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LETTER TO THE EDITOR

First detection of an invertebrate iridovirus in the daphnid, *Daphnia pulex*

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Summary. – *Daphnia pulex* from a small pond were infected by an iridescence virus. Infected daphnia differed from healthy ones due to the intense characteristic pink reflected iridescent color. Infected individuals collected in the field died in laboratory as a result of the iridoviral infection. Ultrastructural analysis using electron microscopy revealed highly abundant icosahedral virions in the cytoplasm of multiple types of tissue in the infected daphnids. The mean particle sizes were approximately 200 nm (n = 150) edge-to-edge and 185 nm point-to-point.

Keywords: daphnia; Daphnia pulex; iridovirus; electron microscopy

Iridoviruses are large double-stranded DNA viruses with comparatively large genomes. *(1)* These viruses are members of the order *Megavirales*, the so-called nucleocytoplasmic large DNA viruses (NCLDVs) that are globally distributed and cause lethal infections in mollusks, insects, mites, annelids, nematodes as well as in poikilothermic vertebrates *(2, 3)*. As iridoviruses are economically and ecologically significant, they are increasingly receiving research attention *(4, 5)*. In crustaceans the first representative iridoviral disease has been described in *Simocephalus expinosus* collected in Florida, USA *(6)*. Bergoin *et al.* (1984) then described diseased *Daphnia magna* (*Branchiopoda: Cladocera*) from salt-marshes in southern France *(7)*. It was shown that *D. magna* were infected with a virus most likely belonging to the family *Iridoviridae*. In 2016, Vávra *et al.* (2016) published light and electron micrographs of an iridovirus in *D. curvirostris* in the Czech Republic *(8)*. Infected crustaceans have an elongated body with extremely enlarged fat cells that are white and nontransparent. Because of this, the disease was called White Fat Cell Disease (WFCD) *(9)*. The disease has been reported in Belgium, England, France and the Netherlands *(10, 11)*. The full sequence of the genome of Daphnia iridescent virus 1 (DIV-1) causing in *D. magna* disease called WFCD was determined *(9)*. This short communication describes the first detection of an iridovirus in *Daphnia pulex* in Ukraine.

Diseased *Daphnia pulex* were collected near Kyiv city from a small pond, which was inhabited by mosquito *Aedes cantans* larvae. Pieces of the cut diseased *D. pulex* were fixed in 1% osmium tetroxide for 1 h 20 min and dehydrated in an increasing ethanol series (50–100%). Staining with 2% uranyl acetate was performed in 70% ethanol. Specimens were treated with acetone and embedded in Epon 812 resin. Ultrathin sections were examined using the JEMB-100 B electron microscope.

Daphnia pulex individuals infected with the iridovirus differed from healthy ones due to the intense characteristic iridescent pink color of the body. The iridescence was clearly visible under the binocular microscope throughout the carapace at all stages of development. Infected individuals died in laboratory as a result of a viral infection, but the iridescent color they had persisted for two to four hours after death.

Ultrastructural analysis using electron microscopy revealed highly abundant icosahedral virions in the cytoplasm of multiple types of tissue in the infected animals.

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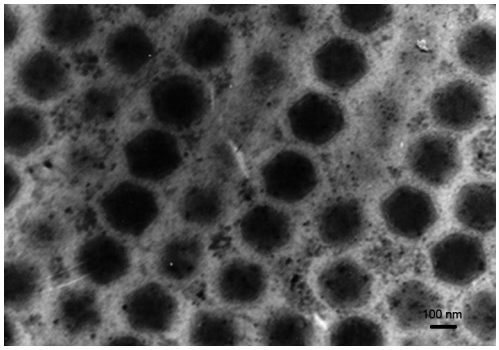

Fig. 1

Iridescent virus from Daphnia pulex.
Diameter of virions is 200 nm.

Paracrystalline arrays of virions were observed (Fig. 1). The virions consisted of an electron-dense core surrounded by an icosahedral capsid. These virions were similar to those observed in *D. magna* from Mediterranean salt-marshes *(7)*, as well as those identified in *D. curvirostris* in Czech Republic, as well as in other hosts infected with viruses of the family *Iridoviridae (1, 8)*.

The mean particle sizes were 200 nm (n = 150) edge-to-edge and 185 nm point-to-point. The virions in *D. pulex* were larger than those found in *S. expinosus* (136 nm edge-to-edge and 154 nm point-to-point) *(6)*, but smaller than the virions observed in *D. curvirostris* from the Czech Republic (about 243 nm) *(8)*.

Thus, the morphological features (large size of virions), the nature of the cytopathology, and the characteristic iridescence of iridoviruses indicate that this virus is an iridovirus. Nevertheless, the more precise taxonomic position of the virus will be known only after analysis of its genome. To date, 25 complete iridovirus genome sequences have been reported (NCBI, https://www.ncbi.nlm.nih.gov/). Only three of these are associated with crustaceans, the *Armadillidium vulgare (12)*, *Cherax quadricarinatus (13)* and *D. magna (9)* iridescent viruses. There is a necessity to investigate full genome of *D. pulex* iridovirus to show phylogenic relatedness among iridoviruses infecting crustaceans.

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