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First Report of Bismarck Palm Anthracnose Caused by *Colletotrichum siamense* in China

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Abstract: Bismarck palm (*Bismarckia nobilis* Hildebr. & H. Wendl.) is one of the most sought after palms. A severe disease of Bismarck palm was discovered in Wenchang, Hainan Province, China, in July 2010. The main symptoms were russet to gray-white lesions at the tip of leaflets, and the irregular-shaped lesions surrounded by reddish to brown halos. The lesions gradually extended to base of the compound leaf, and entire infected leaves dried up at the advanced stage. A fungus was consistently obtained on the potato dextrose agar medium and the colonies were grayish white, cottony aerial mycelia, with peach to orange conidial masses. The conidia were hyaline, one-celled, and cylindrical with obtuse to slightly rounded ends. A multi-locus approach was utilized to identify the casual pathogen. Molecular phylogenetic trees were constructed using six combined loci, and strain BWZ2 clustered with the ex-type strains of *C. siamense* (CBS 112983) in the maximum likelihood phylogenetic tree. The pathogenicity test indicated that typical gray-white lesions surrounded by brown halos were observed on all the inoculated leaflets at 7 days post inoculation. On the basis of the biological characteristics, pathogenicity, and analyses of the concatenated sequences of the *ACT*, *CHS1*, *GAPDH*, *HIS*, ITS, and *TUB2* genes, the causal agent was identified as *Colletotrichum siamense* Prihastuti, L. Cai & K. D. Hyde. This is the first report of Bismarck palm anthracnose caused by *C. siamense*.

Keywords: Bismarck Palm, Anthracnose, *Colletotrichum siamense*

1. Introduction

Bismarckia palm (*Bismarckia nobilis* Hildebr. & H. Wendl.) is a popular ornamental plant, which is usually used for landscapes, green belts and home gardens [1]. *B. nobilis* is one of the most sought after palms grown in the Coconut Grant View Garden (CGVG), which is located in Wenchang, Hainan Province, China. There are more than 100 species of palm that are grown as both palmae germplasms and ornamental plants in the CGVG. In July 2010, a severe leaf disease emerged on *B. nobilis* in the CGVG). The aim of this study was to determine the etiological agent of the disease of Bismarck palm.

2. Materials and Methods

2.1. Fungal Isolation and Morphological Characterization

Diseased (mature and typically symptomatic) Bismarck palm leaves were collected from the CGVG. Pieces (3-5 × 3-5 mm) of leaflet tissue from the margins of lesions were immersed in 70% ethanol and rinsed twice with sterile distilled water. They were placed on potato dextrose agar (PDA) and incubated at 25°C in the dark.

2.2. PCR Amplification, Sequencing of Genes and Phylogenetic Analysis

A revised CTAB method [2] was used to extract the genomic DNA from representative strain BWZ2 (China

Center for Type Culture Collection No. CCMCC AF 2014008) that had been grown from a single conidium. It was used as template to amplify the following regions (*ACT*: actin [ACT512F and ACT783R]; *CHS1*: chitin synthase A gene [CHS1-79F and CHS1-354R]; *GAPDH*: glyceraldehyde-3-phosphate dehydrogenase [GDF1 and GDR1]; *HIS*: histone H3 [CTLH3F and H3-1b], ITS: ITS1-5.8S-ITS2-28S [ITS1 and ITS4]; and *TUB*2: chitin synthase A gene [CHS1-79F and CHS1-354R]; *GAPDH*: glyceraldehyde-3-phosphate dehydrogenase [GDF1 and GDR1]; *HIS*: histone H3 [CTLH3F and H3-1b], ITS: ITS1-5.8S-ITS2-28S [ITS1 and ITS4]; and *TUB*2: β-tubulin 2 [T1/Bt2b]. PCR was performed as previously [2-6]. The DNA of the amplicons was sequenced by Sangon Biotech (Shanghai) Co., Ltd. (Shanghai, China).

A BLAST search of the nucleotide database of GenBank indicated that the related amplicons of the *ACT*, *CHS1*, *GAPDH*, *HIS*, ITS and *TUB*2 genes of the representative isolate BWZ2. Molecular phylogenetic trees were constructed based on the close-neighbor-interchange algorithm (Maximum Parsimony) by MEGA version 7 [7] using six combined loci. *Colletotrichum novae-zelandiae* was used as an outgroup.

2.3. Pathogenicity Testing

To fulfill Koch's postulates, three isolated leaflets (about 10 cm in length) cut from 3-year-old Bismarck palms were wounded with a sterilized scalpel, and 6 × 6 mm mycelial plugs cut from the edge of colonies and grown on PDA at 25°C for 7 d were attached to the wound. Control leaflets were inoculated with plain PDA plugs. All the leaflets were then incubated in petri dishes (Φ = 15 cm) with a relative humidity of approximately 90% at 25°C. The pathogenicity was tested twice.

3. Results

3.1. Symptoms, Morphological and Cultural Characterization

In July of 2010, this severe leaf disease was observed with an incidence of 100% (4 out of 4 plants were infected; 19.5489ºN, 110.7909ºE) and 89.2% (37 of 41 plants were infected; 19.5490ºN, 110.7904ºE) on *B. nobilis* in the CGVG (Figure 1A). The initial visible symptoms were russet to gray-white lesions at the tip of leaflets (Figure 1B), and the irregular-shaped lesions surrounded by reddish to brown halos then gradually extended to base of the compound leaf (Figure 1C and D). At the advanced stage, entire infected leaves dried up (Figure 1B).

A fungus was consistently obtained. It produced grayish white, cottony aerial mycelia (Figure 1E), with peach to orange conidial masses (Figure 1F) at the inoculum point on the PDA plates. The colonies were initially white and later turned pale brownish to pinkish, while the bottom of plates was pale yellowish to pinkish. The conidia were hyaline, one-celled, and cylindrical with obtuse to slightly rounded ends that were aseptate and ranged from 12.5 to 17.5 × 5.0 to 7.5 µm (n =100) (Figure 1G). The hyphae grew at a temperature of 10-35°C, and the optical temperature was 25-30°C. The morphological characters were consistent with those of *Colletotrichum siamense* ([3, 8-10].

Figure 1. *Symptoms of anthracnose on Bismarck palm (Bismarckia nobilis Hildebr. & H. Wendl.) and the causal pathogen Colletotrichum siamense. The field symptoms of leaf anthracnose on Bismarck palm at an advanced stage (A), Field symptoms of leaf anthracnose on Bismarck palm at the initial stage (B), Field symptoms of leaf anthracnose on Bismarck palm at an advancing stage (C), Leaf symptoms of Bismarck palm anthracnose (D), Colony pattern of Colletotrichum siamense isolate BWZ2 on potato dextrose agar medium (PDA) (E), Peach to orange conidial masses produced at the inoculum point in the PDA plates (F), Conidia of C. siamense isolate BWZ2 (Bar=20 µm) (G), infection symptoms (H) Left, leaf symptoms of isolate BWZ2 developed 7 days after inoculation and Right, the control.*

3.2. Phylogenetic Analysis

A multi-locus approach [2, 8-9] was utilized to further identify this pathogen. A BLAST search of the nucleotide database of GenBank indicated that the related amplicons of the *ACT* (OP573254), *CHS1* (OP573255), *GAPDH* (OP573256), *HIS* (OP573257), ITS (KJ685390), and *TUB*2 (OP573258) genes of the representative isolate BWZ2 shared 100%, 99.3%, 98.8%, 99.7%, 100%, and 99.6% of sequence identity with those of *C. siamense* (MW696003, MF188858, HM038326, MG561765, MK880376, and JF811021,

respectively). Molecular phylogenetic trees were constructed based on the close-neighbor-interchange algorithm using six combined loci that included *ACT*, *CHS1*, *GAPDH*, *HIS*, rDNA-ITS, and *TUB2* regions (Figure 2, Table 1), and strain BWZ2 lustered with the ex-type strains of *C. siamense* (CBS 112983) in the maximum likelihood phylogenetic tree.

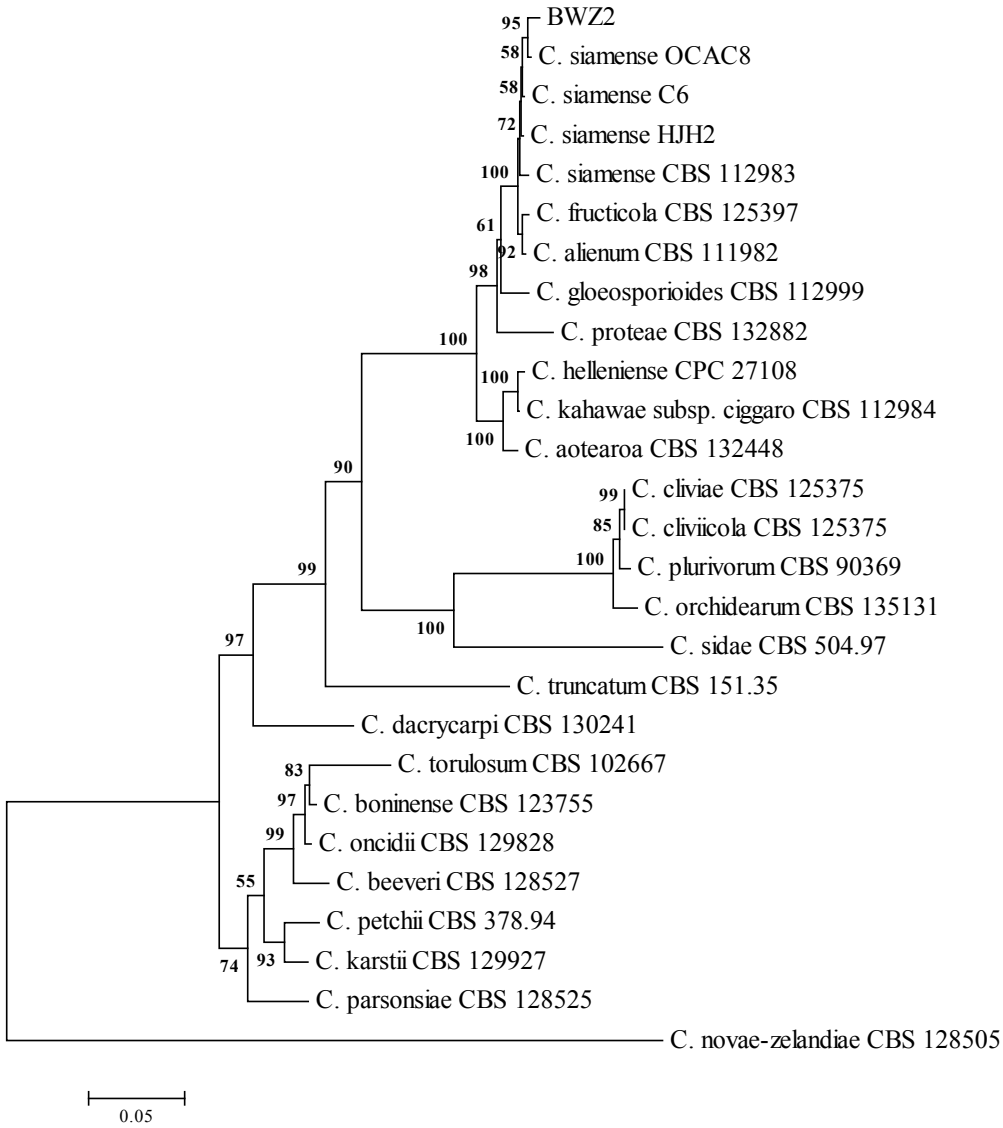

Figure 2. *A Bayesian inference phylogenetic tree was built using concatenated sequences of the ACT, CHS-1, GAPDH, HIS3, ITS, and TUB2 genes. Colletotrichum novae-zelandiae is used as an outgroup. The scale bar indicates the number of expected changes per site.*

3.3. Pathogenicity Test

After 7 d, typical gray-white lesions surrounded by brown halos were observed on all the inoculated leaflets but not on the control leaflets (Figure 1F). The fungus was reisolated from the diseased leaflets and was morphologically identical with strain BWZ2. No visible symptoms were observed in the control leaflets.

4. Discussion and Conclusion

Bismarckia palm adapts well to the changing environments [1]. So far no major disease has been reported on it. The causal agent was identified as *C. siamense* Prihastuti, L. Cai & K. D. Hyde based on morphological [2, 8-10] and molecular results. In the genus *Colletotrichum*, *C. siamense* is the most widely distributed species, being observed on nearly all continents with up to 228 records in references. In addition, *C. siamense* presents the most diverse host range, being found in at least 103 wild and cultivated hosts [11].

Colletotrichum siamense is the primary type of anthracnose disease in tropical fruits [4, 9]), and it has been cited as a pathogen of areca palm (*Areca catechu* L.) [12] (Cao *et al*, 2020), *Cornus hongkongensis* [13] and *Pharbitis purpurea* [14] in China. However, it has not been reported on Bismarck palm anywhere in the world [15]. To the best of our knowledge, this is the first report of Bismarck palm anthracnose caused by *C. siamense* in China.

Table 1. *Colletotrichum isolates used in this study and GenBank accession numbers.*

	Taxon	Isolate designation	*act*	*chs1*	*gapdh*	*His3*	ITS	*tub2*
1	*Colletotrichum truncatum*	CBS:151.35*	GU227960	GU228352	GU228254	GU228058	GU227862	GU228156
2	*Colletotrichum gloeosporioides*	CBS:112999	JQ005500	JQ005326	JQ005239	JQ005413	JQ005152	JQ005587
3	*Colletotrichum boninense*	CBS 123755*	JQ005501	JQ005327	JQ005240	JQ005414	JQ005153	JQ005588
4	*Colletotrichum torulosum*	CBS:128544*	JQ005513	JQ005339	JQ005252	JQ005426	JQ005164	JQ005599
5	*Colletotrichum oncidii*	CBS:129828*	JQ005517	JQ005343	JQ005256	JQ005430	JQ005169	JQ005603
6	*Colletotrichum beeveri*	CBS:128527*	JQ005519	JQ005345	JQ005258	JQ005432	JQ005171	JQ005605
7	*Colletotrichum karstii*	CBS:129927	JQ005554	JQ005380	JQ005293	JQ005467	JQ005206	JQ005640
8	*Colletotrichum petchii*	CBS:378.94*	JQ005571	JQ005397	JQ005310	JQ005484	JQ005223	JQ005657
9	*Colletotrichum novae-zelandiae*	CBS 128505*	JQ005576	JQ005402	JQ005315	JQ005489	JQ005228	JQ005662
10	*Colletotrichum parsonsiae*	CBS:128525*	JQ005581	JQ005407	JQ005320	JQ005494	JQ005233	JQ005667
11	*Colletotrichum dacrycarpi*	CBS:130241*	JQ005584	JQ005410	JQ005323	JQ005497	JQ005236	JQ005670
12	*Colletotrichum fructicola*	CBS 125397*	JX009581	JX009874	JX010032	KY856315	JX010173	JX010409
13	*Colletotrichum cliviae*	CBS:125375	JX519240	JX519232	JX546611	JX560963	JX519223	JX519249
14	*Colletotrichum kahawae*	CBS 112984	KC296923	KC296966	KC296989	KC297048	KC297059	KC297082
15	*Colletotrichum aotearoa*	CBS 132448	KC296921	KC296974	KC296997	KC297054	KC297064	KC297089
16	*Colletotrichum siamense*	CBS 112983	KC296929	KC296984	KC296998	KC297043	KC297065	KC297100
17	*Colletotrichum alienum*	CBS 111982	KC296932	KC296975	KC297007	KC297034	KC297069	KC297091
18	*Colletotrichum proteae*	CBS 132882	KC296940	KC296986	KC297009	KC297045	KC297079	KC297101
19	*Colletotrichum sidae*	CBS 504.97*	KF178569	KF178521	KF178497	KF178545	KF178472	KF178593
20	*Colletotrichum helleniense*	CPC 27108	KY856022	KY856189	KY856273	KY856364	KY856449	KY856531
21	*Colletotrichum plurivorum*	CBS 90369	MG600928	MG600844	MG600784	MG600890	MG600721	MG600988
22	*Colletotrichum cliviicola*	CBS 125375*	MG600939	MG600850	MG600795	MG600892	MG600733	MG601000
23	*Colletotrichum orchidearum*	CBS 135131*	MG600944	MG600855	MG600800	MG600897	MG600738	MG601005
24	*Colletotrichum siamense*	BWZ2	OP573254	OP573255	OP573256	OP573257	KJ685390	OP573258
25	*Colletotrichum siamense*	OCAC8	KJ813458	KJ813508	KJ813558	KJ813533	KJ813608	KJ813483
26	*Colletotrichum siamense*	C6	MF627909	MF627929	MF627899	MF627939	MF627889	MF627949
27	*Colletotrichum siamense*	HJH-2	MH370528	MH370521	MH370514	MH370542	MH370507	MH370549

* = ex-type or authentic culture.

Abbreviations

PDA: potato dextrose agar; CGVG: Coconut Grant View Garden; CTAB: cetyltrimethylammonium Ammonium Bromide.

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References

[1] Tariq, R. M. S., Raza, S., & Ahmad, T. (2020). Fungal Diseases of Bismarckia Palm (*Bismarckia nobilis*). In: Ul Haq, I., Ijaz, S. (eds) Etiology and Integrated Management of Economically Important Fungal Diseases of Ornamental Palms. Sustainability in Plant and Crop Protection, vol 16. Springer, Cham. doi: 10.1007/978-3-030-56330-1_9.

[2] Yang, Y. L., Liu, Z. Y., Cai, L., Hyde, K. D., Yu, Z. N. & Mckenzie, E. H. C. (2009). *Colletotrichum* anthracnose of Amaryllidaceae. Fungal Diversity, 39: 123-146.

[3] Weir, B. S., Johnston, P. R., & Damm, U. (2012). The Colletotrichum gloeosporioides species complex. Studies in Mycology, 73: 115-180.

[4] Sharma, G., Kumar, N., Weir, B. S., Hyde, K. D. & Shenoy, B. D. (2013). The ApMat gene marker can resolve Colletotrichum species: a case study with Mangifera indica. Fungal Diversity, 61: 117-138.

[5] Xu, G. (2020). Pathogen identification and whole genome cloning and structural analysis of mycovirus in pathogen of *Areca catechu* Diaporthe leaf spot (Master thesis). Haikou: Hainan University.

[6] Vieira, W. A. S., Bezerra, P. A., Silva, A. C., Veloso, J. S., Câmara, M. P. S., & Doyle, V. P., (2020). Optimal markers for the identification of *Colletotrichum* species. Molecular Phylogenetics and Evolution, 143: 106694.

[7] Kumar, S., Stecher, G., & Tamura, K. (2016). MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Molecular Biology and Evolution, 33: 1870-1874.

[8] Prihastuti, H., Cai, L., Chen, H., McKenzie, E. H. C., & Hyde, K. D. (2009). Characterisation of *Colletotrichum* species associated with coffee berries in northern Thailand. Fungal Diversity, 39: 89-109.

[9] Dwarka, D. J., Sharma, G., & Rajasab, A. H. (2016). *Colletotrichum siamense* causes anthracnose on the fruits of Pongamia pinnata in India. Mycosphere, 7: 492-498.

[10] Larran, S., Vera Bahima, J., Dal Bello, G., Franco, E. & Balatti, P. (2015). *Colletotrichum siamense* causing anthracnose in *Bauhinia forficata* subsp. *pruinosa* in Argentina. Australasian Plant Disease Notes, 10: 7.

[11] Talhinhas, P., & Baroncelli, R., (2021). *Colletotrichum* species and complexes: Geographic distribution, host range and conservation status. Fungal Diversity, 110: 109-198.

[12] Cao, X., Xu, X., Che, H., West, J. S., & Luo, D. (2020). Eight *Colletotrichum* species, including a novel species, are associated with areca palm anthracnose in Hainan, China. Plant Disease, 104: 1369-1377.

[13] Wang, Q. H., Zhang, Y., Zhang, Y. T., Li, D., Lin, X. L., & Lyu, J. (2021). First report of *Colletotrichum siamense* causing anthracnose on *Cornus hongkongensisin* China. Plant Disease, 105: 1860-1860.

[14] Dai, X. K., Liu, T., Li S. J., Chen, G, Chen, X. Y., Deng, Z. Y., & Zhu, T. H. (2022). First report of leaf spot caused by *Colletotrichum siamense* on *Pharbitis purpurea* in Sichuan, China. Plant Disease, Aug 30. Online ahead of print. doi: 10.1094/PDIS-05-22-1051-PDN.

[15] Farr, D. F., & Rossman, A. Y. (2023). Fungal Databases, Syst. Mycol. Microbiol. Lab., Online publication. ARS, USDA. Retrieved April 12, 2023, from https://nt.arsgrin.gov/fungaldatabases/