



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

First Report of Brown Rot Caused by *Porogramme epimiltina* on *Gastrodia elata* in China



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Susceptibility and yield response of commercial corn hybrids to maize dwarf mosaic disease

Mark W. Jones and Erik W. Ohlson

Date: 22 January 2024

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First Report of Leaf Spot of Pineapple (*Ananas comosus* (L.) Merr Caused by *Neoscytalidium dimidiatum* in Malaysia



Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Brown Rot Caused by *Porogramme epimiltina* on *Gastrodia elata* in China

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Gastrodia elata Blume is a valuable medicinal plant in China with great significance in medicine (Li et al. 2023). From 2022 to 2023, *G. elata* tuber rot occurred in about 50 households in the main cultivation areas (27°39'N, 104°16'E) of *G. elata* in Yiliang County, Zhaotong, Yunnan Province, southwest China. The planting area of *G. elata* was 776 ha, and the disease incidence rate was 10%. Symptoms presented as light brown, sunken, soft, and foul-smelling lesions on the surface of the tubers. Infected *G. elata* tubers were randomly collected from each household and packed in transparent plastic bags, and strains were isolated in the laboratory as follows. Fifteen infected *G. elata* tubers were surface sterilized with 0.5% NaOCl for 2 min, rinsed five times with sterile water, and dried. Symptomatic tissues from the margin between necrotic and healthy tissues were cut into 5 × 5-mm pieces, placed onto potato dextrose agar (PDA), and incubated at 28°C in the dark for 3 days. Hyphal tips of fungi growing from the samples were transferred onto new PDA plates and incubated until they produced conidia. Two fungal strains (Charliezhao 425 and 433) with the same morphological characteristics were obtained from the samples. Colonies were whitish, grew rapidly, irregularly turned pale orange at the edge or center of the mycelium pad on a 2-week-old Petri dish, and finally dark red,

with oval to spherical spores, measuring 2.7 to 5.3 × 2.3 to 3.5 μm ($n = 50$). The morphological characteristics of the isolates resembled *Porogramme epimiltina* (Kubayashi et al. 2001; Mao et al. 2023). Genomic DNA of the two representative isolates (Charliezhao 425 and 433) was extracted using the DN14 cetyltrimethylammonium bromide rapid plant genome extraction kit (Aidlab Biotechnologies, Beijing). The internal transcribed spacer (*ITS*) and translation elongation factor (*TEF1*) genes were amplified by polymerase chain reaction using the primers ITS1/ITS4 (White et al. 1990) and EF1-983F/EF1-2218R (Rehner and Buckley 2005), respectively. All sequences were deposited in GenBank (accession nos. OR905803 and OR905804 for *ITS* and OR939812 and OR939813 for *TEF1*). A BLASTn homology search with the *ITS* nucleotide sequences showed that they had 98.99 to 99.15% identity with the *P. epimiltina* isolates OP997539 (588/594 bp) and OP997539 (584/589 bp), and the *TEF1* sequences had 95.41 to 95.59% identity to the isolates OP556566 (540/565 bp) and OP556566 (542/567 bp). To fulfill Koch's postulates, the surfaces of five mature and healthy *G. elata* tubers were disinfected with 1% NaClO solution for 1 min, rinsed with sterile water five times, and dried at 25°C for 30 min. Conidial suspensions (10^6 spores/ml) were prepared from the two isolates (Charliezhao 425 and Charliezhao 433) and sprayed on *G. elata* tubers, and the controls were treated with distilled water. All *G. elata* tubers were incubated at 25°C with 80% relative humidity. The experiment had three replicates. After 7 days of culture, the inoculated tubers were rotten and smelly. No symptoms were observed in the control groups. The pathogen was reisolated from all inoculated birch tubers and confirmed as *P. epimiltina* by morphological and molecular analysis, thus fulfilling Koch's postulates. *G. elata* is a valuable and extensively used herbal traditional Chinese medicine with a wide range of clinical applications. As far as we know, this is the first report of *P. epimiltina* causing brown rot of *G. elata* in China.

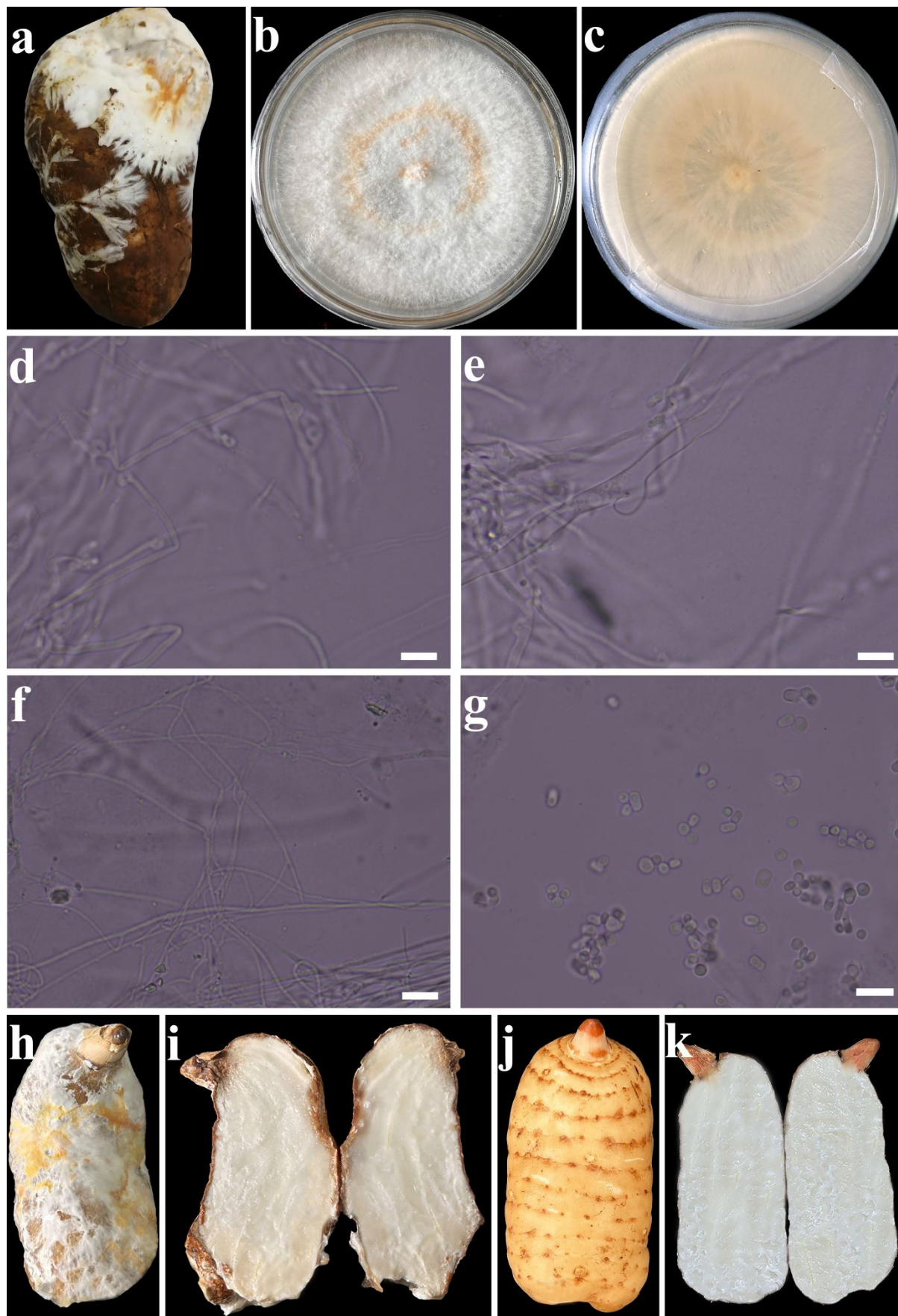
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The author(s) declare no conflict of interest.

e-Xtra

Keywords: China, *Gastrodia elata*, *Porogramme epimiltina*, rot



Supplementary Figure S1. a: Rot symptom on *Gastrodia elata*; **b-c:** Colonies on PDA (front and reverse); **d-e:** Simple clamped thin-walled generative hyphae; **f:** branched fibre hyphae; **g:** Basidiospores; **h-i:** Pathogenicity test, infection symptoms observed on *Gastrodia elata* after 7 days; **j-k:** The control. Scale bars: d-g=10 μm

>OR905803.1 Porogramme epimiltina voucher Zhaochanglin 425 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence

GATGACTGCGGAGGACATTAACGAGTTGAACGGGGTTGTGGCTGGCCTTC
ACGGGCATGTGCACACCTCACTCATCCACTCTACACCTGTGCACTTACTGT
GGGTTTCGAGAGGCCGCGCTTGCGTGGTCGATCGGGCTCACGTCTATTACA
AACTCTTCAGTATCAGAATGTGTATCGCGATGTAACGCATCTATATACTT
TCAGCAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGC
GATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCA
CCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCGTGTAAT
TCTCAACCTATAAATCCTTGCGGTTTTTAGGCTTGGACTTGGAGGCTTTTGC
TGGCTTTACCGTCGGCTCCTCTTAAATGCATTAGCTCGATTCCTTGCGGATC
GGCTCTCAGTGTGATAATTATCTGCGCTGTGACCGTGAAGCGTTTGGCGAG
CTTCTAACCGTCTCTCTGAGACAAACACTTTGACATCTGACCTCAAATCAG
GTAGGACTACCCGCTGAACTTAAGCATAAAAAAGGGGGGGGGGAAAAAA
TTTTA

>OR905804.1 Porogramme epimiltina voucher Zhaochanglin 433 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence

TAGGTGACTGCGGAGGATCATTAACGAGTTGAACGGGGTTGTAGCTGGCCT
TCACGGGCATGTGCACACCTCACTCATCCACTCTACACCTGTGCACTTACT
GTGGGTTTCGAGAGGCCGCGCTTGCGTGGTCGATCGGGCTCACGTCTATTA
CAAACCTCTTCAGTATCAGAATGTGTATCGCGATGTAACGCATCTATATACT
CTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAA
TGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAAC
GCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCGTGT
AATTCTCAACCTATAAATCCTTGCGGTTTTTAGGCTTGGACTTGGAGGCTTT
TGCTGGCTTTACCGTCGGCTCCTCTTAAATGCATTAGCTCGATTCCTTGCGG

ATCGGCTCTCAGTGTGATAATTATCTGCGCTGTGACCGTGAAGCGTTTGGCG
AGCTTCTAACCGTCTCTCTGAGACAAACACTTTGACATCTGACCTCAAATC
AGGTAGGACTACCCGCTGAACTTAAGCATATAAAAAGGGGGGGGGGAAAA
AATGTGG

>OR939812 [organism=Porogramme epimiltina] Porogramme epimiltina strain
Zhaochanglin 433 translation elongation factor 1-alpha (tef1) gene, partial cds

ATACTGATACTTCCAGGCCGACTGCGCCATCCTCATCATCGCCGGTGGTACC
GGTGAGTTCGAGGCCGGTATCTCCAAGGACGGCCAGACTCGCGAGCACGC
CCTCCTCGCCTTCACCCTCGGTGTCCGTCAGCTCATCGTCGCCGTCAACAA
GATGGACACGACCAAGGTTCGTCCACCTCGTGGTCGTCTTATCACGTTCAA
TATACTAACTCTTACTTCAGTGGTCCGAGGACCGTTTCAACGAAATCGTCA
AGGAGACGTCCACCTTCATCAAGAAGGTCGGCTACAACCCCAAGGCGGTT
GCGTTCGTCCCCATCTCCGGCTGGCACGGCGACAACATGCTCGAGGAGTCC
AGCAAGTATGTGCATGCGCTCTTGTCGTGAGGAGTTGATGCTTATATGTATT
CCCTTCAGCATGCCCTGGTACAAGGGCTGGACCAAGGAGACCAAGGCGGG
TGTCGTCAAGGGCAAGACCCTCCTCGATGCCATCGACGCCATCGAGCCCCC
CGTCCGTCCCTCGGACAAGCCCCTCCGTCTTCCTCTCCAGGATGTTTACAA
GATCGG

>OR939813 [organism=Porogramme epimiltina] Porogramme epimiltina strain
Zhaochanglin 425 translation elongation factor 1-alpha (tef1) gene, partial cds

ATACTGGTACTTCCAGGCCGACTGCGCCATCCTCATCATCGCCGGTGGTACC
GGTGAGTTCGAGGCCGGTATCTCCAAGGACGGCCAGACTCGCGAGCACGC
CCTCCTCGCCTTCACCCTCGGTGTCCGTCAGCTCATCGTCGCCGTCAACAA
GATGGACACGACCAAGGTTCGTCCACCTCGTGGTCGTCTTATCACGTTCAA
TATACTAACTCTTACTTCAGTGGTCCGAGGACCGTTTCAACGAAATCGTCA
AGGAGACGTCCACCTTCATCAAGAAGGTCGGCTACAACCCCAAGGCGGTT
GCGTTCGTCCCCATCTCCGGCTGGCACGGCGACAACATGCTCGAGGAGTCC
AGCAAGTATGTGCATGCGCTCTTGTCGTGAGGAGTTGATGCTTATATGTATT

CCCTTCAGCATGCCCTGGTACAAGGGCTGGACCAAGGAGACCAAGGCGGG
TGTCGTCAAGGGCAAGACCCTCCTCGATGCCATCGACGCCATCGAGCCCCC
CGTCCGTCCCTCGGACAAGCCCCTCCGTCTTCCTCTCCAGGATGTTTACAA
GATCGGTGGTATCGGCACGGTGCCCGTCGGTCGTGTCGAGACTGGTGTCAT
CAAGGCCGGCATGATCGTCACGTTTCGCGCCTACGAACGTGACCACTGAGG
TCAAGTCCGTGAGATGCACCACGAGCAGCTTGAGCAGGGTGTTCCCGGT
GACAACGTTGGTTTCAACGTCAAGTACGTTTACCTGCTTACCTTCACTTGTC
TAGTTCTCGTCACTGACCTCTGTAATAGGAACGTCTCCGTCAAGGATATCCG
CCGTGGCAACGTTCGCCTCCGAATCGAAAAACGACCCCGCCAAGGAGG