Pathogen Isolation and Identification of Three Diseases in Understory *Dendrobium devonianum* Paxt. in Longling

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Abstract [**Objectives**] This study was conducted to identify the disease types and pathogen composition of understory *Dendrobium devonianum* Paxt. in Longling County, Yunnan Province. [**Methods**] A disease investigation was carried out by the field survey method combined with the quadrat sampling method. [**Results**] Through tissue isolation, pathogenicity testing, and morphological as well as molecular identification, three fungal diseases were identified: black spot, round spot, and brown spot. In specific, black spot had the highest incidence (40.98%), and was caused by *Nigrospora chinensis*. Round spot was caused by *Neopestalotiopsis foedans*. Brown spot was caused by *Pseudopestalotiopsis camelliae-sinensis*. [**Conclusions**] This study provides a scientific basis for green disease control.

Key words *Dendrobium devonianum* Paxt., Understory cultivation, Black spot, Round spot, Brown spot, Pathogen identification

0 Introduction

Dendrobium devonianum Paxt. is a perennial herbaceous plant of *Dendrobium* in the Orchidaceae family. Its stem is rich in bioactive components such as flavonoids, alkaloids, and polysaccharides, exhibiting pharmacological effects including stomachnourishing, antioxidant, and immunomodulatory activities[1-3]. Longling County in Yunnan Province is known as the "Hometown of *Dendrobium*". The understory bionic cultivation model offers both ecological and economic advantages and represents an important development direction for the local Dendrobium industry[4-5]. However, the frequent occurrence of diseases in understory cultivation severely restricts the development of the industry[6]. Currently, few studies have been conducted on diseases in the understory environment. This study aimed to identify the pathogenic species in this region, providing a scientific basis for green disease control.

1 Materials and methods

1.1 General situation of the survey area The survey was conducted in Longling County, Baoshan City, Yunnan Province. Eight understory cultivation areas of *D. devonianum* were selected. This region has a subtropical monsoon climate, with an average annual temperature of 14.9 ℃, annual precipitation of 2 100 mm, and a relative humidity of 83%. From 2024 to 2025, a disease survey was carried out in the main understory cultivation bases of *D. devonianum*. Detailed information is shown in Table 1.

Table 1 Locations of disease investigation and sampling from 2024 to 2025

Sampling location	Epiphytic host tree	Longitude and latitude	Altitude//m
Yangmei Mountain, Longshan Town	Tea grove	24°35′45″ N, 98°42′41″ E	1 642.5
Yangmei Mountain, Longshan Town	Mixed forest	24°59′76″ N, 98°71′19″ E	1 635.2
Dendrobium Research Institute	Mixed forest	24°64′88″ N, 98°65′74″ E	1 350.4
Yangbu Village, Pingda Township	Tea grove	24°18′41″ N, 98°56′5″ E	1 702.0
Tianning Village, Bizhai Township	Mixed forest	24°28′10″ N, 98°58′15″ E	1 901.0
Potou Village, Bizhai Township	Mixed forest	24°28′1″ N, 98°56′30″ E	1 784.8
Bangmai Village, Zhen'an Town	Mixed forest	24°46′14″ N, 98°50′38″ E	1 658.5
Xiaotianba, Zhen'an Town	Mixed forest	24°43′9″ N, 98°47′9″ E	1 910.9

1.2 Disease survey method A random sampling method combined with a five-point sampling method was adopted. In each surveyed location, five 50 m × 50 m quadrats were established. Within each quadrat, 10 trees were randomly selected, and five *Dendrobium* plants were surveyed on each tree, resulting in a total of 250 plants surveyed. The number of diseased plants and symptom characteristics were recorded to calculate the disease incidence and disease index[7].

1.3 Isolation, purification and preservation of pathogens The tissue isolation method was adopted[8]. Tissues from the junction of diseased and healthy areas were collected, surface-sterilized with 75% ethanol and 1% NaClO, and then inoculated onto potato dextrose agar (PDA) medium. The cultures were incubated at 28 ℃ in the dark. Mycelia were picked for purification and preserved at −80 ℃.

1.4 Pathogenicity test of isolates The test was conducted following Koch's postulate. The tested isolates were cultured on PDA for 5 - 7 d. Micro-wounds were made on the leaves of healthy plants, and a 6 mm mycelial disk was inoculated onto each

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wound. Sterile PDA was used as the control. Each treatment was repeated three times. The plants were kept under humid conditions and observed regularly.

1.5 Morphological and molecular identification of pathogens The morphology of colonies and conidia was observed[9-10]. DNA was extracted using the CTAB method, and the rDNA ITS region was amplified using the ITS1/ITS4 primers. The PCR products were sequenced, and the resulting sequences were compared using BLAST, followed by phylogenetic tree construction[11].

1.7 Data processing Statistical analysis of the data was performed using SPSS 26.0 software, with Duncan's multiple range test for comparison ($P<0.05$). Figures and charts were generated using GraphPad Prism 9 and Excel.

2 Results and analysis

2.1 Occurrence of three diseases on understory *D. devonianum* Three fungal diseases were identified. The average incidence of black spot disease was 40.98%, with a disease index of 25.63. The incidence of round spot disease was 11.00%, with a disease index of 7.82. The incidence of brown spot disease was 17.50%, with a disease index of 12.36 (Table 2).

Table 2 Occurrence of the three diseases on understory *Dendrobium devonianum* in Longling County

Disease	Incidence//%	Disease index	Main affected part	Peak occurrence period
Black spot	40.98±5.50	25.63±5.60	Leaves and stems	June – August
Round spot	11.00±1.50	7.82±0.91	Leaves	June – September
Brown spot	17.50±5.50	12.36±3.20	Leaves	June – October

NOTE The data in the table are presented as mean±standard deviation (SD) of the eight sampling locations.

2.2 Black spot disease The pathogen was identified as *Nigrospora chinensis*. The initial lesions were yellowish-green or light brown (1 – 3 mm). They later expanded to dark brown or purplish-black with a grayish-white center (Fig. 1). The disease first appeared from April to May, and reached its peak from June to August (Fig. 2). Black spots appeared 5 d after inoculation and expanded with a halo after 10 d (Fig. 3). The colonies were initially white and cottony, and later turn gray-black to black. Conidia were spherical (10 – 20 μm) with an umbilicate base (Fig. 4). The ITS sequences showed 99% identity to *N. chinensis* (Fig. 5).

NOTE a – c: Disease incidence in the understory; d – f: Diseased leaves.

Fig. 1 Understory symptoms of black spot disease on *Dendrobium devonianum*

2.3 Round spot disease The pathogen was identified as *Neopestalotiopsis foedans*. The lesions had dark reddish-brown to purplish-black edges with grayish-white centers, surrounded by concentric rings (Fig. 6). Typical lesions appeared 15 d after inoculation (Fig. 7). Colonies were initially white and later turned grayish-white in the center. The conidia were fusiform, 4-septate, with 2 apical appendages (Fig. 8). ITS sequence analysis showed 99% identity with *N. foedans* (Fig. 9). This disease occurred mildly in the understory cultivation areas of Longling County, only observed in Yangbu Village, Pingda Township (7%) and the Dendrobium Research Institute (15%), and was more likely to occur under high humidity conditions.

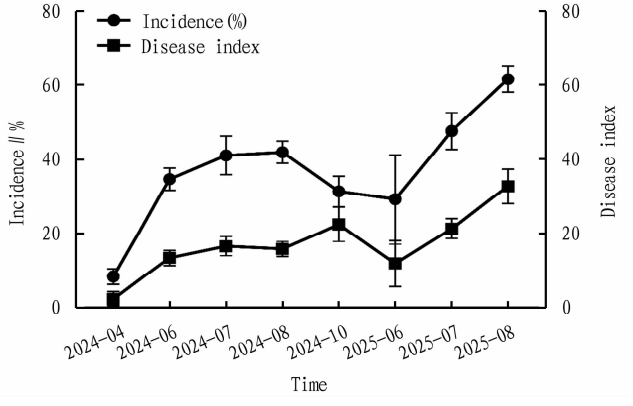
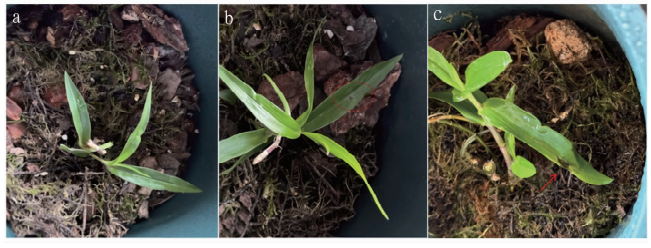
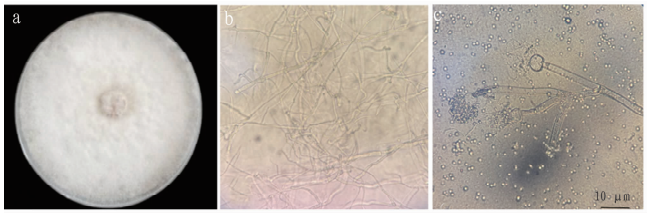


Fig. 2 Occurrence of black spot disease on understory *Dendrobium devonianum* in Longling County



NOTE a. Healthy plant; b. Symptoms at 5 d after inoculation; c. Symptoms at 10 d after inoculation.

Fig. 3 Pathogenicity test results of black spot disease on *Dendrobium devonianum*



NOTE a. Colony surface (front view); b. Mycelial morphology; c. Conidia and conidiophores.

Fig. 4 Colony and microscopic characteristics of the pathogen causing black spot disease on *Dendrobium devonianum*

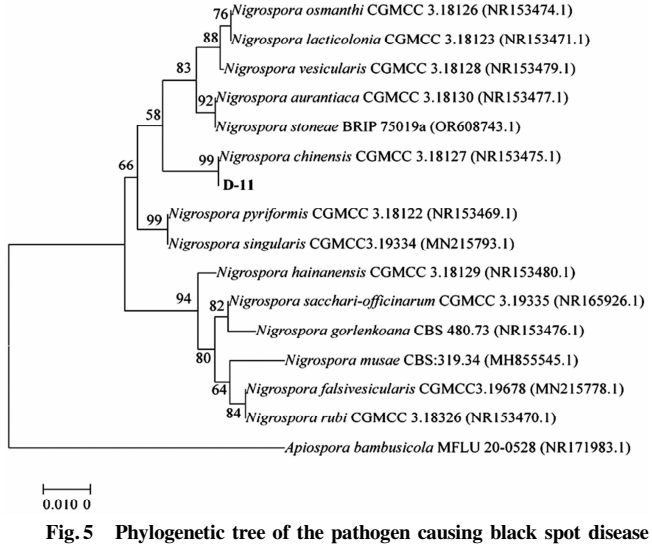


Fig. 5 Phylogenetic tree of the pathogen causing black spot disease based on ITS sequences

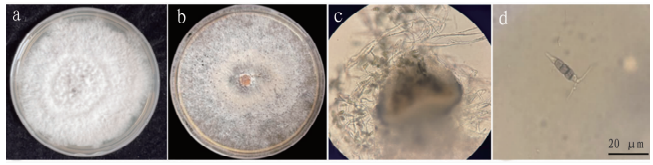
NOTE a – b, Symptoms on infected plants.

Fig. 6 Understory symptoms of round spot disease in *Dendrobium devonianum*

NOTE a. At 1 d after inoculation; b. Symptoms at 5 d after inoculation; c. Symptoms at 15 d after inoculation.

Fig. 7 Pathogenicity test results of round spot disease on *Dendrobium devonianum*

2.4 Brown spot disease The pathogen was identified as *Pseudopestalotiopsis camelliae-sinensis*. The infection mostly started from the leaf tip or leaf edge. The lesions had dark brown edges and grayish-white centers (Fig. 10). Concentric ring-like lesions appeared 15 d after inoculation. The colony centers turned black due to sporulation (Fig. 11). The conidia were fusiform, with 2 – 4 apical appendages (Fig. 12). The ITS sequences exhibited 100% support rate (Fig. 13).



NOTE a. Colony morphology; b. Sporulating colony; c. Acervulus; d. Conidia.

Fig. 8 Morphological characteristics of the pathogen causing round spot disease on *Dendrobium devonianum*

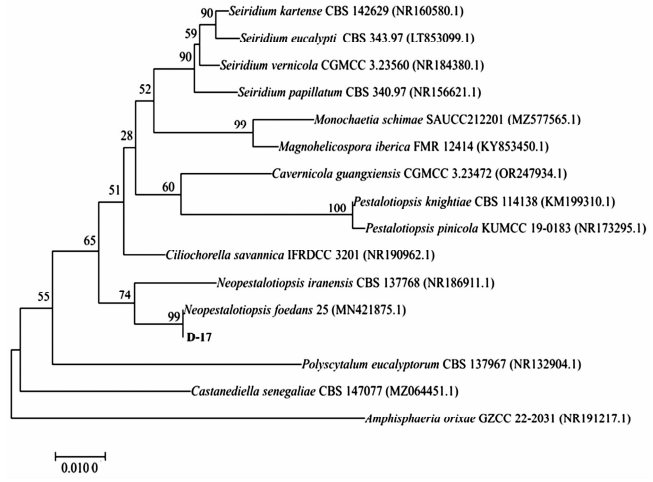
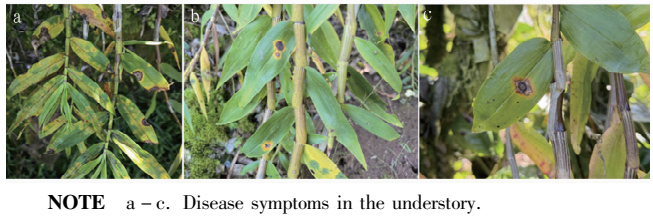
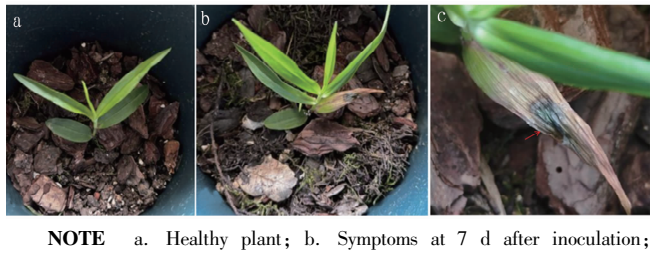


Fig. 9 Phylogenetic tree of the pathogen causing round spot disease based on ITS sequences



NOTE a – c. Disease symptoms in the understory.

Fig. 10 Understory symptoms of brown spot disease on *Dendrobium devonianum*

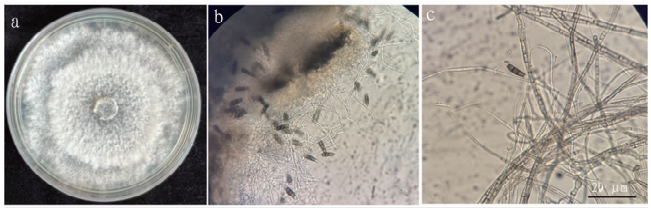


NOTE a. Healthy plant; b. Symptoms at 7 d after inoculation; c. Symptoms at 15 d after inoculation.

Fig. 11 Pathogenicity test results of brown spot disease on *Dendrobium devonianum*

This disease occurred in clusters in Longling County, and ob-

served only in Yangbu Village, Pingda Township (30%) and under the tea tree forest of Yangmei Mountain, Longshan Town (5%).



NOTE a. Colony morphology; b. Conidia; c. Conidia with appendages.

Fig. 12 Morphological characteristics of the pathogen causing brown spot disease on *Dendrobium devonianum*

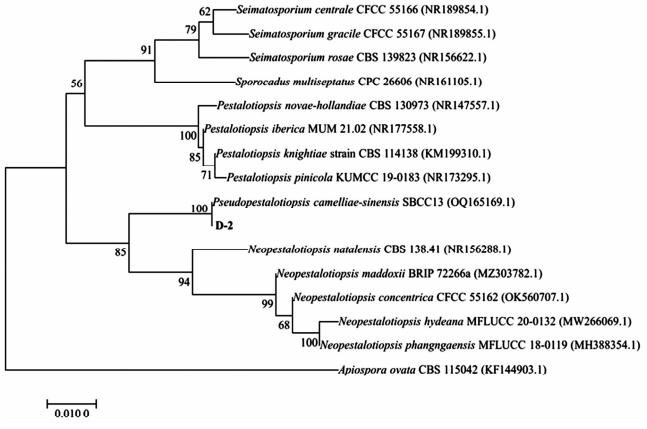


Fig. 13 Phylogenetic tree of the pathogen causing brown spot disease based on ITS sequences

3 Discussion and conclusions

In this study, a systematic disease survey was conducted in the understory *D. devonianum* cultivation areas of Longling County. A total of three fungal diseases were identified: black spot, round spot, and brown spot. Among them, black spot was the most prevalent. The pathogen causing black spot disease was identified as *N. chinensis*, which belongs to the same genus, but is a different species from *Nigrospora sphaerica* isolated by Zhao *et al.*[12], indicating the diversity of *Nigrospora* fungi in *Dendrobium* plants. This genus (*Nigrospora* spp.) has been reported to cause leaf spot in *Dendrobium* and olive trees[13-14] and is widely distributed in various plant diseases. The pathogen responsible for round spot disease was identified as *N. foedans*, and the pathogen causing brown spot disease as *P. camelliae-sinensis*. Both are pestalotiopsis-like fungi. Li *et al.*[15] reported that round spot disease on *Dendrobium officinale* was caused by *Myrothecium roridum*, which differs from the findings of this study. This difference may be attributed to differences in host species, cultivation methods, and environmental conditions. Understory and greenhouse environments differ in temperature, humidity, light, and ventilation, which may influence the distribution and composition of dominant pathogen populations. The brown spot pathogen identified in this study, *P. camelliae-sinensis*, was initially discovered to cause gray blight on tea leaves[16].

Furthermore, these three pathogens represent either the first reports or new records on *D. devonianum*, enriching our understanding of the pathogen species and revealing the pathogen diversity associated with the understory cultivation model. This study provides a theoretical basis for developing targeted integrated management strategies. It is recommended to establish a comprehensive disease control system for understory cultivation based on ecological regulation, combining biological control with precise application of fungicides, to achieve sustainable cultivation of *D. devonianum*.

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