylogenetic classification of Cordyceps and the clavicipitaceous fungi *Studies in mycology* **57** 5-59.
- [20] Trinh T K 2014 The list of macro fungi in Vietnam *Vietnam National University Press Ha Noi Vietnam*.
- [21] Dang V S *et al* 2019 *Lasianthus bidoupensis* (Rubiaceae) a New Species from Southern Vietnam *Annales Botanici Fennici*. **56** 1-3 191-195.
- [22] Vu T L *et al* 2017 Analysis of nrLSU gene to support identification of fungus belonging to Cordyceps genus and Clavicipitaceae family *Journal of Science and Technology* **55** 1A 93-98.

- [23] Wang X-L and Yao Y-J 2011 Host insect species of *Ophiocordyceps sinensis* A review *ZooKeys* **127** 43-59.
- [24] Wu Z-H *et al* 2001 A simplified method for chromosome DNA preparation from filamentous fungi *Mycosystema* **20** 575-577.
- [25] Xiao Y-P *et al* 2019 Two new entomopathogenic species of *Ophiocordyceps* in Thailand *MycoKeys* **47** 53-74.