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Short communication

Taxonomic identity of a *Phakopsora* fungus causing the grapevine leaf rust disease in Southeast Asia and Australasia

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ABSTRACT

Three distinct groups were revealed among the grapevine leaf rust fungi from Japan, Taiwan, Thailand, Malaysia, Indonesia, East Timor and Australia in phylograms generated from sequence analyses of the internal transcribed spacer 2 and the large-subunit rRNA gene (D1/D2 region). A group of Thai, Malaysian-Indonesian and East Timorese-Australian fungi was distinct from two other groups of grapevine leaf rust fungi, *Phakopsora meliosmae-myrianthae* and *P. montana*, distributed in temperate East Asia. Although complete life cycle and native host plants are unknown for the Southeast Asian and Australasian fungus, it is likely to be a biologically distinct species.

The grapevine leaf rust (GLR) was believed to be caused by *Phakopsora ampelopsidis* sensu auct (Hiratsuka 1935), non Dietel & P. Syd. (Dietel 1898), until Ono (2000) reported that a GLR fungus in Asia was *P. euvitis* Y. Ono, distinct from *P. ampelopsidis* Dietel & P. Syd. on *Ampelopsis* plants and *P. vitis* P. Syd. on *Parthenocissus* plants. A GLR fungus widely distributed in Southeast Asia and Australasia was identified as *P. euvitis* (Weinert et al. 2003), a name that has been corrected as *P. meliosmae-myrianthae* (Henn.) Y. Ono (Ono et al. 2012). A subsequent GLR study in Japan revealed an additional fungus, *P. montana* Y. Ono & Chatasiri, on *Vitis coignetiae* Planch. *Phakopsora montana* differs from *P. meliosmae-myrianthae* in the spermogonial and aecial host preference and aeciospore morphology (Ono et al. 2012). Identification of the Southeast Asian and Australasian GLR fungus with the East Asian *P. meliosmae-myrianthae* was based on the uredinial and telial morphology. However, since the uredinial and telial morphology does not distinguish between *P. meliosmae-*

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myrianthae and *P. montana*, the taxonomic identity of the Southeast Asian and Australasian GLR fungus with *P. meliosmae-myrianthae* becomes inconclusive. Furthermore, only cultivars derived from *V. vinifera* L. have been known as the hosts of the Southeast Asian and Australasian GLR fungus and, therefore, comparisons in the whole life cycle have been impossible.

Currently leaf rust is a minor fungal foliar disease of commercial table, wine and raisin grapes in temperate East Asia. It is important to realize, however, that the incidence and severity of major fungal foliar diseases of grapevines may change in the traditional viticulture regions under global climate change (Hayman et al. 2009; Fraga et al. 2012) and in newly developing tropical viticulture regions like Southeast Asia [Possingham 2008; Commins et al. 2012; Truong 2012; FAO database, http://faostat3.fao.org/faostat-gateway/go/to/download/Q/QC/E (accessed on 20 May 2013)]. Leaf rust is likely to become a major disease of grapevines grown under warm climate condition, particularly in Southeast Asia where commercial cultivars and their root-stocks are often severely infected by a GLR fungus (Fig. 1A, B).

Because the taxonomic identity is inconclusive and because the biological nature is largely unknown, we set up a study to morphologically and molecularly characterize the Southeast Asian and Australasian GLR fungus and to elucidate its native vitaceous host(s) and full life cycle. Results would benefit effective GLR management in the Southeast Asian and Australasian viticulture. This paper reports the similarity between the East Asian, Southeast Asian and Australasian GLR fungi in uredinial and telial morphology and the difference between these three fungi in the molecular property. It also discusses a possible life cycle of the Southeast Asian and Australasian GLR fungus in its native distribution range.

Samples of the GLR fungi for morphological and molecular systematic studies were taken from the specimens deposited in the Herbarium of Systematic Mycology, Ibaraki University (IBAR). The selected samples were from Japan, Thailand, Malaysia, Indonesia, East Timor and Australia. Seventy-five samples were used for the morphological study and fifty-nine for the molecular analysis (Fig. 3, Table S1).

Uredinial and telial morphology was examined, and measurements made, by the method described by Ono (2000) and Pota et al. (2013). Slide preparations were examined and photographed under an Olympus BH2 microscope (Olympus, Tokyo, Japan) with both bright-field and differential interference contrast (DIC) equipment. Mean value of each morphological measurement was calculated and Tukey Multiple Means Comparison test was performed using the software package SPSS (SPSS Japan, Tokyo, Japan) run on Microsoft Windows XP.

Uredinial and telial morphology were similar between the samples from different localities in Southeast Asia and Australasia (Tables 1 and 2). Uredinia were produced on the abaxial leaf surface, minute, loosely or densely grouped, and surround by paraphyses (Fig. 2A, B). The paraphyses were cylindrical, weakly to moderately incurved, 23–67 μm high and 6–24 μm wide. The wall was colorless, dorsally 0.4–3.0 μm thick and apically 0.9–3.6 μm thick (Table 1). The size and shape of paraphyses varied within individual specimens as well as among specimens. Urediniospores were short-pedicellate, obovoid to ellipsoid, or pyriform and 15–28 × 13–25 μm in size (Table 1, Fig. 2C). The wall was colorless to pale yellow, evenly 0.6–2.1 μm thick and completely echinulate. Urediniospore germ pores were hardly observable. When observed, they were six (rarely five or seven) and scattered over the wall (Fig. 2D, E); and rarely four germ pores were distributed in an equatorial zone. Telia were observed only in the Thai specimens. They were scattered or grouped on the abaxial leaf surface, subepidermal and composed of 2–6 layers of more or less linearly arranged teliospores (Fig. 2F). The teliospores at the uppermost layer were ellipsoid to oblong, angular and 8–22 × 5–16 μm in size. The apical wall was 0.9–2.3 μm thick and light brown; the lateral wall was 0.6–2.6 μm thick and almost colorless. The teliospores at the second layer and below were 9–23 × 6–15 μm in size. The apical wall was 0.9–2.1 μm thick and lateral wall was 0.9–2.3 μm thick (Table 2).

As previously noted, the Southeast Asian and Australasian GLR fungus was similar to *P. meliosmae-myrianthae* and *P. montana* in uredinial and telial morphology. In this study, uredinial paraphyses of the Southeast Asian and Australasian GLR fungus appeared thinner than those of the East Asian GLR fungi (Table 1, Fig. 2B), however, the apparent difference was not statistically significant. Tukey Multiple Means Comparison test of the urediniospore and teliospore measurements was not able to separate geographically defined groups among the samples of the Southeast Asian and Australasian GLR fungus (data not shown). That the Southeast Asian and Australasian GLR fungus was not separable from *P. meliosmae-myrianthae* and *P. montana* by uredinial and telial morphology

Fig. 1 – Grapevine leaf rust symptoms in Thailand. A: A rusted wine grape cultivar (infected leaf enlarged at bottom right). B: A root stock variety defoliated due to sever rust infection (infected leaf enlarged at bottom left).