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# *Diaporthe kyushuensis* sp. nov., the teleomorph of the causal fungus of grapevine swelling arm in Japan, and its anamorph *Phomopsis vitimegaspora*

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***Diaporthe kyushuensis* sp. nov. is described and named as the teleomorph of causal fungus of grapevine swelling arm. The anamorph of the fungus is *Phomopsis vitimegaspora*.**

Key Words——*Diaporthe kyushuensis*; grapevine swelling arm; *Phomopsis vitimegaspora*; taxonomy.

In the 1960s, a new canker disease of the grapevine cultivar Kyohou (*V. vinifera* × *V. labrusca*) was noticed within a part of the grape-cultivation area in Kyushu, Japan. In the 1970s, the disease spread to many grape fields in Kyushu accompanying the increase in cultivated area of the table grape cv. Kyohou (Sadamatsu, 1988; Kajitani et al., 1991). The disease was often confused with Phomopsis cane and leaf spot caused by *Phomopsis viticola* (Sacc.) Sacc., because critical research into this new disease was not started until the 1980s.

In 1982, Yamato reported that the grapevine canker was caused by *Phomopsis* sp., which had much larger conidia (alpha conidia, 13–24 × 3.6–6.0 μm; beta conidia, 25–42 × 1–1.2 μm) than *P. viticola* (alpha conidia, 6–10 × 2.5–3 μm; beta conidia, 18–30 × 0.5–1 μm) (Punithalingam, 1979). Mikuriya and Sadamatsu (1987) named this new disease "grapevine swelling arm". From the late 1980s to early 1990s, the epidemiology of the disease was investigated critically (Nakao, 1989; Tashiro, 1989; Kajitani et al., 1991; Tashiro, 1992, 1994; Nakao et al., 1995.) The typical symptoms of the disease are: (1) tiny black spots appear at the base of the green shoot and coalesce to form a blackened zone (Fig. 2A), (2) oblong to elliptic black lesions appear independently in the middle of the shoot, (3) if the inoculum concentration is low, the fungus cannot develop black spots on green shoots, although it can cause latent infection on the shoots. In two or more years, flat and slightly hypertrophied nodes first appear on canes (Fig. 2B). Canker is noticed on 4-yr-old or older arms. The name of the disease, grapevine swelling arm, came from these symptoms.

Similar symptoms were observed on grapevine cv. Kyohou in Taiwan, and the causal *Phomopsis* was reported as a new species, *P. vitimegaspora* Kuo et Leu (Kuo and Leu, 1998). The conidial morphology of *P. vitimegaspora* in Taiwan (alpha conidia, (10–)13–18(22) × (3–)4–5(–6) μm; beta conidia, (21–)26–34(–40) × 0.5–1 μm) is very similar to that of the grapevine swelling arm fungus in Kyushu (alpha conidia, 14.5–21.8 × 4.5–7.3 μm; beta conidia, 30.8–45.5 × 0.9–1.6 μm) (Sadamatsu, 1988).

The teleomorph of *Phomopsis* spp. is usually *Diaporthe* (Uecker, 1988). We collected some pruned grapevine canes that showed typical symptoms of grapevine swelling arm from Fukuoka Agricultural Research Center in Kyushu in 1992. After incubating the canes under high moisture conditions, *Diaporthe* perithecia appeared on them. The perithecia contained large ascospores. A monoascosporic isolate (ch-D-1) from the *Diaporthe* developed *Phomopsis* pycnidia on potato dextrose agar, and the conidial morphology was almost the same as grapevine swelling arm fungus observed on diseased canes of grapevine. We made inoculation tests to confirm the pathogenicity of the *Diaporthe*. One layer of gauze was dipped in water suspensions of either ascospores ($2.6 \times 10^5$/ml) collected from dead canes, or alpha conidia ($2.4 \times 10^5$/ml), which were produced on potato dextrose agar by the isolate ch-D-1. The gauze was placed on green shoots of grapevine (cv. Kyohou), then the shoots and the gauze were wrapped with Parafilm. In one month, both suspensions gave rise to spot lesions on the inoculated shoots, producing the symptoms of grapevine swelling arm. It was proved, therefore, that the *Diaporthe* we collected was really the teleomorph of grapevine swelling arm *Phomopsis*.

Internal transcribed spacer (ITS) regions of ribosomal DNA (rDNA) are useful to infer the phylogeny of *Phomopsis* species and varieties (Zhang et al., 1998; Kanematsu et al., 2000). To investigate critically the relationship of the *Diaporthe* we collected in Kyushu and *P. vitimegaspora* from Taiwan, we compared the sequences of ITS regions of their rDNA. The DNA of Taiwan *Phomopsis vitimegaspora* isolate (CSS0071) was extracted by the method of Lee and Taylor (1990). To sequence the ITS regions of rDNA, the PCR products were amplified using

two primers (ITS1 and ITS4; White et al., 1990). The PCR product was directly sequenced using a BigDye terminator cycle sequencing ready reaction kit (Applied Biosystems) and the extension products were analyzed using a 310 genetic analyzer (Applied Biosystems) according to the manufacturer's instructions. The sequences were determined on both strands with primers ITS1, ITS2, ITS3, and ITS4 (White et al., 1990). The sequences of ITS regions of rDNA of Taiwan *P. vitimegaspora* isolate (CSS 0071) (accession no. on DDBJ: ITS1, AB032610; ITS2, AB032611, present study) and of the *Diaporthe* isolate (ch-D-1) (accession no. on DDBJ: ITS1, AB017730; ITS2, AB017762, Kanematsu et al., 2000) were the same. From these molecular data, in addition to the similarity of conidial morphology, we concluded that *P. vitimegaspora* is the anamorph state of the *Diaporthe* we collected from Kyusyu.

The new *Diaporthe* seemed to be unique in comparison with the hitherto known *Diaporthe* species in its large ascospores and conida. In Wehmeyer's monograph (1933), small-spored species, such as *D. eres* Nitschke and *D. arctii* (Lasch) Nitschke, have a large number of synonyms and a wide host range, whereas the concept of the large-spored species was relatively definitive with a limited host habitat because the host range and fungal morphology were well correlated (Kobayashi, 1970). Three *Diaporthe* species have been reported from *Vitis*: *D. medusaea* Nitschke, *D. silvestris* Sacc. & Berl. and *D. vitis* Ellis & Everh. (Wehmeyer, 1933). However, all these species have much smaller ascospores than the *Diaporthe* of this study. Thus no *Diaporthe* species having similar conidial and ascospore morphology to the present *Diaporthe* was found, and the grapevine swelling arm fungus was described as a new species of the genus *Diaporthe*, with the following description.

**Description**

***Diaporthe kyushuensis*** Kajitani et Kanematsu, sp. nov. Figs. 1, 2

anamorph: *Phomopsis vitimegaspora* K. C. Kuo et L. S. Leu, Mycotaxon 66: 498, 1998.

Entostromatibus effusis, circumcinctis cum zona nigricanti; peritheciis immersis, subglobosis, 310–860 μm diam, collo apicali erumpenti cylindrico nigro usque ad 3 mm longo praeditis; ascis unitunicatis, hyalinis, subclavatis vel cylindricis, apice incrassatis et annulatis, 88–117 × 13–20 μm, octosporis; ascosporis hyalinis, bicellularibus, ellipsoideis, apice utrinque rotundatis et interdum cum appendice inconspicuo hyalino, ad septum constrictis, 15.5–21.5 × 8.5–11 μm.

Anamorphosis in agaro decocto tuberorum: alpha-conidiis hyalinis, fusoideis, unicellularibus, 15.5–24 × 4.5–8 μm; beta-conidiis hyalinis, filiformibus, hamatis, unicellularibus, 25–55 × 1–2 μm.

**Materials examined**: on dead twigs of *Vitis vinifera* L. × *V. labrusca* L. cv. Kyohou – Fukuoka Agric. Res. Cent., Chikushino City, Fukuoka Pref., March 1992, by YK (TMF: 7494, holotype), 30 July 1999, by UK (TMF: 7495).

Entostromata effuse, usually formed over wide areas, surrounded by a blackened zone; perithecia solitary or in small groups, embedded in the bark tissue and partly in wood, subglobose, 310–860 μm in diam, with long necks at the top; necks cylindrical, black, up to 3 mm long under moist conditions, collectively or solely erumpent through the bark periderm; asci are clavate or cylindric-clavate, with apical rings at the tip, 89–117 × 13–20 μm, hyaline, 8-spored, arranged irregularly in the perithecium; ascospores are hyaline, two-celled, ellipsoid, rounded at both ends, straight or slightly constricted at the septum, sometimes with a large globule in each cell, 15.5–21.5 × 8.5–11 μm, sometimes with faint hya-

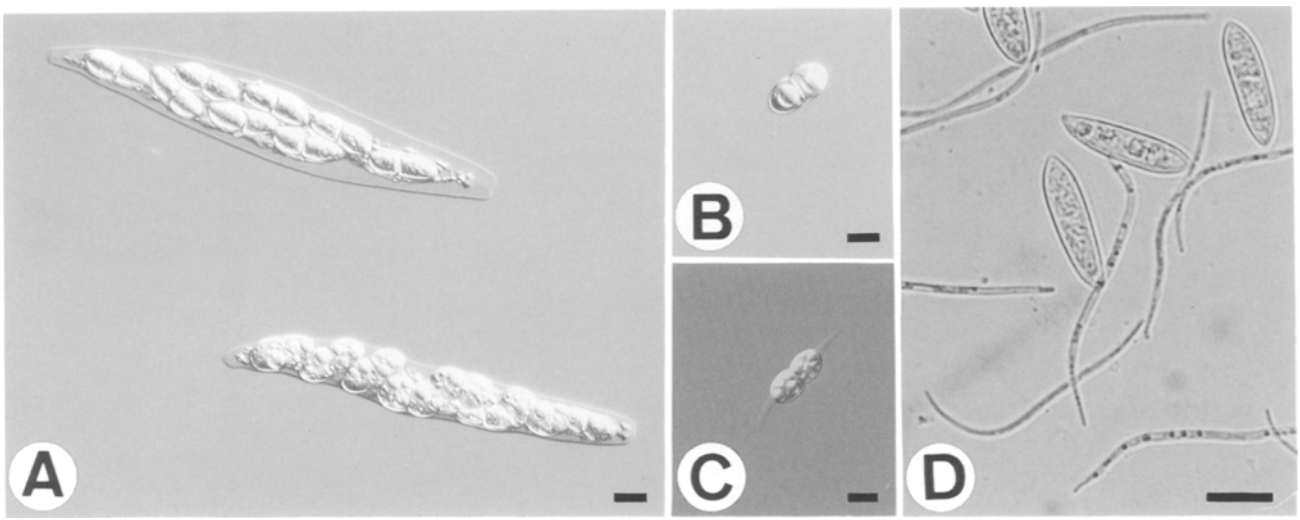


Fig. 1. *Diaporthe kyushuensis*. A: asci and ascospores, B: ascospore without appendage, C: ascospore with appendages, D: alpha conidia and beta conidia. Scale bars: 10 μm.

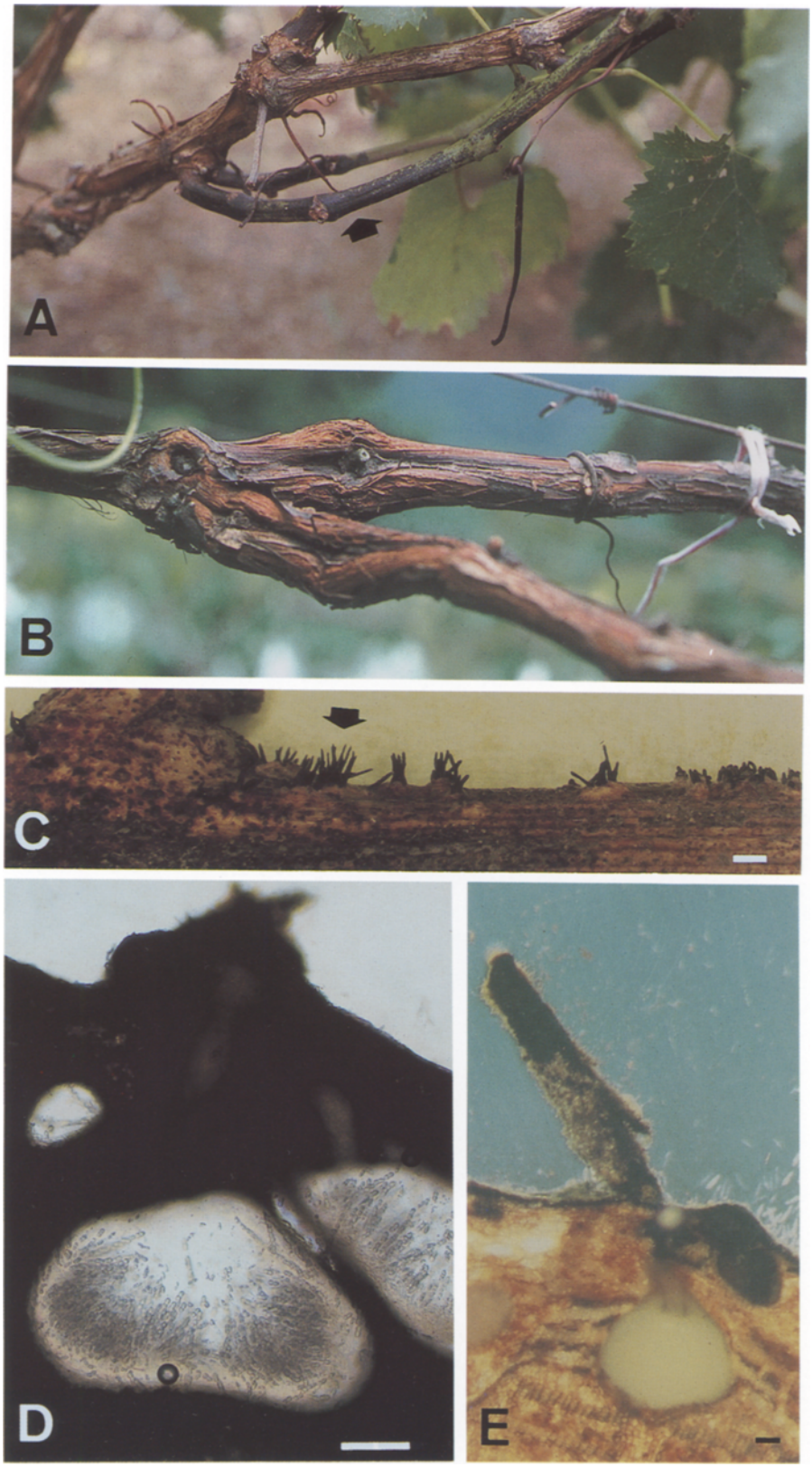


Fig. 2. Symptoms of grapevine swelling arm. A: tiny black spots appearing at the base of green shoots, which soon coalesce to form blackened zones (black arrow). B: flat and slightly hypertrophied nodes. C: Perithecial necks (black arrow) on grapevine cane. D and E: Perithecium. Scale bars: C=2 mm; D, E=100 $\mu$m.

line appendages on both ends of the ascospore.

A monoascosporic isolate (ch-D-1) produced conidia on potato dextrose agar: alpha conidia, hyaline, fusoid, unicellular, 15.5–24 × 4.5–8 μm; beta conidia, hyaline, filiform, hooked, unicellular, 25–55 × 1–2 μm.

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