

A new tobamovirus infecting Hoya spp.

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Received: 20 Aug 2020. Published: 23 Sep 2020. Keywords: Hoya tobamovirus 2, ornamental plant import

During 2019/20, a batch of 6,000 young plants of different *Hoya* spp. (family: *Asclepiadaceae*) was grown in a German nursery. The plants had been originally imported from Thailand and a large proportion developed symptoms consistent with viral infection, e.g. ringspots, necrotic lesions, and irregular chlorosis or chlorotic leaf spots (Fig. 1).

The plants were analysed by electron microscopy and non-enveloped rodshaped particles were observed, suggesting infection with a tobamovirus (Fig. 2A). Virions showed a cross-reaction with a *Youcai mosaic virus* antiserum (DSMZ, Germany) in immunosorbent electron microscopy (Fig. 2B) but there was no reaction with other tobamovirus antisera. Following mechanical transmission, *Nicotiana benthamiana* and *N. tabacum* developed chlorotic lesions four weeks post inoculation. Total RNA was extracted from infected leaf material and a generic tobamovirus RT-PCR was performed using the Tob-Uni 1 and Tob-Uni 2 primers of Letschert *et al.* (2002). Resulting amplicons of c. 800 bp were Sanger sequenced in both directions (Microsynth Seqlab, Germany). The sequences showed 69.4% nucleotide (nt) identity to *Hoya chlorotic spot virus* (HoCSV; KX434725) (Adkins *et al.*, 2018) indicating the presence of a novel tobamovirus, provisionally named Hoya tobamovirus 2 (HoToV2).

Two of the samples (JKI2001336 and JKI2001337) were sequenced using MinION sequencing as described by Gaafar et al. (2019). In sample JKI2001336, HoToV2 was detected whilst in sample JKI2001337, HoToV2 and Tomato spotted wilt virus were detected in a mixed infection. Pairwise alignment using Clustal Omega (1.2.2) showed that the amino acid (aa) sequences of the movement protein (MP) of the two isolates of HoToV2 share 99.7% aa identity to each other and 52.3% identity to HoCSV. The coat protein sequences of the two isolates are identical and share 67.3% aa identity to HoCSV. Phylogenetic analyses grouped both proteins within the tobamovirus genus close to HoCSV (Fig. 3). Comparison of the nt sequences of HoToV2 isolates with a previous sequence from a hoya plant infected with a tobamovirus collected in 2014 showed 98.8% and 98.9% nt identities (Richert-Pöggeler et al., 2018), indicating that this virus has been present in hoya for some time in Germany. The partial sequences of HoToV2 isolates JKI2001336, JKI2001337 and 2014 were deposited in GenBank (Accession Nos. MT750216, MT901191 and MT750217,

respectively).

According to ICTV species demarcation criteria a new species can be considered when the nt sequence identity is <90% based on the whole genome nt sequence (Adams *et al.*, 2017). Based on the current results, we propose HoToV2 as a new species within the genus *Tobamovirus*, family *Virgaviridae*.

Acknowledgements

Y. Gaafar and K. Richert-Pöggeler contributed equally to this report. The authors would like to thank Joe Ehlers for excellent technical assistance.

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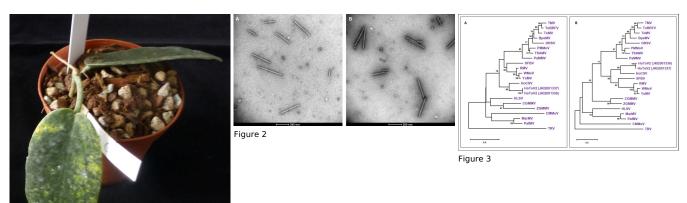


Figure 1

 To cite this report: Gaafar YZA, Richert-Pöggeler KR, Hartrick J, Lüddecke P, Maaß C, Schuhmann S, Wilstermann A, Ziebell H, 2020. A new tobamovirus infecting Hoya spp.. New Disease Reports 42, 10. http://dx.doi.org/10.5197/j.2044-0588.2020.042.010

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