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First Report of Nerine latent virus Infecting Green-Tinge Spiderlily (*Hymenocallis speciosa*) in Indonesia

Laporan Pertama *Nerine latent virus* yang Menginfeksi Bunga Bakung Laba-laba (*Hymenocallis speciosa*) di Indonesia

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ABSTRACT

In 2024, a composite leaf sample showing irregular yellowing symptoms was collected from green-tinge spiderlily (*Hymenocallis speciosa*) in Bogor Regency, Indonesia. The sample (isolate SLPB2) was tested positive for carlavirus infection using a degenerate primer pair targeting the partial coat protein and nucleic acid binding protein regions, and then the amplicon was sequenced. BLAST analysis of the sequence indicated that the isolate belongs to the species Nerine latent virus (NeLV, Carlavirus latensnerinis). This solate shared 83.5%–99.8% nucleotide identity with other NeLV isolates available in GenBank, confirming its assignment to the species. The genus *Carlavirus* (family *Betaflexiviridae*) comprises several economically important spesies that infect a wide range of horticultural crops, including ornamental plants. Further disease surveillance and biological characterisation are needed to assess the distribution and impact of NeLV on *H. speciosa*, and to inform the development of effective management strategies. To the best of our knowledge, this is the first report of NeLV infecting green-tinge spiderlily, both in Indonesia and worldwide.

Keywords: carlavirus, degenerate primer, NeLV, ornamental plant, RT-PCR

ABSTRAK

Pada tahun 2024, sampel daun komposit yang menunjukkan gejala menguning tidak teratur dikumpulkan dari tanaman bunga bakung laba-laba (*Hymenocallis speciosa*) di Kabupaten Bogor, Indonesia. Isolat sampel (SLPB2) dideteksi menggunakan *degenerate primer* carlavirus dengan target sebagian gen protein selubung dan *nucleic acid binding protein.* Sampel yang terdeteksi positif kemudian disekuen dan dianalisis. Analisis BLAST pada sekuens yang diperoleh menunjukkan bahwa isolat tersebut merupakan anggota dari nerine latent virus (NeLV, Carlavirus latensnerinis). Isolat SLPB2 memiliki identitas nukleotida sebesar 83.5%–99.8% dengan isolat NeLV lain yang tersedia di GenBank, sehingga mengonfirmasi identitas sampel tersebut. Genus Carlavirus (famili Betaflexiviridae) beranggotakan virus-virus penting secara ekonomi yang menginfeksi berbagai macam tanaman hortikultura, termasuk tanaman hias. Survei penyakit dan karakterisasi biologi lebih lanjut diperlukan untuk menilai sebaran serta dampak NeLV pada *H. speciosa*, sekaligus menyusun strategi pengelolaan yang komprehensif. Studi ini merupakan laporan pertama mengenai NeLV yang menginfeksi bunga bakung lili di Indonesia dan di tempat lain.

Kata kunci: carlavirus, *degenerate primer*, NeLV, RT-PCR, tanaman hias

Spiderlilies (*Hymenocallis* spp.; Asparagales: Amaryllidaceae) are herbaceous, bulbous perennials widely grown as ornamental plants in public parks and along main roadsides. Previous studies have reported viruses from three genera infecting spiderlilies: Orthotospovirus (Calla lily chlorotic spot virus, Capsicum chlorosis virus, Hippeastrum chlorotic ringspot virus, Impatiens necrotic spot virus, and Tomato spotted wilt virus), Carlavirus (Lily symptomless virus), and Potyvirus (Lycoris mild mottle virus and Narcissus degeneration virus) (Liu *et al.* 2012; Sastry *et al.* 2019; Chao *et al.* 2025). Foliar ringspot symptoms caused by orthotospovirus in spiderlilies are typically distinctive and can be diagnosed visually in the field (Chao *et al.* 2025). By contrast, carlavirus infections are frequently latent or associated with only mild symptoms, although yellowing, chlorotic striping, and leaf distortion may occur (Singh *et al.* 2005; Sastry *et al.* 2020).

In 2023, a symptomatic leaf sample was collected from a green-tinge spiderlily (*Hymenocallis speciosa*) growing near a railway station in Bogor, Indonesia, and the resulting isolate was designated SLPB2. Symptomatic leaves showed irregular yellowing (Figure 1). Total nucleic acid was extracted using CTAB method (Doyle and Doyle 1987), with a minor modification in which the RNAse treatment step was omitted (Nurulita *et al.* 2025). The degenerate carlavirus primer pair, AlcarF (5'-TGC TGC YTT TGA TAC YTT CGA T-3') / Poty1 (5'-GGA TCC CGG GTT TTT TTT TTT TTT V-3') was used to amplify the partial coat protein and nucleic acid binding protein regions of carlaviruses with an expected amplicon size of 715 bp (Langeveld *et al.* 1991; Cremer *et al.* 2021). One-step RT-PCR cocktail consisted of 12.5 µL of 2× DreamTaq Green PCR master mix (Thermo-Fisher Scientific, Memphis, US), 1 µL of 10 µM of each primer, 1 µL of ribonuclease inhibitor (40 U μL^{-1}), 0.5 µL (200 U μL^{-1}) of reverse transcriptase (NZYtech, Portugal), 2 µL of total RNA, and nuclease-free water to make the final volume to 25 µL. Thermocycling conditions were 45 °C for 60 min (cDNA synthesis), 94 °C for 1 min (pre-denaturation), 35 cycles of 94 °C for 30 s (denaturation), 56 °C for 60 s (annealing), and 72 °C for 60 s (extension), and a final extension step of 72 °C for 10 min. PCR amplicons with expected size were sequenced by 1st BASE Malaysia through PT Genetika Science Indonesia.

Raw sequences were trimmed and assembled into a consensus contig, which was compared against the GenBank nucleotide database using BLAST. Selected reference sequences were retrieved and aligned with the consensus using MUSCLE algorithm in Geneious Prime® 2025.1.3 software (Biomatters Ltd., New Zealand). Pairwise sequence identities were calculated and visualized using the Sequence Demarcation Tool (SDT) v1.3 (Muhire *et al.* 2014). A maximum-likelihood phylogenetic tree was inferred in MEGA 12 software (Kumar *et al.* 2024) using the adaptive bootstrap (5.50 thresholds and Tamura-Nei substitution model).

The contig from the SLPB2 isolate comprised 651 nucleotides (nt) spanning partial coding regions of the coat protein (CP) and nucleic acid binding protein (NABP) genes. The CP gene is one of the regions used to distinguish species within the family *Betaflexiridae* (ICTV 2011). The SLPB2 isolate shared 83.5%–99.8% nucleotide sequence identity with nerine latent virus (NeLV) isolates from several countries (Figure 2), well above the species demarcation threshold for *Betaflexiridae* (> 72% nt or > 80% aa identity in the CP or polymerase gene; ICTV 2011), confirming its assignment to NeLV. The SLPB2 sequence was deposited in GenBank under accession number LC860499.

In the maximum-likelihood phylogenetic tree, the SLPB2 isolate clustered within the NeLV clade (Figure 3) and grouped most closely with an NeLV isolate previously reported from *Hippeastrum* sp. in Indonesia, suggesting that NeLV may be established across multiple Amaryllidaceae hosts in the region. One NeLV isolate from South Africa (KY769699.1) formed a separate sub-clade, indicating some geographic genetic structure within NeLV that warrants further investigation.

NeLV has a documented host range largely confined to the Amaryllidaceae, with natural infections previously reported in *Nerine bowdenii*, *Narcissus* sp., *Hippeastrum* sp., *Lycoris* sp., *Crinum asiaticum*, *Haemanthus albiflos*, *Scadoxus multiflorus, Agapanthus* sp., and *H. littoralis* (Maat *et al.* 1978; Wylie and Jones 2012; Chen *et al.* 2016; Monger and Eden 2019; Sastry *et al.* 2020; Heo and Choi 2021; Nurulita *et al.* 2025). The detection of NeLV in *H. speciosa* in Bogor extends this host range to a further Amaryllidaceae species and is consistent with the close phylogenetic affinity between the SLPB2 isolate and the *Hippeastrum* isolate previously reported from Indonesia (Nurulita *et al.* 2025), pointing to the likely circulation of NeLV among co-located amaryllid

Figure 1 Symptom of Nerine latent virus infecting *Hymenocallis speciosa* leaves.

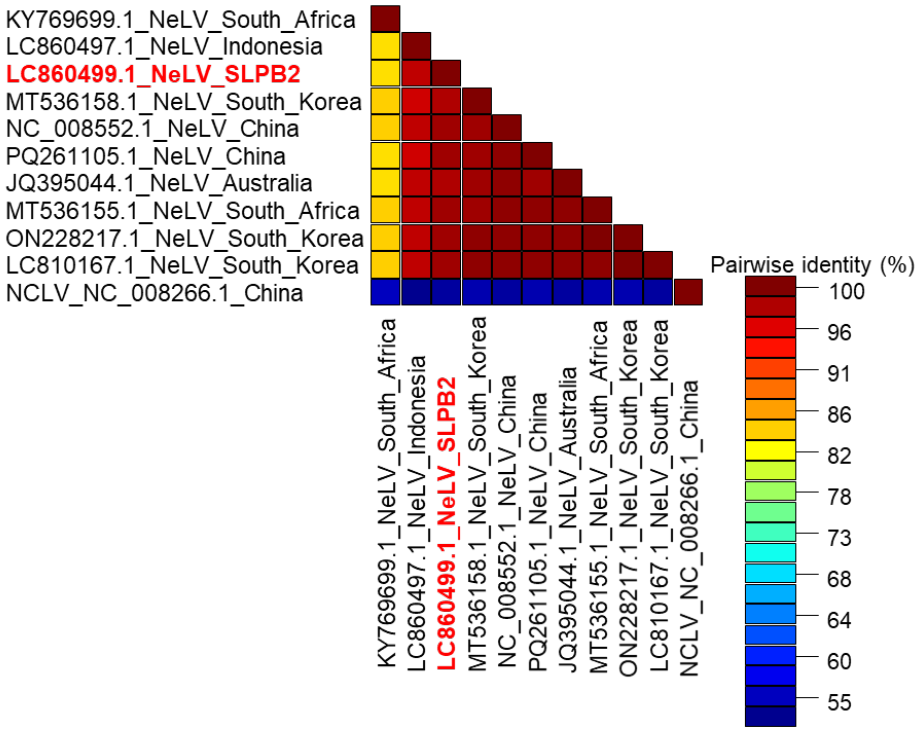

Figure 2 Pairwise identities plots of Nerine latent virus (NeLV) aligned by MUSCLE and displayed by Sequence Demarcation Tool software. Colored heat map of pairwise identities corresponds to the colors on the scale. Narcissus common latent virus (NCLV; genus Carlavirus) was used as an outgroup.

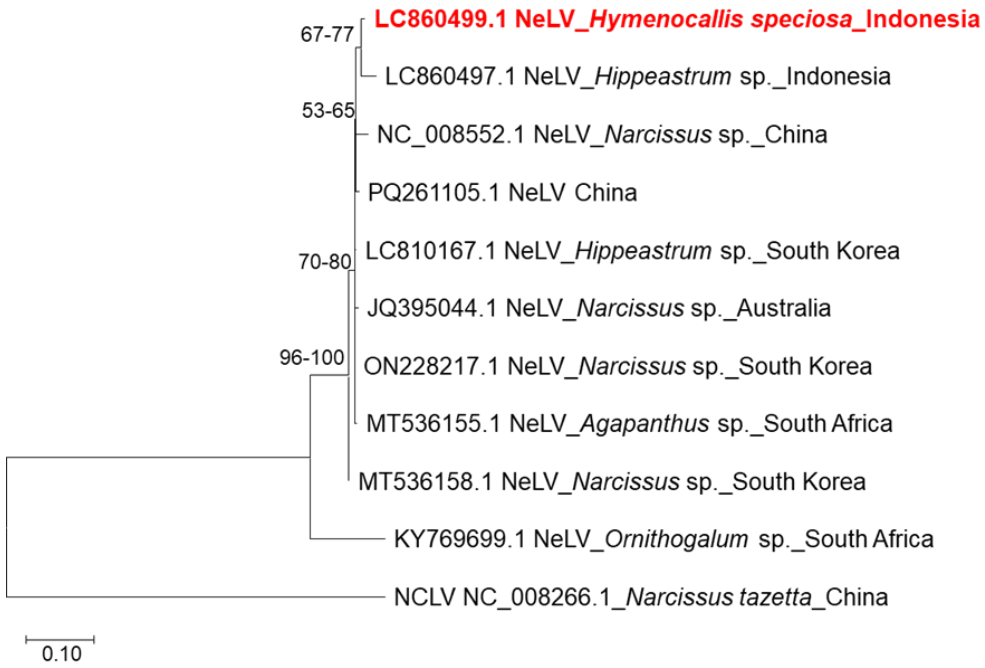

Figure 3 Maximum-likelihood phylogram of Nerine latent virus (NeLV) infecting *Hymenocalis speciosa* from Indonesia and other NeLV isolates infecting various hosts in several countries. The scale bar indicates the number of substitutions per site. Narcissus common latent virus (NCLV; genus Carlavirus) was used as an outgroup to root the trees.

hosts in the region. Although carlavirus infections in ornamental amaryllids are generally latent or associated with only mild foliar symptoms (Sastry *et al.* 2020), they are epidemiologically significant. For example, NeLV is transmitted in a non-persistent manner by aphids and is readily disseminated through vegetatively propagated planting material such as bulbs and offsets (Maat *et al.* 1978; Ng and Perry 2004), both of which are common pathways in the international ornamental trade. Because *H. speciosa* is widely planted as an amenity species along roadsides and in public parks in Indonesia, infected plants in these environments

may act as inoculum reservoirs for nearby cultivated amaryllid collections and commercial bulb production. Targeted surveys across geographic regions and amaryllid hosts, combined with whole-genome characterisation of Indonesian isolates and biological assays to assess symptom expression and yield or quality impacts under local conditions, are therefore required to clarify the epidemiology of NeLV and to inform virus-tested propagation and phytosanitary measures for the Indonesian ornamental sector.

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