Report

Leaf Blister on *Leucothoe grayana* var. *venosa* Caused by an *Exobasidium* Species

Exobasidium 属菌によるハコネハナヒリノキ平もち病（新称）

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Leucothoe grayana Maxim. var. *venosa* Nakai is localized in bright, open, nutrient-poor soil in mountainous areas, such as wind-swept areas and rocky areas of Yamanashi, Shizuoka, and Kanagawa Prefectures. It grows in Hakone and Tanzawa areas in Kanagawa Prefecture (Sasaki, 2018). Chlorotic spots on leaves of this plants were found for the first time near the peak of Mt. Komagatake, Hakone, Kanagawa Prefecture in July 2021. Hymenia of these spots suggested that they are caused by *Exobasidium* species. Leaf blisters caused by *Exobasidium bisporum* Sawada ex Ezuka have been recorded on *Leucothoe grayana* var. *oblongifolia* (Miq.) Ohwi (Sawada, 1950; Ito, 1955; Ezuka, 1991), *L. grayana* var. *glabra* Komatsu ex Nakai (Sawada, 1950; Ito, 1955; Ezuka, 1991), *L. grayana* var. *glaucina* (Koidz. ex Komatsu) Koidz. ex Nakai (Ezuka, 1991), *Vaccinium axilare* Nakai (syn. *V. ovalifolium* Sm.) (Sawada, 1950), and *V. oldhamii* Miq. (Ezuka, 1991). Although Sawada (1950) described *E. bisporum* Sawada on three varieties of *Leucothoe grayana* and *Vaccinium axilare* in Japan, the species name was treated as a nomen nudum because it lacked a Latin description. Ezuka (1991) validated this species as *E. bisporum* Sawada ex Ezuka and added *V. oldhamii* as a new host plant of this species because the morphology of basidia, sterigmata, and basidiospores produced on the plants mentioned above were within the range of this species. In addition, the mode of basidiospore germination was budding (Table 1).

Piątek et al. (2012) reported phylogenetic analyses of *Exobasidium* species using the concatenated sequences of the internal transcribed spacer (ITS) region and large subunit (LSU) of nuclear rDNA. These analyses indicated a cluster with high to moderate support was composed of *E. bisporum* on *Eubotryoides grayana* (Maxim.) H. Hara var. *oblongifolia* (Miq.) Ohwi (syn. *L. grayana* var. *oblongifolia*), *E. grayana* (Maxim.) H. Hara var. *glabra* (Komatsu ex Nakai) H. Hara (syn. *L. grayana* var. *glabra*), and five *Exobasidium* spp. on five *Vaccinium* spp., while the analyses of the LSU dataset of these six species could not resolve the relationships. Although Shibata & Hirooka (2022) recently showed a phylogenetic tree using the concatenated sequences of ITS-LSU regions including the two *E. bisporum* sequences used in Piątek et al. (2012), specimens of *E. bisporum* identified on *V. oldhamii* and *V. ovalifolium* were not included in their analysis. In this report, we examined the morphology and cultural characteristics of specimens on *L. grayana* var. *venosa* to identify its causal fungal species. To clarify its genetical relationship with *E. bisporum*, we also examined the homology of the ITS region of rDNA.

Fresh materials of leaf blister on *L. grayana* var. *venosa* collected on 28 July 2021 and 30 June 2022 were used for morphological observation. Hymenia of the specimens were examined with a light microscope

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as described previously (Nagao et al. 2003). Dried specimens were deposited in the Mycological Herbarium, Kanagawa Prefectural Museum of Natural History, Japan (KPM-NC). Several cultural strains were isolated from a single germinated basidiospore obtained from fresh materials and kept on Pearlcore: Potato dextrose agar 'Eiken' (PDA) as described previously (Nagao et al., 2003). Morphology of one of the cultures was examined, and size of conidia obtained from a pure culture on PDA were measured under a light microscope. A representative isolate of KPM-NC 26934 was deposited in NARO GeneBank (No. MAFF 247751). Genomic DNA was extracted from a colony on PDA using the protocol of Izumitsu et al. (2012). PCR amplification of ITS and the sequencing process were followed by Orihara et al. (2012). Alignment was done with Clustal Omega (Madeira et al. 2022) through the European Bioinformatics Institute (EMBL–EBI) web site (https://www.ebi.ac.uk/Tools/msa/clustalo/) under default settings, and the results were output in the NCBI Standard Nucleotide BLAST for the homology search (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The newly obtained ITS sequence for the isolate is available from NCBI GenBank under Acc. No. LC735739.

Description of *Exobasidium* species on *Leucothoe grayana* var. *venosa* (Fig. 1)

Spots were produced on the abaxial surface of leaves (Fig. 1I). Fresh spots with hymenium appeared pale white to pale yellow. The hymenium often had a farinose appearance. Later, the spots turned brown, formed dry lesions and finally their centers dropped out to form shot holes. Lesions were observed infrequently. The hymenium is composed of basidia with 2 sterigmata and conidia. Basidia are cylindrical, 10.3–35.9 × 4.8–6.9 µm, emerging directly from the host surface. Sterigmata are (2.1–)4.8–6.9(–8.3) × (0.7–)1.3(–2.1) µm, developing outwardly and tapering toward the tip (Fig. 1A, B). Basidiospores are ellipsoid, (12.4–)15.2–20.7 × 4.1–6.9 µm, hyaline, smooth, one–celled when formed, and septate with (2–)3–4(–5) septa (Fig. 1C) after formation. Septate basidiospores germinated after 24 h when released onto an agar surface. They were also observed to germinate in hymenia on leaves of the plant. The mode of germination is budding. Direct budding from several cells of the basidiospores was predominant (Fig. 1D, E) but short germ tubes sometimes emerged from cells of the basidiospores and produced conidia at the tip of the germ tubes in the hymenium (Fig. 1F). The short germ tubes didn't elongate into pseudohyphae. Colonies on PDA were composed of partially elongated pseudohyphae and conidia. The surface of the colonies was corrugate without a farinose appearance and pink to pale pink, the same as the reverse of the colonies. Colonies were glutinous and did not fix on the agar surface (Fig. 1G). Conidia on PDA were lacrimiform, subfusiform or clavulate, 3–6 × 0.7–1.7 µm. Conidia polarly budded or germinated to produce pseudohyphae (Fig. 1H).

Specimens examined: JAPAN: Kanagawa Pref., Hakone, near the peak of Mt. Komagatake, 28 July 2021 (KPM-NC 26934) and 30 June 2022 (KPM-NC 26936), W. Ohnishi and H. Nagao.

The sizes of basidia, sterigmata, and basidiospores of *E. bisporum* Sawada ex Ezuka are 40 × 6–7 µm, 4–6 µm length, and 15–24 × 5–7 µm, respectively (Ezuka, 1991), which are mostly in the same range of the *Exobasidium* specimens on *L. grayana* var. *venosa* collected in Kanagawa Prefecture (Table 1). The shape of basidiospores of the *Exobasidium* specimens on *L. grayana* var. *venosa* are ellipsoid. Sawada (1950) showed combined spore measurements from *L. grayana* varieties and *V. ovalifolium*, whereas Ezuka (1991) reported the spore measurements separately from *L. grayana* varieties and *V. oldhamii*. The range of septal number of basidiospores isolated from *V. oldhamii* was broader (1–7) than those from *L. grayana* varieties (1–4). The specimens obtained from *L. grayana* var. *venosa* showed slightly broader range of septal number (i.e., (2–)3–4(–5)) than *E. bisporum.* In addition, the mode of germination of basidiospores is budding, the same as that of *E. bisporum* reported by Ezuka (1991). Thus, the *Exobasidium* species on this host plant is morphologically close to *E. bisporum*.

The top three homology search results by NCBI indicated that *Exobasidium* sp. ON787635 isolated from Iceland moss, *Cetraria islandica*, *E. bisporum* AB180368 isolated from *E. grayana* var. *oblongifolia*, and *E. bisporum* AB180364 isolated from *E. grayana* var. *glabra* showed 96% (523 bp/546 bp identical), 96% (508 bp/530 bp identical), and 94% (499 bp/532 bp identical) identities, respectively, while the homology search between *E. bisporum* AB180368 and *E. bisporum* AB180364 showed only 94 % (500 bp/531 bp identical) identity. This homology search result suggests that each *E. bisporum* isolate from the two varieties of *E. grayana* are not identical in ITS sequences, and that the ITS region of representative isolate MAFF 247751 is different from *E. bisporum*.

In conclusion, *Exobasidium* species on *L. grayana* var.

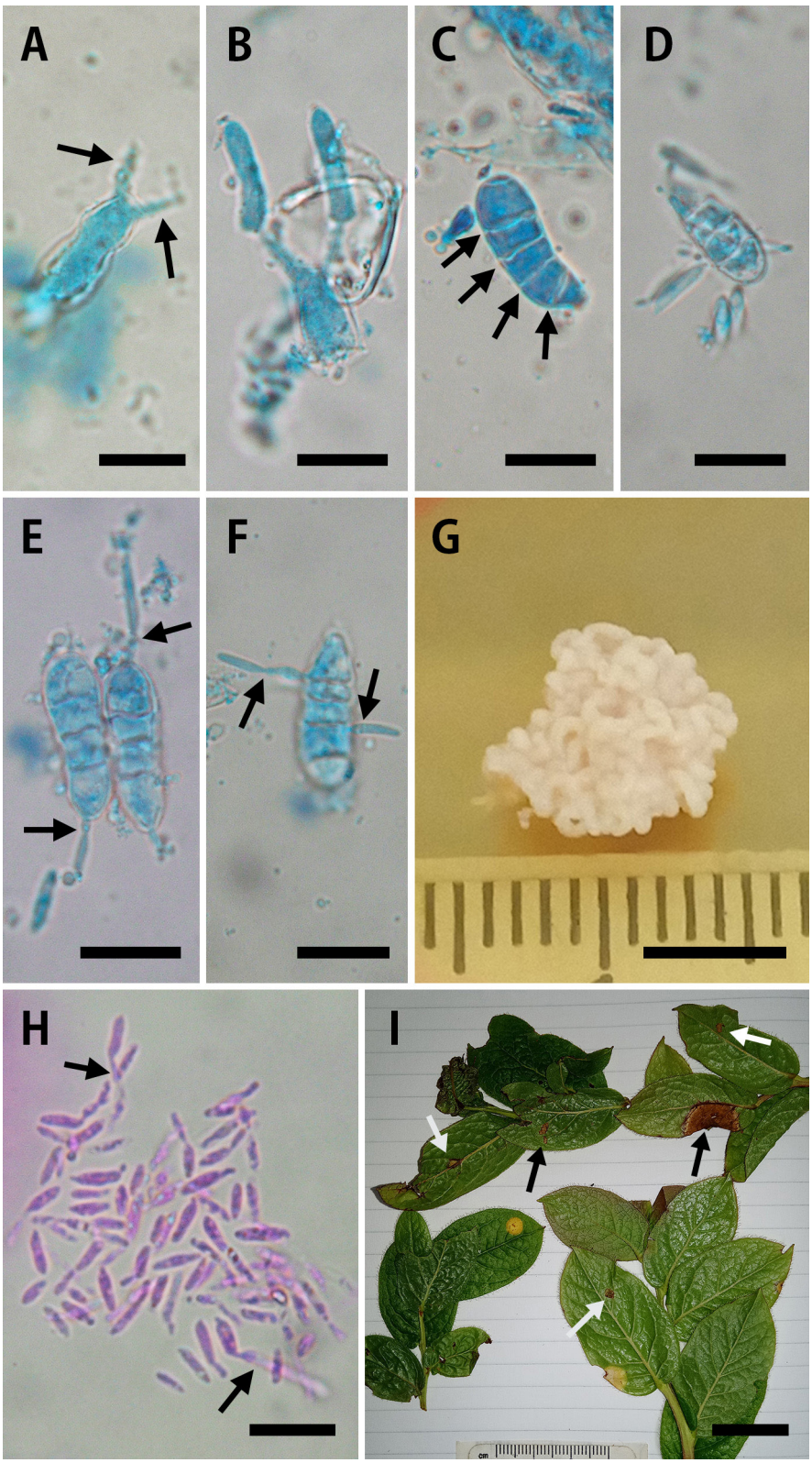

Fig. 1. Morphology and symptoms of *Exobasidium* sp. on *Leucothoe grayana* var. *venosa* collected on Mt. Hakone Komagatake, Kanagawa Prefecture (KPM-NC 26934). Basidium with 2 sterigmata (arrows) (A), a sterigma bearing an immature basidiospore (B), ellipsoid basidiospore, one–celled when formed, becoming septate with 3–4 septa (arrows) (C), basidiospore with directly budded conidia (D), or germinated by a short germ tube (arrows) producing a conidium at the tip (E, F). Colony from a single basidiospore of *Exobasidium* sp. on PDA after 21 days incubation (G); colony was composed of conidia and pseudohyphae (arrows) (H). Chlorotic spot. Arrows indicate the old spots (I). Scale bars: A–F, H = 10 µm, G = 5 mm, I = 20 mm. Photographed by Hideyuki Nagao.

Table 1. Morphological comparison of *Exobasidium bisporum* on the different hosts and *Exobasidium* sp. on *Leucothoe grayana* var. *venosa*

Host plant	Isolate deposited in	Herbarium deposited in	Basidium[1] [No. of sterigma]	Sterigma[1]	Basidiospore[1] [No. of septum]	Mode of germination	Conidia[1]	GenBank Acc. No.		Reference
								LSU	ITS	
Leucothoe grayana Maxim. var. *glabra* Konatsu ex Nakai	N/A[3]	5411[4]	40 × 6–7 [2]	4–6	15–24 × 5–7 [0–7]	Budding	7–14 × 1–2.5	N/A	N/A	Ezuka (1991)
	IFO 9942	N/A	N/A	N/A	N/A	N/A	N/A	AB177598	AB180364	NBRC 9942[5]
Leucothoe grayana[2] & *Vaccinium ovalifolium* Sm.	N/A	N/A	40 × 6–7 [2]	4–6	15–24 × 5–7 [0–7]	Budding	7–14 × 1–2.5	N/A	N/A	Sawada (1950)
Leucothoe grayana Maxim. var. *hypoleuca* Nakai	N/A	N/A	40–55 × 6–9 [2]	N/A	15–24 × 6–7 [3]	Budding	7–14 × 1–2.5	N/A	N/A	Ito (1955)
	N/A	N/A	60–80 × 5–7 [2]	N/A	15–22 × 5–8 [1–4]	Budding	7–15 × 1–2.4	N/A	N/A	Ezuka (1991)
Leucothoe grayana Maxim.	IFO 30152	N/A	N/A	N/A	N/A	N/A	N/A	AB177596	AB180368	NBRC 30152[6]
Vaccinium oldhamii Miq.	N/A	N/A	40–60 × 6–8 [2(–3)]	N/A	14–24(–27) × 4–7 [1–7]	Budding	5–12 × 1–2	N/A	N/A	Ezuka (1991)
	MAFF 238671	NIAES 20540	15–35(65) [2–3]	4–7(–8) × (1.5–)2–3	17–24 × 4–5 [(3–)4–7(–8)]	Budding (6% by germ tube)	3–7.2(9) × (0.6–)1.2–1.8	N/A	N/A	nagao-14196
Leucothoe grayana Maxim.var. *venosa* Nakai	MAFF 247751	KPM-NC 26934	10.3–35.9 × 4.8–6.9 [2]	(2.1–)4.8–6.9(–8.3) × (0.7–)1.3(–2.1)	(12.4–)15.2–20.7 × 4.1–6.9 [(2–)3–4(–5)]	Budding	3–6 × 0.7–1.7	N/A	LC 735739	This study

[1] Unit of measurement : μm

[2] Including *E. grayana* var. *glabra* : syn. of *L. grayana*

[3] Not available

[4] The Herbarium of the University Museum, Iwate University

[5] https://www.nite.go.jp/nbrc/dbrp/dataview?dataId=STNB0000000009942

[6] https://www.nite.go.jp/nbrc/dbrp/dataview?dataId=STNB0000000030152

venosa, MAFF 247751 is morphologically similar to *E. bisporum*, but genetically different. Therefore, further investigations are required to identify this species.

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摘　要

長尾英幸・大西　亘・折原貴道 , 2023. *Exobasidium* 属菌によるハコネハナヒリノキ平もち病（新称）. 神奈川県立博物館研究報告（自然科学）, (52): 1–5. [Nagao, H., W. Ohnishi & T. Orihara, 2022. Leaf Blister on *Leucothoe grayana* var. *venosa* Caused by an *Exobasidium* Species. Bull. Kanagawa Pref. Mus. (Nat. Sci.), (52): 1–5.]

2021 年 7 月神奈川県箱根町駒ケ岳頂上付近でハコネハナヒリノキの葉に黄白化した病斑が見つかった。病斑の葉裏側は粉状物で覆われていた。これらは植物病原菌によるものと考えられ、形態学的特徴と宿主情報からこの植物病原菌はハナヒリノキ平もち病菌に近縁のもち病菌と考えられた。ITS 領域による相同性検索の結果、登録されているハナヒリノキ平もち病菌が上位 3 位以内の相同性を示したが、本菌はもち病菌の 1 種だがハナヒリノキ平もち病菌とは同定できなかった。以上のことより、本病害は *Exobasidium* 属菌によるハコネハナヒリノキ平もち病（新称）とした。