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Gnomoniopsis smithogilvyi: identification, characterization and incidence of the main pathogen causing brown rot in postharvest sweet chestnut fruits (*Castanea sativa*) in Chile

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Abstract

We describe the first identification of *Gnomoniopsis smithogilvyi* causing brown rot on chestnut fruits in Chile, with an incidence of 4.8%. Previously, *Phomopsis castanea* (IMI 278057) was reported as the cause of the disease in Chile, but a molecular re-identification revealed that it corresponded to *G. smithogilvyi*. All chestnut fruits inoculated with the isolate *G. smithogilvyi* RGM 2903 developed brown rot symptoms on fruits.

Keywords Sweet chestnut · Incidence · Postharvest disease · *Phomopsis castanea*

The relative occurrence of brown rot in sweet chestnut fruits (*Castanea sativa*) was evaluated during March to August 2018 to 2020, in Chile. A total of 31851 fruits, from the cultivars: Bouche Rouge, Marrone di Castel Borello, Marrone di Chiusa Pesio, Marrone di Città di Castello, Marrone di Cuneo, Marrone di Marradi, and Marrone di Val di Susa were collected from two packing houses located in Ñuble, Chile, representing orchards in the center-south growing areas. Asymptomatic fruits were surface sterilized in 70% (v/v) ethanol for 1 min, cut in halves, and incubated in moist chambers (5 d) at 24 °C under light-darkness cycles of 12:12 h. After incubation, kernel tissues that developed brown rot and white to brown mummification (Fig. 1A) and light-brown cirri emerging from the acervuli (Fig. 1B) were cultured on potato dextrose agar (Difco™ PDA) and incubated at 24 °C for 7 d. On PDA, cultures grew as white cottony colonies with concentric rings that turned yellowish over time (Fig. 1C); black conidiomata (158–200 µm) (Fig. 1D) and ellipsoidal hyaline conidia of 6.3 ± 0.6 (5.3–7.8) $\times 2.5 \pm 0.3$ (1.9–2.9) µm (Fig. 1E) were exuded in gelatinous matrices forming light-brown cirri on the colony surface. These characters agree with the morphological descriptions for *G. smithogilvyi* Shuttlew (Synon. *Gnomoniopsis castaneae* Tamietti) (Maresi et al. 2013; Lione et al. 2019; Shuttleworth et al. 2012, 2018). The average incidence of this postharvest pathogen among cultivars in Chile was 4.8% (Table 1). Similar studies carried out in other countries point to wide variability in that there is a wide variability in the incidence of brown rot caused by *G. smithogilvyi* (reported as *G. castaneae*), ranging from from 3–93%, varying with the orchard and year of evaluation and influenced by climatic conditions, such as the maximum or average monthly temperatures (Dennert et al. 2015; Lione et al. 2015; Shuttleworth et al. 2013).

Two representative isolates from this study were deposited in the Chilean Collection of Microbial Genetic Resources (CChRGM) and registered with the collection codes RGM 2903 and RGM 2904. Molecular identification of these isolates was carried out by isolating their genomic DNA using the Wizard® Genomic DNA Purification Kit (Promega), followed by sequencing of the ITS and EF-1α DNA regions with primers ITS1/ITS4 (White et al. 1990) for ITS and EF1-728F (Carbone and Kohn 1999) and EF2 (O'Donnell et al. 1998) for EF-1α. Sequencing was performed at Macrogen (Seoul, Republic of Korea). Sequences were assessed for quality and consensus sequences assembled using Sequencher v5.4.6 software (Gene Codes Corporation, MI) and submitted to GenBank (ITS: MT413428-MT413429; EF-1α:

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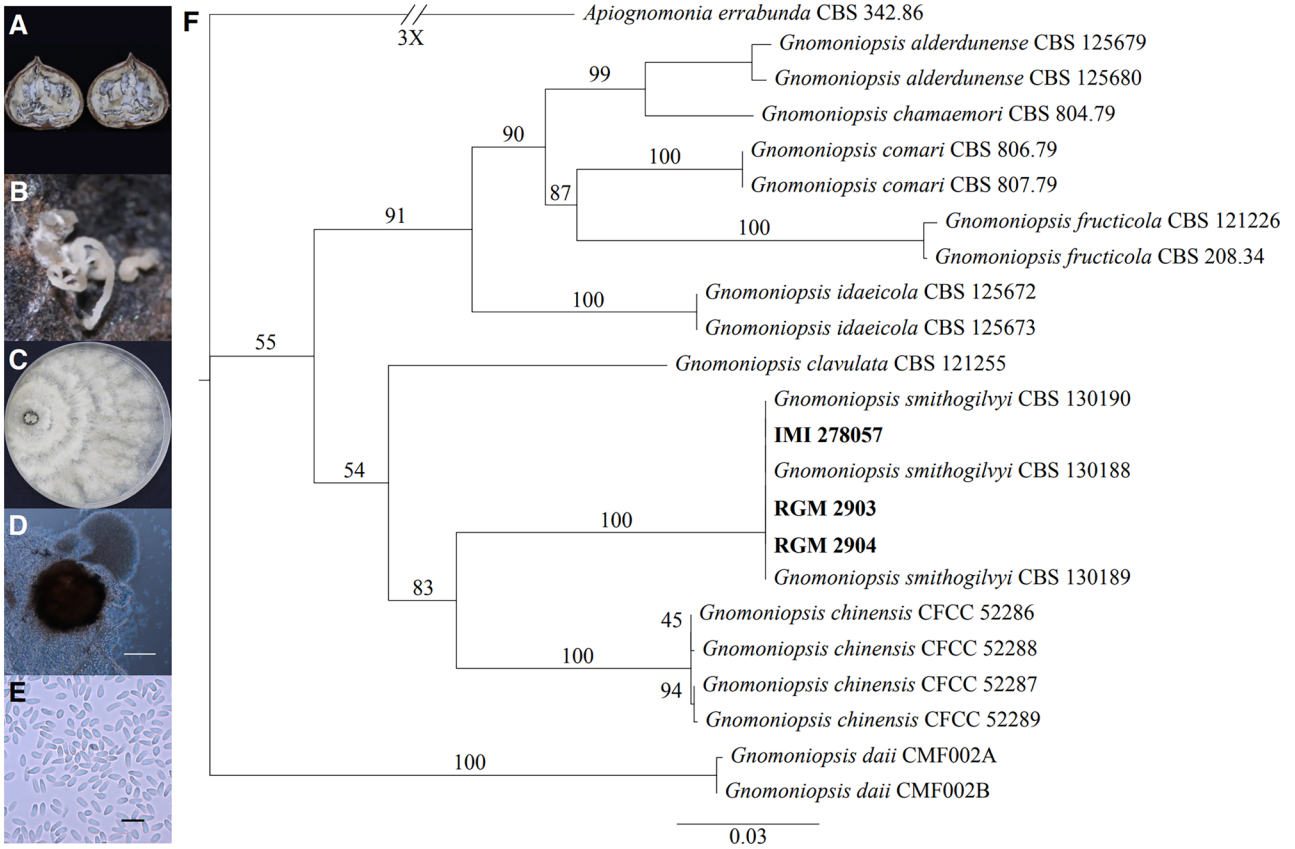

Fig. 1 Isolation and identification of *Gnomoniopsis smithogilvyi* from sweet chestnut fruits in packing houses in Ñuble Region, Chile. (**A**) fruits showing brown rot; (**B**) cirrus emerging from fruits; (**C**) colony of *Gnomoniopsis smithogilvyi* RGM 2903 growing on PDA; (**D**) microscopic observation of an acervulus showing conidia exudates (40×magnification; bar = 100 µm); (**E**) microscopic observation of conidia (100×magnification; bar = 10 µm); (**F**) phylogenetic tree resulting from a maximum likelihood analysis of combined sequence data from ITS and EF-1α DNA regions for *Gnomoniopsis* species; numbers in the nodes represent ultrafast bootstrap values. The strain *Phomopsis castanea* described by Montealegre and González (1986) was reported in their publication with the code IMI 278059, which has a typographical error. The correct code according to CABI is IMI 278057, which was used throughout this work

MT435530-MT435531, respectively). The consensus tree was obtained from a maximum likelihood analysis in the IQ-TREE webserver (Trifinopoulos et al. 2016) using concatenated sequence data from the ITS and EF-1α DNA regions for the *Gnomoniopsis* isolates and those of the reference strains (Dennert et al. 2015). The substitution model was set in automatic for each partition dataset (ITS and EF-1α) and the analysis was run with 1000 ultrafast bootstrap replicates (substitution model for each partition was ITS: K2P + I and EF-1α: TIM2 + F + G4). Both isolates grouped together with *G. smithogilvyi* CBS 130190 (*ex-type*) in a single clade, showing 100% of ultrafast bootstrap support in the maximum likelihood-derived tree. Thus, morphological and molecular information confirmed the affiliation of strains RGM 2903 and RGM 2904 to *G. smithogilvyi* species (Fig. 1F), making the first identification of this species in Chile. However, this is not the first report of this postharvest pathogen.

Table 1 Incidence of the postharvest pathogen *Gnomoniopsis smithogilvyi* on seven cultivars of chestnut fruits from packing houses of Ñuble Region, Chile

	Chestnut cultivar							
	Bouche Rouge	M. di Castel Borello	M. di Chiusa di Pesio	M. di Città di Castello	M. di Cuneo	M. di Marradi	M. di Val di Susa	Total
N° fruits analyzed	2430	5849	2245	4417	8356	3594	4960	31851
Incidence (%)	3.7	4.1	5.6	4.6	6.5	4.7	4.4	4.8*

*Average incidence

Previously, *Phomopsis castanea* Sacc. was determined as the causal agent of brown rot symptoms on sweet chestnut fruits in Chile (Montealegre and González 1986), but due to the similarity of the symptoms caused by *P. castanea* (Washington et al. 1999) and *G. smithogilvyi*, there was confusion about which was the main causal agent of brown nut rot (Smith and Ogilvy 2008; Maresi et al. 2013). The isolate of *P. castanea* reported by Montealegre and González (1986) was morphologically identified and deposited under the code IMI 278057 in the CABI culture collection (Surrey, UK). It remained there for 35 years without further revision of its taxonomical affiliation until this present study. The ITS region of IMI 278057 was sequenced with primers TW81/AB28 (Curran et al. 1994) and the nucleotide sequence (MZ854073) showed 100% identity with each of the *G. smithogilvyi* strains obtained in this work (RGM 2903 and RGM 2904). It also had phylogenetic relationships with *G. smithogilvyi* species in a single clade with 100% of ultrafast bootstrap support (Fig. 1F) and consequently was re-identified as *G. smithogilvyi*.

Pathogenicity testing was undertaken on thirty asymptomatic sweet chestnut fruits collected directly from sweet chestnut trees in Santa Rosa Experimental Station, Chillán, Chile. The fruits were inoculated with a single disk (1 mm diameter) of freshly growing mycelium which was excised from a 7-day-old PDA culture of the strain RGM 2903. This was inserted in an artificial wound made with a sterile metal cork borer in the insertion zone between the peduncle and involucre. Inoculated fruits were incubated for 7 d at 25 °C in the dark. As a control, 20 fruits were treated similarly, but using 1 mm disks of sterile PDA. Only fruits inoculated with strain RGM 2903 presented brown rot symptoms when examined after 7 days, whilst the control did not. It was possible to re-isolate the fungus from all inoculated fruits. Morphological characteristics of the colonies and conidial morphology were identical to the strain RGM 2903.

In this work, we have reported the average incidence of the postharvest pathogen *Gnomoniopsis smithogilvyi* on seven cultivars of chestnut (*Castanea sativa*) during a three-year survey, confirmed the presence of *G. smithogilvyi* as the causal agent of brown rot in sweet chestnut fruits in storage in Chile, and reassessed the taxonomic position of the strain IMI 278057 by molecular means, thereby identifying it as *G. smithogilvyi*.

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Declarations

Conflict of interest The authors have no conflict of interest.

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