

## EVOLUTIONARY RELATIONSHIP OF UKRAINIAN ISOLATE OF ODONTOGLOSSUM RINGSPOT VIRUS

*Odontoglossum ringspot virus (ORSV) is one of the most dangerous viruses of decorative orchids. Infection caused by this virus can reduce the phenotypic differences of orchids in collections, and as a result lead to their total exhaustion. In this study, the possible origin of the Ukrainian isolate of ORSV from the collection of O. V. Fomin Botanical Garden of the Taras Shevchenko National University of Kyiv was investigated. The properties of nucleotide sequences of the RNA-dependent RNA-polymerase (RdRp) and coat protein gene of ORSV were examined. RNAs of ORSV isolated from leaves of *Cymbidium hybridum* from O. V. Fomin Botanical Garden's collection were amplified through RT-PCR and sequenced. Obtained sequences were compared at the nucleotide level with ORSV isolates accessible within the GenBank. Phylogenetic analysis showed that the studied ORSV isolate may have a common root with a few South Korean isolates.*

**Keywords:** orchids, ORSV, RT-PCR, phylogenetic analysis.

**Introduction.** Tropical and subtropical orchids are cultivated in numerous countries around the world and stay one of the most important commercial crops within the world's botanical industry. Viral infections of orchids are known since the middle of the XX century. Numerous viruses influence the quality of orchids, and, subsequently, significantly decrease their commercial and esthetical value. About 50 viruses responsible for a wide range of orchid diseases have been currently described [1–3]. However, Odontoglossum ringspot virus (ORSV, *Tobamovirus*) is one of the most common as it can be rapidly transmitted, contributing significant spread of infection among susceptible plants [4–7]. This infection is transmitted exclusively in artificial ecosystems basically during vegetative proliferation or mechanically. Viral contamination affects the physiological state of orchids developed in greenhouses [5]. The loss of ornamental flowers can not only damage the development of industrial production in Ukraine, but also reduce the diversity of plants in collections, since tropical orchids are mainly grown in greenhouses as part of botanical gardens collections. Cases of ORSV infection of tropical orchids in the collections of Ukrainian botanical gardens have been known since the late 90s of the last century [8, 9]. The aim of the study is to determine the origin of Ukrainian isolates of ORSV in orchid collection from O. V. Fomin Botanical Garden of the Taras Shevchenko National University of Kyiv.

**Materials and Methods.** Leaves of *Cymbidium hybridum* with necrotic and chlorotic striping symptoms were collected from the O. V. Fomin Botanical Garden of the Taras Shevchenko National University of Kyiv. Virus identification was carried out using DAS-ELISA with polyclonal antisera specific to ORSV (Prime Diagnostics, The Netherlands). Total RNA was extracted using the RNeasy Plant

Mini Kit (Qiagen, Germany). Reverse transcription-polymerase chain reaction (RT-PCR) was accomplished using the SuperScript II kit (Invitrogen, USA). To amplify the coat protein (CP) gene of ORSV, RT-PCR was used in one reaction tube with the pair of primers: ORSV-F-5'AC-GCACAATCTGATTGATTGAA-3', ORSV-R-5'-TATCAA CGTTATTTCTAAATAT-3' (Expected product size 528 bp) [10]. The amplified products from *Cymbidium hybridum* samples were purified using MinElute Gel Extraction Kit (Qiagen, Great Britain). The resulting PCR products were sequenced using Applied Biosystems 3730x1 DNA Analyzer using Big Dye terminators, version 3.1 (Applied Biosystems, USA). Phylogenetic analysis was carried out using Mega X and IQTREE software.

**Results and Discussion.** Orchid plants from the collection of O. V. Fomin Botanical Garden of the Taras Shevchenko National University of Kyiv with symptoms of mosaic, spots, necrosis, and chlorosis have been identified. Mosaic symptoms on leaves were an alternation of dark and light zones, which shaped a mosaic design. Necrotic symptoms or ring spotting were the arrangement of little (0.5–2 mm) and medium-sized (2–4 mm) dark spots of circular and oval shape on the leaf edge, occasionally encompassed by a limit border (Fig. 1 A). Yellowing of the leaf edge in combination with the arrangement of negligible leaf spots was a reasonably common symptom. Symptoms of mosaic, yellowing, and ring necrosis can occur in plants affected by ORSV [11–15]. ORSV was detected by ELISA with appropriate antisera in all selected samples of orchid plants. Subsequently, *Cymbidium hybridum* leaves with symptoms typical for ORSV infection, which was also confirmed by DAS-ELISA, were used to isolate total RNA. Rod-shaped virions were found in the sap of plant leaves with typical viral symptoms (Fig. 1 B).

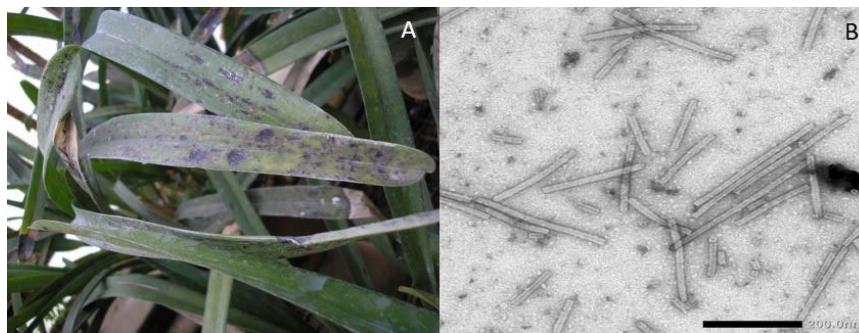
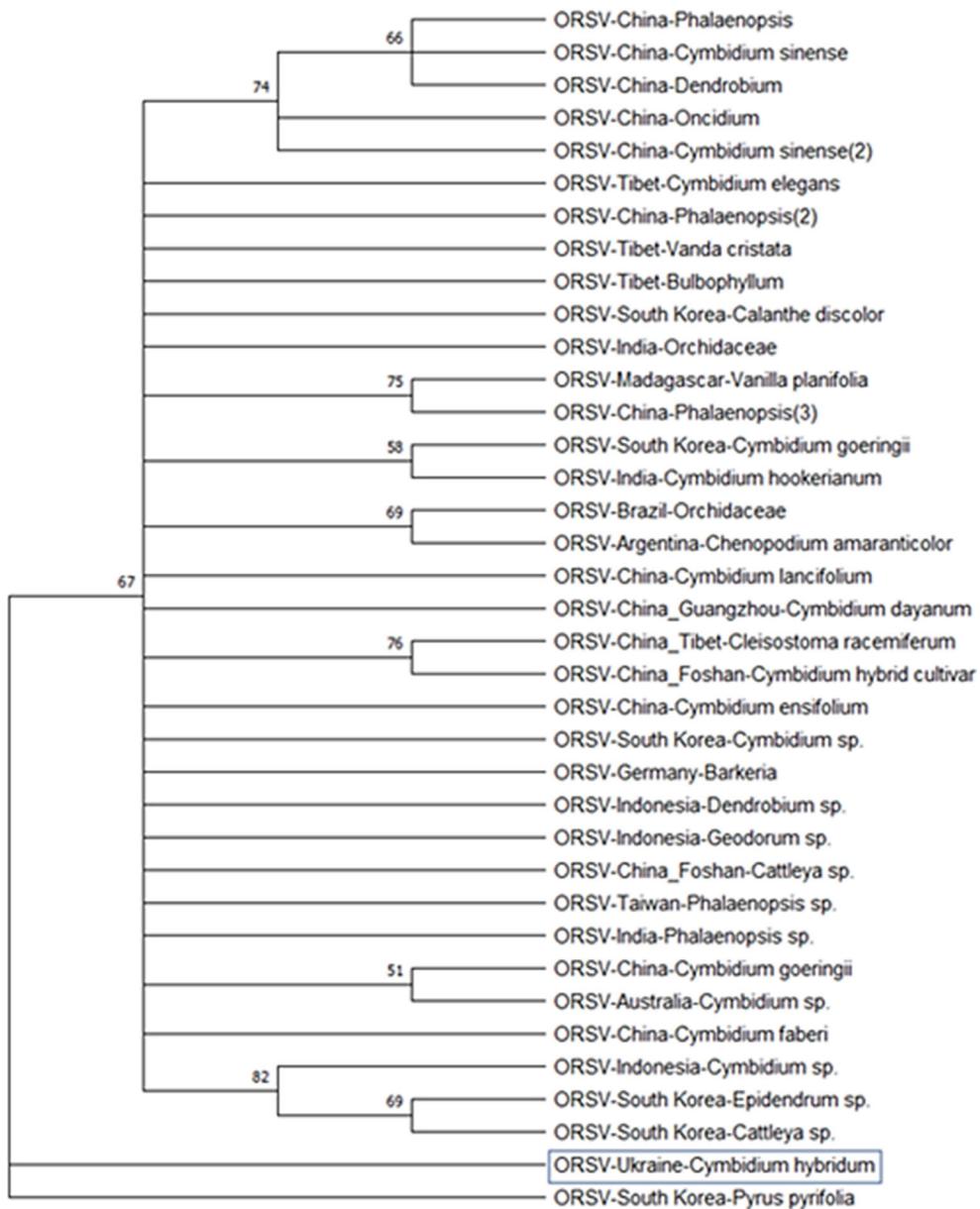


Fig. 1. Leaf of *Cymbidium hybridum* with symptoms of black necrotic spots. (A). Electron micrograph of rod-shaped viral particles in plant sap of *Cymbidium hybridum*. Bar – 200 nm (B)

The Gene sequence of the Ukrainian ORSV isolate (ORSV-Ukraine) was compared with other known isolates of ORSV accessible from the GenBank. To determine the conceivable root of the ORSV isolates tested in Ukraine, a phylogenetic investigation based on nucleotide sequences was conducted. The Maximum Likelihood strategy was chosen as a discrete strategy, which was performed using the Kimura

two-parameter model. As can be seen from the phylogenetic species affinity tree (Fig. 2), Ukrainian isolate (ORSV-Ukraine) and one of the ORSV isolates from South Korea were clustered together proposing their conceivable common root. Also, the Ukrainian isolate was compared with other to-bamoviruses to establish its species similarity (Fig. 3).



**Fig. 2. Phylogenetic analysis of Ukrainian ORSV isolates based on a nucleotide sequence of the coat protein gene.**  
The phylogenetic tree (50 % majority rule consensus tree) is constructed using the ML method  
(Kimura two-parameter model, bootstrap 10000 replications)

The next step was related to the construction of a phylogenetic tree based on ORSV polymerase gene, the results of which revealed the similarity of the Ukrainian isolate with the Mexican one (Fig. 4).

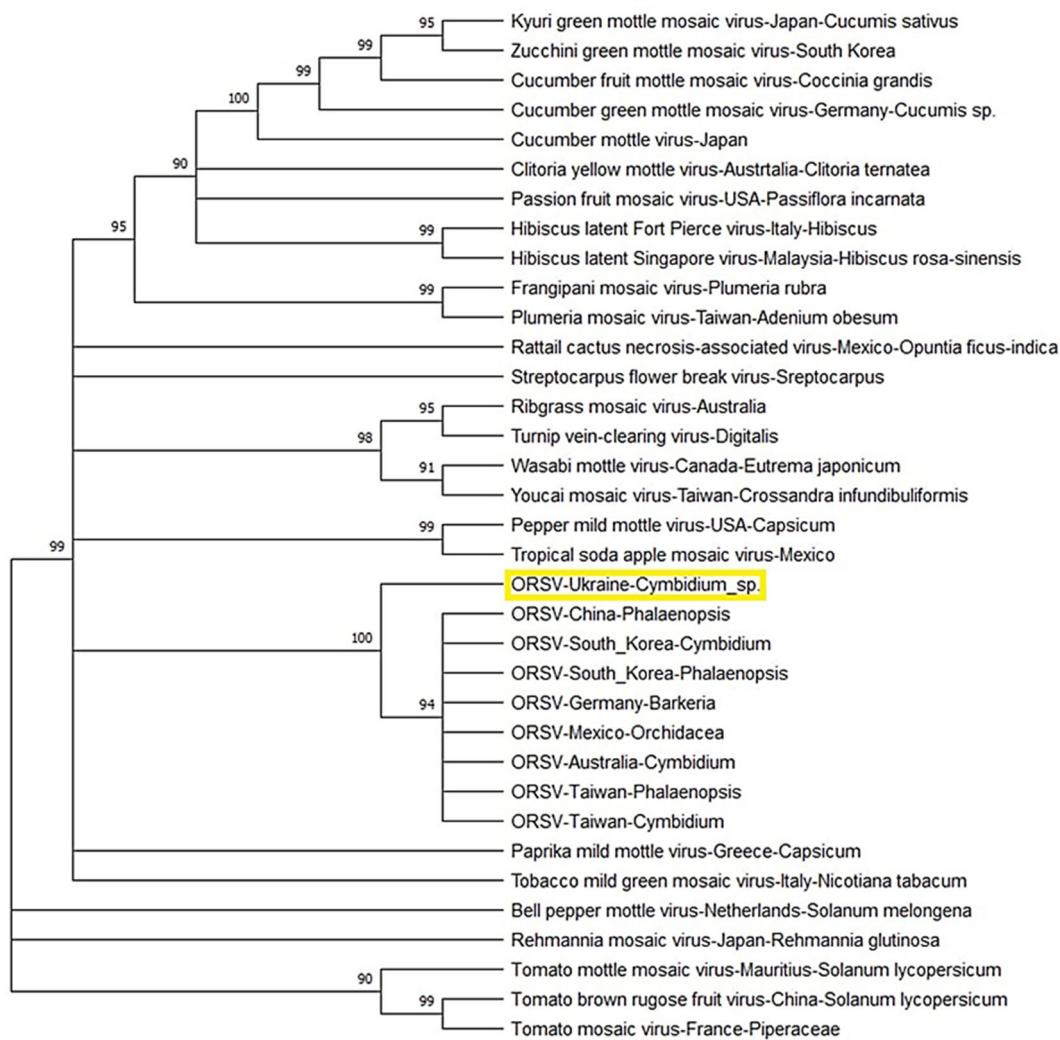


Fig. 3. Phylogenetic analysis of Ukrainian ORSV isolates based on the nucleotide sequence of coat protein gene.

The tree is constructed using the ML method

(90 % majority-rule consensus tree, Kimura 2-parameter model, bootstrap 10000 replications)

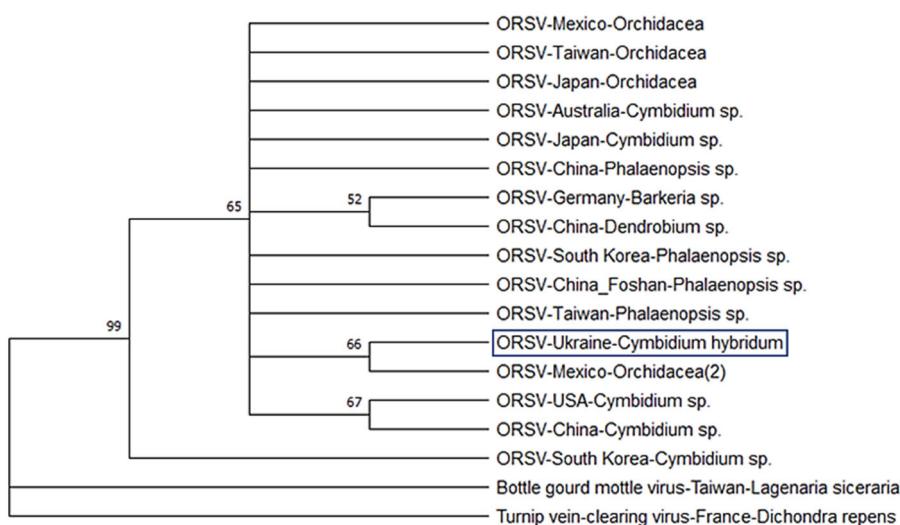


Fig. 4. Phylogenetic analysis of Ukrainian ORSV isolates based on the nucleotide sequence of the RdRp gene.

The tree is constructed using the ML method

(50 % majority rule consensus tree Kimura 2-parameter model, bootstrap 10000 replications)

ORSV is a unique virus [16]. It is a recombinant; its replicase genes are closest to those of from *Brassicaceae*-infecting tobamoviruses, whereas its movement and coat protein genes are closest to those from *Solanaceae* [17]. It's known from previous studies that all existing ORSV isolates share high homology with each other at nucleotide level [18, 19]. Besides this the nucleotide sequence of the ORSV capsid protein is highly conserved [20]. Thus, in subsequent studies the most variable virus sequences such as the C-terminal region of the ORSV should be used as the most informative markers for phylogenetic analysis.

In conclusion, it was determined that ORSV isolates collected from *Cymbidium hybridum* in Ukraine shared high homology at the nucleotide level with other known ORSV isolates based on CP and RdRp genes/protein sequences. ORSV isolates showed similarity to the virus isolates from South Korea. Since the plants from tropical orchids' collections in botanical gardens in Ukraine were originally imported from eastern countries, we suggest that ORSV could invade orchid collections with imported plant material with a further spread in Ukraine.

#### References

- Zettler FW, Wistler GC, Elliot MS, Ko N-J. Viruses of orchids and their control. *Plant. Disease.* 1990; 74: 621-626.
- Lee Ch-H, Zheng Y-X, Jan F-J. The Orchid-Infecting viruses found in the 21st century Orchid Biotechnology III. 2017; 145-164 : [https://doi.org/10.1142/9789813109223\\_0009](https://doi.org/10.1142/9789813109223_0009)
- Ahmad S, Chen G, Huang J, Yang K, Hao Y, Zhou Y & Peng D. Beauty and the pathogens: A leaf-less control presents a better image of *Cymbidium* orchids defense strategy. *Frontiers in Plant Science*, 2022; 1; 1-13.
- Pant RP, Baljeet Kaur. Virus diseases of orchids. *RASSA Journal of Science for Society.* 2022.; 4 (1): 1-15.
- Ajijikutira PA, Lim-Ho CL, Woon MH, Ryu KH, Chang CA, Loh CS, Wong SM. Genetic variability in the coat protein genes of two orchid viruses: *Cymbidium* mosaic virus and *Odontoglossum* ringspot virus. *Arch. Virol.* 2002; 147: 1943-1954.
- Wong SM, Chng CG, Lee YH, Tan K, Zettler FW. Incidence of *Cymbidium* Mosaic and *Odontoglossum* Ringspot Viruses and Their Significance in Orchid Cultivation in Singapore. *Crop Prot.* 1994; 13:235-239, [https://doi.org/10.1016/0261-2194\(94\)90084-1](https://doi.org/10.1016/0261-2194(94)90084-1)
- Cánovas SE, Ballari MC and Nome CF. First report of *Cymbidium* mosaic virus and *Odontoglossum* ring spot virus in Argentina. *Australasian Plant Dis. Notes.* 2016; 11: 2. <http://dx.doi.org/10.1007/s13314-015-0189-7>.
- Koroteyeva HV, Polischuk VP. [Viral diseases in the orchid collection of Academician O. V. Fomin Botanical Gardens of Taras Shevchenko Kyiv National University] [Scientific Notes of NaUKMA]. 2001; 19(2): 399-400. Ukrainian
- Polischuk V, Koroteyeva G, Bysov A, Lavrentieva A. Spreading of virus infection in the orchid collection in Ukraine. *Plant science.* Sofia. 2007; XLIV (3) : 213-216.
- Chng CG., Wong SM., Mahtani PH, Loh CS, Goh CJ, Chung MC-M, Watanabe Y. The complete sequence of a Singapore isolate of *Odontoglossum* ringspot virus and comparison with tobamoviruses. *Gene.* 1996; 171: 155-161.
- Panjaitan MVT, Mahfut, Handayani TT, Wahyuningsih S. Various ORSV infection symptoms on *Ceratostylis* induction in orchid plants. *Journal of Sustainability Science and Technology.* 2021.1(2): 64-71.
- Manfut Identification and Efforts to Control Infection *Odontoglossum* ringspot virus (ORSV) on Orchid. *International Journal of Engineering, Science and Information Technology.* 2021, 1(1):25-29.
- Pearson MN, Cole JS. Further observations on the effects of *Cymbidium* mosaic virus and *Odontoglossum* ringspot virus on the growth of *Cymbidium* orchids [Electronic resource]. *Journal of Phytopathology.* 1991; 131(3); 193-198. – Available at: <https://doi.org/10.1111/j.1439-0434.1991.tb01187.x>
- Yamane K, Oyama K, Iuchi E, Ogawa H, Suzuki T, Natsuk T. RT-PCR detection of *Odontoglossum* ringspot virus, *Cymbidium* mosaic virus, and Tospoviruses and association of infections with leaf yellowing symptoms in *Phalaenopsis*. *Journal of Phytopathology.* 2008; 156: 268-273. <http://dx.doi.org/10.1111/j.1439-0434.2007.01352.x>
- Hu JS, Ferreira S, Xu MQ. Detection of *Cymbidium* Mosaic Virus, *Odontoglossum* Ringspot Virus, Tomato Spotted Wilt Virus, and Potyviruses Infecting Orchids in Hawaii, Department of Plant Pathology. 1993:464-468. [https://www.apsnet.org/publications/PlantDisease/BackIssues/Documents/1993Articles/PlantDisease77n05\\_464.PDF](https://www.apsnet.org/publications/PlantDisease/BackIssues/Documents/1993Articles/PlantDisease77n05_464.PDF)
- Dubs M C, Van Regenmortel MH. *Odontoglossum* ringspot virus coat protein: sequence and antigenic comparisons with other tobamoviruses. *Arch. Virol.* 1990; 115: 239-249.
- Gibbs A. Evolution and origins of tobamoviruses. *Phil. Trans. R. Soc. Lond.* 1999; B 354: 593-602. <http://doi.org/10.1098/rstb.1999.041>
- Sherpa A, Tusa B, Hallan V, Zaidi A. A Detection of *Odontoglossum* ringspot virus in orchids from Sikkim, India. *Australasian Plant Pathology – Australasian plant pathol.* 2006; 35: 69-71.
- Rashmi ER, Pant R, Baranwal V, Jain R. Detection of orchid viruses and molecular characterization of *odontoglossum* ringspot virus (ORSV) isolates. *Indian Council of Agricultural Research.* 2021; 91(8):
- Yoon JY, Chung BN, Choi SK. High sequence conservation among *Odontoglossum* ringspot virus isolates from orchids. *Virus Genes.* 2011; 42(2):261-7.

Надійшла до редколегії 12.05.22  
Отримано виправлений варіант 14.06.22  
Підписано до друку 14.06.22

Received in the editorial 12.05.22  
Received version on 14.06.22  
Signed in the press on 14.06.22

А. Пономаренко, студ.,  
Г. Коротєєва, канд. біол. наук,  
О. Андрійчук, канд. біол. наук  
Київський національний університет імені Тараса Шевченка, Київ, Україна

## ЕВОЛЮЦІЙНІ ВЗАЄМОВІДНОСИНИ УКРАЇНСЬКОГО ІЗОЛЯТУ ВІРУСУ КІЛЬЦЕВОЇ ПЛЯМИСТОСТІ ОДОНТОГЛОССУМУ

**Вірус кільцевої плямистості одонтоглоссуму (ВКПО)** є одним із найнебезпечніших вірусів декоративних орхідей. Захворювання, викликані цим вірусом, можуть призвести до зниження фенотипових відмінностей колекцій орхідей і, як наслідок, – їхнього повного виснаження. У цій роботі досліджувалося можливе походження українського ізоляту ВКПО, виділеного з рослин колекції Ботанічного саду імені О. В. Фоміна Київського національного університету імені Тараса Шевченка. Досліджено властивості нуклеотидних послідовностей РНК-залежної РНК-полімерази (RdRp) та капсидного білка ВКПО. РНК ВКПО виділяли з листків *Cymbidium hybridum*, відібраних із колекції Ботанічного саду імені О. В. Фоміна. Далі послідовності РНК вірусу були ампліфіковані за допомогою RT-PCR та секвеновані. Отримані послідовності порівнювали на рівні нуклеотидів із послідовностями ізолятів ВКПО, доступними в GenBank. При проведенні філогенетичного аналізу було виявлено, що досліджуваний ізолят ВКПО може мати спільній корінь із кількома південнокорейськими ізолятами.

Ключові слова: орхідні, ВКПО, ЗТ-ПЛР, філогенетичний аналіз.