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Short Communication

Occurrence, pathology, and ultrastructure of iridovirus and cytoplasmic polyhedrosis viruses in daphnids from the Czech Republic





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1. Introduction

Iridoviruses (IVs; family Iridoviridae, genera Iridovirus and Chloriridovirus) and cytoplasmic polyhedrosis viruses (CPVs; family *Reoviridae*, genus *Cypovirus*) are well known virus types that cause diseases in insects, and in a few cases in other invertebrates. Iridoviruses derive their name from the iridescent blue/green color imparted to infected larvae by the accumulation of paracrystalline arrays of virions in infected tissues (Xeros, 1954). The IVs have been reported from larvae of many species belonging to the Lepidoptera, Coleoptera, and Diptera, as well as Orthoptera and Hymenoptera (Chinchar et al., 2005). Reports from crustaceans are rare, but IVs have been reported from terrestrial isopods (Isopoda), a few daphnids (Crustacea, Branchiopoda) and other non-insect invertebrates (Federici and Hazard, 1975; Federici, 1980; Williams et al., 2005). IVs typically have a broad tissue tropism that includes the epidermis, fat body, muscles, nerves, and reproductive tissues, but not the midgut epithelium, which most insect viruses infect. Following their discovery in insects, it was later realized that certain viruses that cause diseases in vertebrates, (certain frogs and fish), also are members of

ABSTRACT

Iridescent (IVs, family Iridoviridae, genus Iridovirus) and cytoplasmic polyhedrosis viruses (CPVs; family Reoviridae, genus Cypovirus) are well known in insects, with thirteen IV species recognized from various orders, and sixteen CPV species known from lepidopterans. In 1975, an IV and CPV were reported in the daphnid, Simocehpalus expinosus, in Florida, but other reported daphnid virus infections seem to be rare. Here we report infected daphnids from woodland and carp ponds in the Czech Republic, Daphnia curvirostris with an IV, and D. pulex and D. ambigua, with CPVs. This suggests these viruses are more common in daphnids, the rarity of reports due to few surveys.

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the family Iridioviridae (Williams et al., 2005). In contrast, CPVs are almost exclusively known from the midgut epithelium of lepidopteran larvae. There are no accepted CPV species other than those that attack lepidopteran larvae, for which sixteen species are recognized (Mertens et al., 2005), even though this type of virus is known to occur in chironomids (Federici et al., 1973), twenty species of mosquito larvae representing nine genera (Shapiro et al., 2005; Becnel and White, 2007), black fly larvae (Green et al., 2007), as well as the freshwater daphnid, Simocephalus expinosus (Federici and Hazard, 1975).

The IV infections are easily recognized in infected larvae by their typically blue/green iridescent color. CPVs on the other hand generally require dissection of infected larvae and examination of midgut epithelial cells by bright-field or phase microscopy, which reveals numerous polyhedral occlusion bodies in the cytoplasm (Payne, 1981). Virus presence in IV infections is confirmed by the presence of large (125-160 nm) icosahedral virions in the cytoplasm of infected cells, whereas in the case of CPVs, the virions are also icosahedral, but only ~60 nm in diameter, and typically occur free or occluded within a matrix of polyhedral protein coded for by the viral genome. Of interest is that mosquito larvae and daphnids typically breed in similar habitats, but there is no evidence that the same IV or CPV can infect both hosts.

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During a survey of crustaceans for microsporidian infections in woodland and carp ponds in the Czech Republic, we used the above signs of disease and techniques and detected IV and CPV infections in three species of daphnids. Because reports of these two virus diseases in daphnids are extremely rare, we describe our findings here, along with virion structural properties, revealed by conventional and advanced microscopy techniques.

2. Material and methods

2.1. Site, collection of infected specimens, microscopy methods

2.1.1. Daphnids with an iridovirus infection

The IV-infected daphnids were found in *Daphnia curvirostris* Eylmann 1878, inhabiting a permanent forest pool fed by ground-water near the town Přerov nad Labem, Central Bohemia Region, Czech Republic (50°.167N, 14.810E) (Fig. 1A). Specimens that appeared by eye to slightly opaque or were opalescent were investigated.

2.1.2. Daphnids infected with cytoplasmic polyhedrosis virus (CPV)

Two species of daphnids were found infected with CPVs (referred to here as CPV-I and CPV-II). CPV-I was found in *Daphnia pulex* Leyidig 1860, collected in October–November, 2013 in a semipermanent forest marsh (drying rarely in some years), near Běleč, Central Bohemia region, Czech Republic (50094 3'12.240"N, 14095 1'4.221"E; Fig. 2A). CPV-II was found in *Daphnia ambigua* Scourfield 1947, collected in the carp-breeding pond Dvorský, near Kařez, Central Bohemia, Czech Republic (490 97 49'0"N, 13 47' 0"E) in October 2015.

2.2. Transmission electron microscopy (TEM), tomography (ET) and Atomic force microscopy (AFM)

Live daphnids were examined under $100-200 \times$ power of the light microscope (LM) for the presence of viral crystals in the gut

or fat body. TEM, ET and AFM observations were done using sections of materials fixed in 2.5% glutaraldehyde in 2% cacodylate buffer, post-fixed with osmium, and embedded in Epon-Araldite blocks. Additional information is given in Supplementary Files (SF) 1–4.

3. Results

3.1. Tissue infection, prevalence and host reaction

Iridovirus. Infected specimens were slightly opaque with a bluish tint, but their behavior was normal. Such specimens represented an estimated 1% of the population based on macroscopic appearance of specimens in a dense population of daphnids sampled in June 2014. Enlarged fat cells were the site of infection in *D. curvirostris.*

CPV-I and CPV-II. These two isolates infected the gut epithelial cells of their hosts, and polyhedra could be seen at low magnification in cells of the first third to half of the gut in live hosts (Fig. 2B, SF2-Fig. 3A). CPV-I patent infections occurred in about 1% of adult *D. pulex* sampled (nearly 500 inspected). The CPV-II infection was very rare and only two *D. ambigua* specimens were found infected among hundreds examined. The behavior of CPV-infected specimens was the same as that of healthy daphnids.

3.2. Virus description and identity

3.2.1. Iridovirus infections

Ultrathin sections of fat cells of *D. curvirostris* revealed the presence of stages of iridovirus virogenesis, including the presence of virogenic stromae in which icosahedral virions were present. In more advanced stages of infection large virions, measuring 243.5 \pm 6.0 nm (n = 50) in diameter, were present in paracrystalline arrays (Fig. 1B and C). Developing and mature virions were characteristic of those belonging to the genus *Iridovirus*, being icosahedral with a dense core, and a putative lipid membrane



Fig. 1. Habitat, pathology, and ultrastructure of an iridovirus found in populations of the daphnid, *Daphnia curvirostris*, in a woodland pond in the Czech Republic. (A) Woodland pond from which iridovirus-infected daphnids were collected; (B–F) electron micrographs illustrating characteristic pathology and ultrastructure of the virions: (B) virogenic stroma in an adipose cell (note the small cluster of assembled virions within the stroma); (C) clusters of virions in paracrystalline arrays, which inpart a bluish tint to infected daphnids; (D–F) illustration of the icosahedral ultrastructure of virions that characterize this virus type. (R1 = diameter of capsid – 243.5 ± 6.0 nm; R2 = diameter of the membrane envelope – 208.6 ± 5.9; R3 = central core – 183.6 ± 7.4; n = 50). Bar Fig. B = 2 μ m, C = 2 μ m, D = 500 nm, E and F = 100 nm.

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