

First report of *Pseudoidium* aff. *neolycopersici* in Indonesia

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Abstract Tamarillo was found to be heavily infested with powdery mildew in Bali, February 2012. The cause of the disease, *Pseudoidium* aff. *neolycopersici*, was characterised based on the morphology and molecular phylogenetic analysis of the internal transcribed spacer sequence. This is the first report of *P.* aff. *neolycopersici* on tamarillo in Indonesia.

Keywords Plant pathogen · Powdery mildew · Tamarillo · Taxonomy · Indonesia

Solanum betaceum (syn. *Cyphomandra betacea*) (English: Tamarillo or Tree Tomato, Indonesian: Terung Belanda) is a small tree belonging to the family Solanaceae, originating from South America. The tamarillo tree is 3–7.5 m tall, leaves ovate, heart-shaped at the base and pointed at the apex, bearing egg-shaped berries with pointed ends that are arranged in clusters near the young shoots. In Indonesia, tamarillo is planted as a backyard venture for its edible berries. Tamarillo was probably introduced to Indonesia by the Netherlands in 1941. Australia and New Zealand have been recognised as exporting countries of tamarillo fruit (Morton 1987).

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Powdery mildew (PM), causing serious defoliation, is recognised as a principal disease infecting tamarillo at locations where the plant is grown. Other fungi causing disease on tamarillo include *Sclerotinia sclerotiorum* and *Ascochyta* sp. (Morton 1987). During the survey of phytopathogenic fungi in February 2012, we found high infestation of PM on the leaves of tamarillo in Bedugul Botanic Garden and its surrounding area (Bali). The leaves were colonised by amphigenous, grayish-white, irregular and circular patches of mycelia (Fig. 1). Conidia were abundant on both sides of the leaf surface. The infected leaves became yellow and showed necrotic spots or blight. Heavy infection was mainly found on older leaves, and no PM mycelia or conidia were found on the berries.

Morphological observation was conducted following the method described by Meeboon et al. (2013). *Anamorph* (Fig. 1) — *mycelium* amphigenous, effuse, white; *hyphae* 4–7 μm wide, hyaline, thin-walled; *hyphal appressoria* in opposite pairs, occasionally solitary, moderately lobed, 3–7 μm diam.; *conidiophores* erect from top of mother cell, (50–)54–95(–114) \times (6–)7.5–10(–11) μm , *foot-cells* subcylindrical, straight to often curved at the base, (20–)22–40(–45) \times (6–)7.5–10(–11) μm , followed by 1–2 shorter cells or a cell about as long as the foot-cell or even longer, forming conidia singly or in short false chains; *conidia* ellipsoid-ovoid, subcylindrical, (25.5–)28–35(–38) \times (10.5–)12–17(–17.5) μm ; *germ tubes* simple, short, straight, cylindrical. Teleomorph was not observed.

Genomic DNA was extracted from the PM colony on the leaf using the chelex method as described in Hirata and Takamatsu (1996). Specimens were deposited at the Mie University Mycological Herbarium (MUMH), Japan (MUMH5562). Molecular phylogenetic analysis based on the sequence from the ITS rDNA region was conducted to identify the PM on tamarillo to species level. This region was amplified using primer pairs of ITS5 (White et al. 1990) and p3 (Kusaba

Fig. 1 *Pseudoidium* aff. *neolycopersici*. **(a)** symptoms on leaf (arrows); **(b)** conidium with germination tube (arrow); **(c)** hyphal appressoria (arrow); **(d)** conidiophores and conidia. Bars=15 μ m **(b, c)** and 30 μ m **(d)**

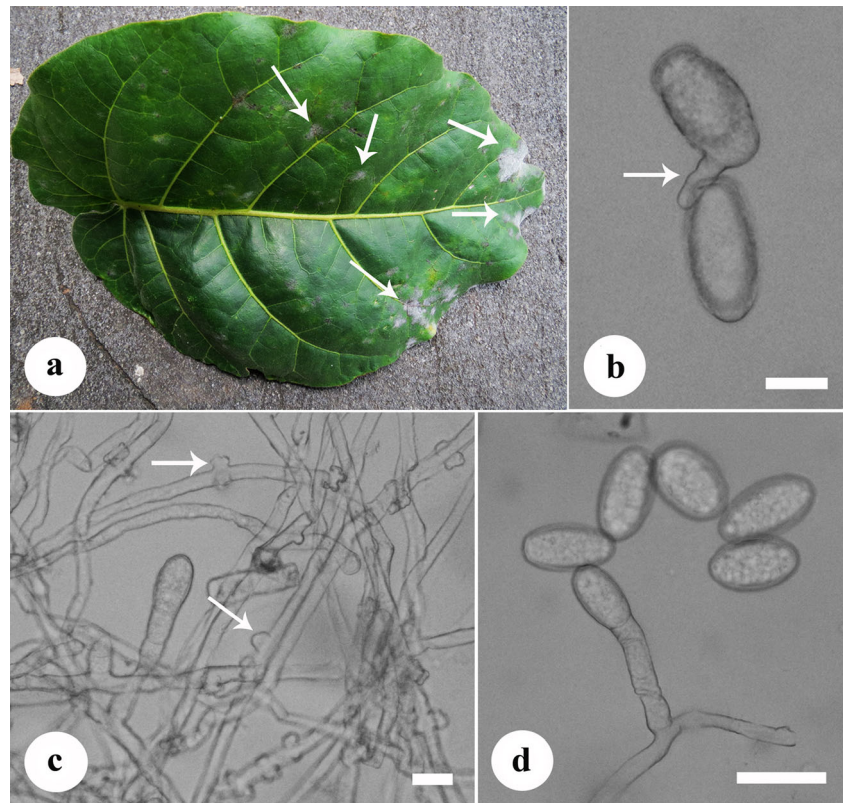
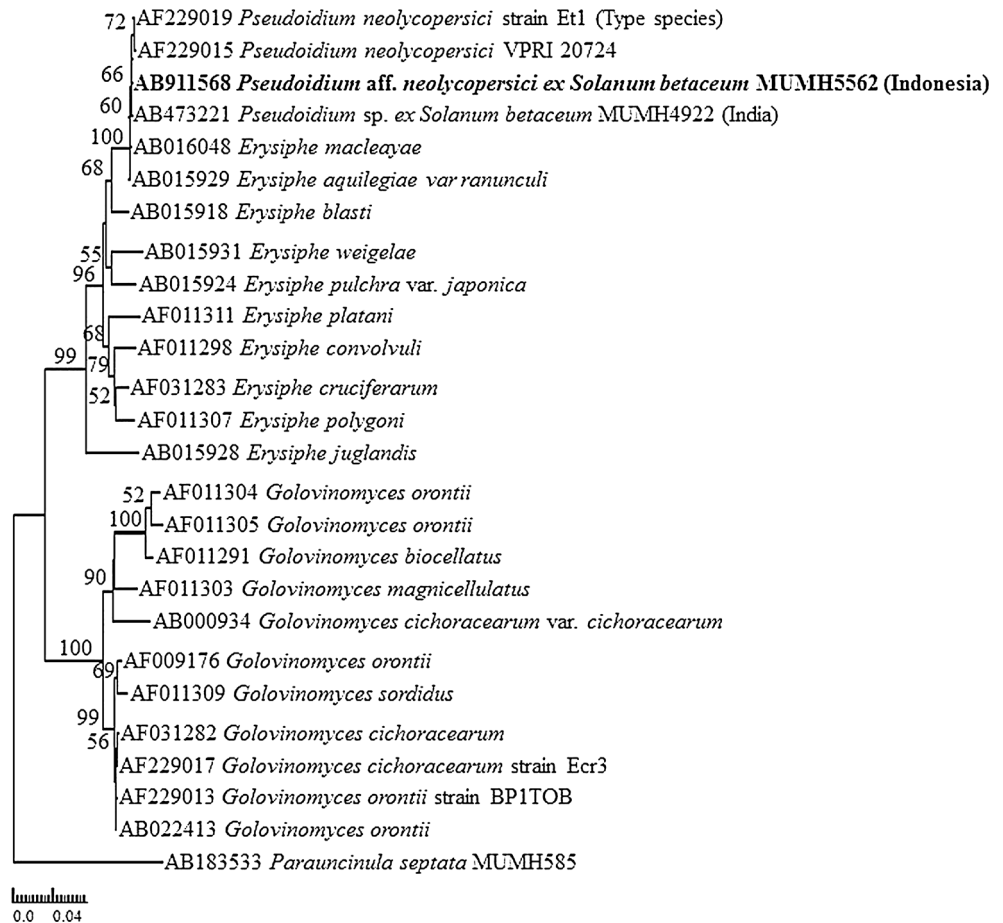


Fig. 2 Phylogenetic tree inferred from ITS rDNA sequence showed the placement of *Pseudoidium* aff. *neolycopersici* (AB911568) among PM species. The tree is rooted with the outgroup sequence of *Parauncinula septata* (AB183533). Branch lengths are indicated (scale bar). Bootstrap values ≥ 50 % are shown above internodes



and Tsuge 1995) for the first amplification, and PM-specific primer sets ITS5/PM6 and PM5/p3 for the second amplification (Takamatsu and Kano 2001). The primer pair of ITS1–ITS4 (White et al. 1990) was used for sequencing. Raw sequences were trimmed and assembled using ChromasPro 1.7.6 (<http://technelysium.com.au/>). The new DNA sequence was deposited to the GenBank/EMBL/DDBJ databases under the accession number AB911568. Multiple alignment of the new sequence with closely related PMs was conducted using MUSCLE (Multiple Sequence Comparison by Log-Expectation) implemented in MEGA (Molecular Evolutionary Genetics Analysis) version 6.0 (Tamura et al. 2013). Phylogenetic analysis was conducted using the Neighbour–Joining (NJ) method in MEGA 6.0. All parameters were set as default. The strength of the internal branches of the phylogenetic tree was tested with bootstrap analysis using 1,000 replications.

Pairwise sequence alignment showed that the ITS sequence of the PMs on tamarillo from Indonesia and India were identical (data not shown). In the phylogenetic tree, both sequences nested together with *Pseudoidium neolycopersici* on *Solanum lycopersicum* (Solanaceae) with 66 % bootstrap support (BS). Sequence alignment of the PM on tamarillo with the holotype of *P. neolycopersici* strain Et1 (AF229019) showed only one base difference (data not shown). This clade nested together with *E. aquilegiae* var. *ranunculi* (AB015929) and *E. macleayae* (AB016048) to form a monophyletic clade with 100 % BS. *Erysiphe aquilegiae* var. *ranunculi* (anamorph: *Oidium clematidis*) was reported worldwide on various genera belonging to the family Ranunculaceae and on *Catharanthus roseus* (Apocynaceae), while *E. macleayae* was only found in temperate regions (China, Japan, Germany, and Switzerland) on *Macleaya cordata* and *Papaver cambricum* (syn.: *Meconopsis cambric*) (Papaveraceae) (Braun and Cook 2012). According to the Systematic Mycology and Microbiology Laboratory Fungus-Host Database (<http://nt.ars-grin.gov/fungal-databases/fungushost/FungusHost.cfm>), four species of PMs were reported on *Cyphomandra*. These include *P. neolycopersici* (Pseudonym: *O. lycopersici*) on *C. betacea* from India; *Golovinomyces cichoracearum* (Syn. *E. cichoracearum*) on *C. betacea* and *C. fragrans* from France, Australia and New Zealand; *G. orontii* (syn.: *E. orontii*) on the same hosts as *G. cichoracearum* from New Zealand; and *Leveillula taurica* (Anamorph: *Oidiopsis sicula*) on *C. betacea* from Guinea (Africa). The current PM on *S. betaceum* found in Indonesia apparently resembles the morphological characteristics of *Pseudoidium* (teleomorph: *Erysiphe*) due to its conidia formed singly or in short false chains with solitary appressoria (Braun and Cook 2012).

The PMs on tamarillo from India were determined by Sharma et al. (2011) and Baiswar et al. (2009, 2013) as *O. lycopersici* and *Oidium* sp. [*neolycopersici*], respectively. However, following the recent taxonomic status of both

names (Braun and Cook 2012), they are now recognised as *P. neolycopersici*. This study clearly showed a close relationship between *P. neolycopersici* and the PM on tamarillo, although with a somewhat low bootstrap support (Fig. 2). It is possible that both PMs diverged recently, and is in the process of speciation. However, evidence is limited to prove that both PMs belong to different species, therefore, we named the PM on tamarillo as *P. aff. neolycopersici*. Braun (2012) discussed the taxonomical status of the PM on tamarillo. Although this fungus was morphologically indistinguishable from *P. neolycopersici*, and was unable to infect tomato and several species of *Solanum*, the taxonomical status of this fungus is still unclear (Braun 2012). Molecular phylogenetic analysis of 28S and IGS (intergenic spacer) rDNA regions may clarify the taxonomical status of the PM on tamarillo (Glawe 2008). This is the first report of *P. aff. neolycopersici* on tamarillo in Indonesia.

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