

## NOTE

### Iridovirus Infection in Mayfly Larvae

**Key Words:** *Ecdyonurus torrentis*; morphology; electron microscopy; Iridovirus; Ephemeroptera.

An iridovirus infection was found in the mayfly larvae of *Ecdyonurus torrentis* Kimmins, 1942 (Ephemeroptera: Heptageniidae) in the Czech Republic. A virus infection with icosahedral particles was found in a group of mayflies collected in the Blanice river in South Bohemia. From larvae collected through October and November 1999, several larvae were dissected and inspected using the electron transmission microscope. Pieces of the infected tissue were fixed in Karnovsky fixative at 4°C overnight. After several washes in PBS (pH 7.4) tissues were postfixed in 1% osmium tetroxide, dehydrated through an acetone series, and embedded in Durcupan. Specimens were examined in a JEOL 1010 TEM at an accelerating voltage of 80 kV. Several of these samples contained the virus particles and are reported here.

Hemocytes adhering to the infected tissue contained multiple virogenic stroma with groups of virus particles and particles in various stage of assembly. Thick-walled tubular structure of up to 3 µm long with a less dense or empty central channel were also observed (Fig. 1). In some virogenic stroma the tubules were closed with rounded ends and the virus particle appeared to be leaving the widened tubules (Fig. 4). Tubules of lesser diameter were also observed in some parts of the virogenic stroma in infected cells (Fig. 2) but the presence of these structures did not appear to be related to the number of virus particles of their degree of maturation.

Fat body appeared to be a principal site of the replication of the virus. Virus particles were hexagonal in cross-section, measuring  $129.6 \pm 0.32$  nm in diameter (range 120–150 nm,  $n = 40$ ) (Fig. 3). The electron-dense central core, 100 nm in diameter, was closed in a lighter staining outer shell, 15 nm thick. Virions were present in irregular groups in the cytoplasm of infected cells where a fine granulation was observed (Figs. 2 and 5). Virus particles showed varying degrees of electron density. Around virions there was an empty area within the cytoplasm of infected tissue (Fig. 5). Some lysosomes, mitochondria and multiple virogenic stroma of varying size with groups of virus particles

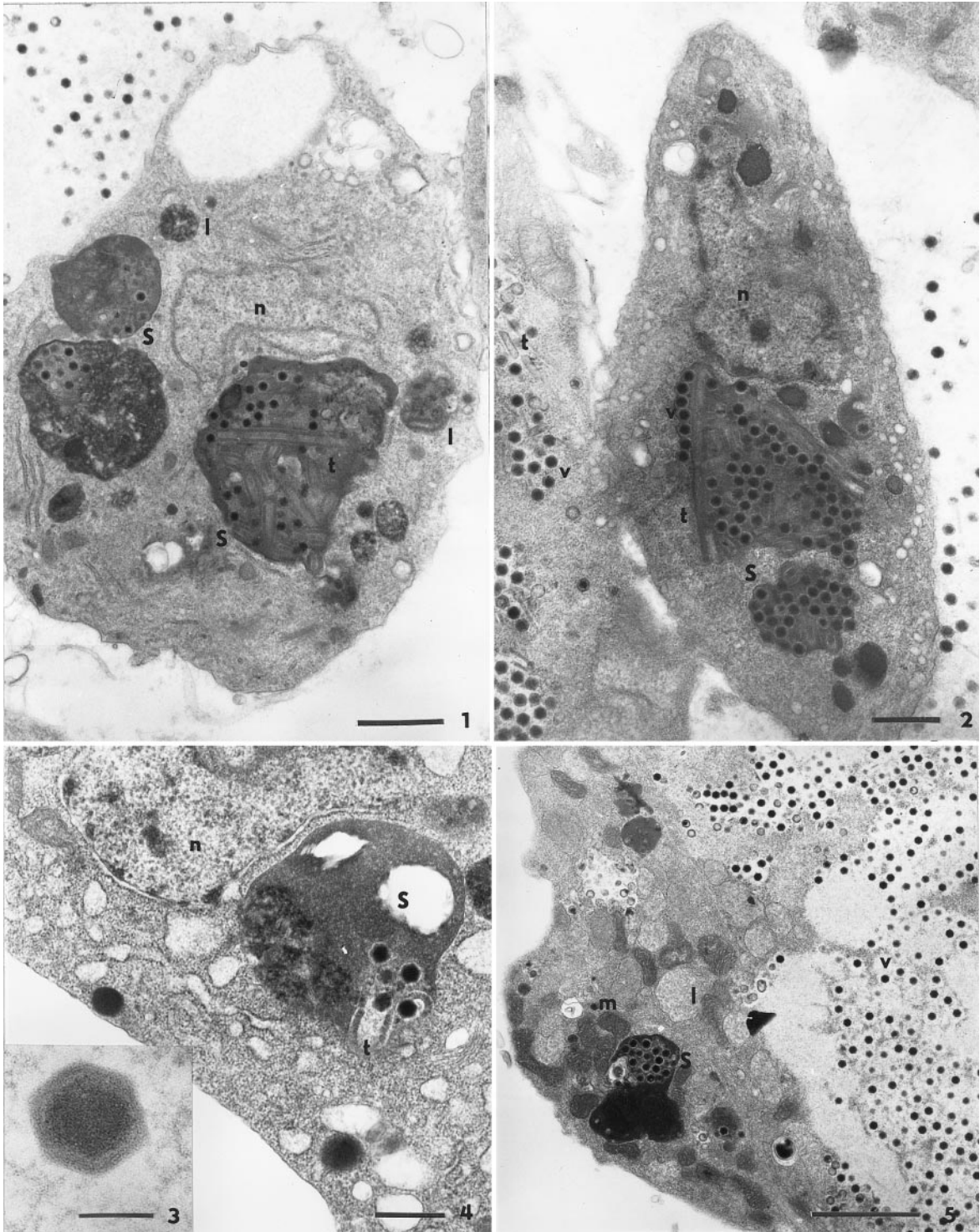
were present in the remaining area of cytoplasm adjacent to the cell wall (Figs. 2 and 5).

Infected animals did not differ from healthy ones. Infected larvae did not show the characteristic iridescent symptom of patent infection as well as laboratory rearing mosquito larvae infected with iridescent virus (Mullens *et al.*, 1999). Inapparent iridescent virus infections have been reported in natural populations of black flies and in laboratory studies with *Aedes aegypti* (Williams, 1993; Marina *et al.*, 1999). It was shown that in large population of black flies larvae only 12 individuals exhibited iridovirus symptoms. PCR and insect bioassay techniques were used to show that up to 37% of apparently healthy larvae were covertly infected; the prevalence of the infection fluctuated during the year (Williams, 1993, 1995). In natural infected *Chironomus* larvae iridovirus infection was described without apparent iridescent symptoms in which the virus particles were surrounded by external fibrils (Stoltz *et al.*, 1968). Authors suggested that the external fibrils prevent formation of a paracrystalline arrays which are responsible for the iridescence. In our material there was not any evidence of the external fibrils.

Invertebrate iridescent viruses have been reported from several insect orders (Weiser, 1965; Kelly, 1985). They can be experimentally transmitted by injection into different insects (e.g., *Galleria mellonella*) and often cause a massive patent infection. According to the size and shape of the virus it is possible that this isolate pertains to the *Iridovirus* genus. Tubular structures (Figs. 1 and 2) described previously in several insect larvae (Stoltz, 1973; Buchatsky and Raikova, 1978; Anthony and Hall, 1970) are very abundant in our material, and they are of different size and shape compared with formerly observed tubules. The reason and fate of these tubules are unclear; we only presume that they are involved in virus development.

As far as we know there is only one iridovirus isolate IV 26 described from mayflies (Williams, 1996). It has been mentioned as not a valid isolate due to lack of characterization informations and this isolate has now been removed from the official list of tentative iridescent viruses (Williams, 1998; Murphy *et al.*, 1995).

We found covert iridovirus infection which does not cause the patent disease and support the idea that the possibility of inapparent infection should not be ig-



**FIG. 1.** Hemocyte of *E. torrentis* with a series of developing virogenic stroma (S) close to the host cell nucleus (n). Other minute centers are in lysosomes (l). The stroma initially show a fine electron-dense mass, later a granulation and finally the formation of mature viral particles. A series of tubules (t) is formed in the stroma. Bar, 1  $\mu$ m.

**FIG. 2.** Part of the connective tissue and adhering hemocyte of an *E. torrentis* larva with iridovirus infection. Virions (v) are in the cytoplasm of infected cells together with tubules (t). In the hemocyte two virogenic stroma (S) with virus particles and tubules (t) close to the nucleus (n). Bar, 1  $\mu$ m.

**FIG. 3.** Mature iridovirus particle with distinct electron-dense core and the capsid shell. Bar, 100 nm.

**FIG. 4.** Hemocyte containing virogenic stroma (S) close to the nucleus (n) with virus particles in close association with the rounded ends of tubules (t). Bar, 500 nm.

**FIG. 5.** Part of the infected tissue with masses of virus particles (v) developing in a thin virogenic stroma (S) and with remnants of the cytoplasm, mitochondria (m) and lysosomes (l). Bar, 2  $\mu$ m.

nored when studying iridescent virus–host interactions. Further work is required to elucidate the identity of this virus and the relationship between the *E. torrentis* iridovirus and the other invertebrate iridoviruses based on the molecular characteristics.

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