Viral gametocytic hypertrophy of the Pacific oyster *Crassostrea gigas* in Ireland

Deborah Cheslett, Frank Mc Kiernan, Cathy Hickey, Evelyn Collins*

Fish Health Unit, Marine Institute, Rinville, Oranmore, Galway, Ireland.

ABSTRACT: Viral gametocytic hypertrophy (VGH) was detected during an investigation of mortalities in Pacific oysters *Crassostrea gigas* from 2 separate Irish production sites. The basophilic inclusions were observed in the gonad tissue of oysters sampled in August and October 2007. The oysters involved did not show any macroscopic disease signs. Transmission electron microscopy demonstrated the presence of viral particles in these intranuclear inclusions. The particles were small, non-enveloped, icosahedral and approximately 50 nm in diameter and thus had characteristics similar to the *Papillomaviridae* and *Polyomaviridae* families. No host defence reaction was observed. The viral particles described here appear to be similar to those described in *C. virginica* from the USA and Canada and to those described in *C. gigas* from Korea and France.

KEY WORDS: *Crassostrea gigas*, viral gametocytic hypertrophy, *Papillomaviridae*, *Polyomaviridae*, Pacific oyster, gonad

Running head: 'VGH in *Crassostrea gigas* in Ireland'

* Corresponding author. Email: evelyn.collins@marine.ie

INTRODUCTION

Numerous viruses can infect molluscs and mortalities have been reported in different bivalve species associated with the presence of viruses belonging to various families (Elston 1997, Renault & Novoa 2004). Viruses described in bivalves have included members of the families *Herpesviridae, Reoviridae, Picornaviridae, Retroviridae, Birnaviridae, Iridoviridae and Papovaviridae*. The family *Papovaviridae* originally comprised the 2 genera *Papillomavirus* and *Polyomavirus* but they are now considered as 2 separate families *Papillomaviridae* and *Polyomaviridae* (Van Regenmortel et al. 2000). These 2 families share morphological characteristics: they are viruses which are non-enveloped, icosahedral and are approximately 40 to 55 nm in diameter (Garcia et al. 2006).

Farley (1985) observed viral gametocytic hypertrophy (VGH) in hypertrophied cells of gonad tubules of *Crassostrea virginica* sampled in various US states but extensively in the state of Maine. He described non-enveloped, icosahedral viral particles 50 to 55nm in diameter in the maturing and mature cells. He also reported on histologically similar lesions seen in *C. gigas* and *C .lurida* from Korea, Japan, Oregon and Washington and similar lesions in *C. rhizophorae* from Puerto Rico. Similar viral particles have also been described from *C. virginica* from the east coast of North America (Sparks 1985), from the Gulf of Mexico (Winstead & Courtney 2003) and from Atlantic Canada (McGladdery & Stephenson 1994).

A papova-like virus associated with VGH was described in the gonad tissue of C. *gigas* in southern Korea (Choi et al. 2004). Viral particles with characteristics similar

to the *Papillomaviridae* and *Polyomaviridae* families have also been reported in *C. gigas* in France (Garcia et al. 2006). Moss et al. (2007) observed VGH histologically in the gonad of wild *C. hongkongensis* during a survey of Asian oysters for pathogens. Watermann et al. (2008) observed VGH in the hypertrophied gametocytes of *C. gigas* during investigations in to the health condition of these oysters along the East Frisian coast of Germany.

The actual impact of papova-like viruses on their hosts has not been fully assessed. Neither is it clearly understood whether one or more viruses are involved in these gonad conditions. In 2007, we observed VGH in *C. gigas* gonad tissue sampled from 2 separate production sites in Ireland. We reprocessed the wax embedded oyster gonad tissue for electron microscopy and describe the ultrastructure of the viral particles observed in these infected oysters.

MATERIALS AND METHODS

From August to October 2007, following reports of increased levels of mortalities, a total of 77 market-sized *Crassostrea gigas* were collected form 2 separate production sites in Ireland (Fig. 1).

Histological examination. Oyster tissue fixed in 10% v/v Formalin solution was processed for routine histology. Sections were cut at 2µm and stained with Haematoxylin and Eosin (H&E).

Ultrastructural examination.

When inclusion bodies were observed during light microscopy, the wax embedded oyster tissue containing the inclusion was reprocessed for transmission electron microscopy (TEM) as follows. With the H&E stained section as a visual guide, the

portion of wax embedded tissue with the inclusion was removed with a scalpel from the wax block and dewaxed overnight in two changes of xylene with agitation. Following rehydration, the tissue was then placed in 3% glutaraldehyde in 0.1M cacodylate buffer (pH7.4) for 2-5 hours, rinsed again in 0.1M cacodylate buffer and finally post fixed in 1% OsO_4 for 2 hours. After dehydration through graded alcohols the tissues were infiltrated with a 1:1 solution of Agar low viscosity resin and 50% ethanol with agitation for 1 hour, followed by 100% resin for 2 hours minimum. Tissues were embedded in resin and cured at 60ºC for 2-3 days. Semi-thin sections were stained with 1% toluidine blue and ultra-thin sections were stained with uranyl acetate and lead citrate. Ultra-thin sections were viewed using a Hitachi H-7500 transmission electron microscope at 75kV.

RESULTS

No gross clinical disease signs were observed in the *Crassostrea gigas* collected from Site A (County Kerry) or Site B (County Donegal) between August and October 2008. In H&E stained sections, basophilic inclusions were observed in hypertrophied nuclei in 2/53 oysters sampled from Site A during August and October and in 1/24 oysters sampled from Site B in August. Infected maturing and mature ovocytes showed hypertrophied nuclei with perinuclear condensed nuclear material (Fig. 2). There was no haemocytic infiltration or other host tissue reaction observed associated with the infection. TEM of reprocessed wax embedded tissue containing the basophilic inclusions demonstrated that the granular inclusions consisted of a homogeneous amalgamation of viral particles. The nuclear membrane of the infected ovocyte was normal and peripherally displaced chromatin could be observed (Fig 3).

The viral particles were approximately 45 to 50nm in diameter and non-enveloped (Fig. 4). They were 5 or 6 sided in section suggesting an icosahedral symmetry (Fig.5). Under TEM the viral particles from both sites appeared to be similar.

During the sampling period, 8 aquaculture sites experienced mortalities in Site A and cumulative mortalities ranged from 10% to 40%. In Site B, 4 operators noted mortalities of approximately 30%. From a total of 77 oysters examined only 3 female oysters were found to have basophilic inclusions with the number of infected cells ranging from 3-14 per section.

Based on the reprocessed TEM material, although of reduced quality, these particles appear similar to those particles described by Winstead & Courtney 2003, Choi et al. 2004, Garcia et al. 2006.

DISCUSSION

Farley (1976, 1985) described a papova-like virus in hypertrophied gametocytes of the eastern oyster *Crassostrea virginica* and since then other authors have reported similar conditions in various oyster species in North America, Asia and Europe (McGladdery & Stephenson 1994, Elston 1997, Choi et al. 2004, Garcia et al. 2006). This is the first report of VGH in *C. gigas* in Ireland. Observations at the ultrastructural level in this study show that the basophilic inclusions seen in histology are in fact large masses of viral particles in the hypertrophied nuclei of gonad tissue. The size and symmetry of these particles suggest similarity to the *Papillomaviridae* and *Polyomaviridae* families (Van Regenmortel et al. 2000). But further studies would be required to formally assign the viral particles to these families.

125 Papilloma-like and papova-like viruses have been described from various bivalve
126 species (Elston 1997). However without the availability of molluscan cell lines, none
127 of these viruses have been isolated and characterised and insufficient knowledge is
128 available from histopathological and ultrastructural studies alone to discriminate
129 between these viruses described from various parts of the world.
130 Although VGH is readily detected in maturing gametes it is more difficult to detect in
131 non-mature oysters (Garcia et al. 2006). A maximum infection level of 350 cells
132 (average 4 infected cells per section) was reported in *C. virginica* by Farley (1985)
133 who also noted that female oysters were more often infected. Garcia et al. (2006)
134 observed up to 16 infected cells per section in *C. gigas*, and also noted that *C. gigas*
135 male and female oysters were equally affected by VGH. But Watermann et al. (2008)
136 observed up to 20 infected cells per section in *C.gigas* and reported that male oysters
137 were more commonly infected. These authors also noted that even though there had
138 been previous surveys carried out along the East Frisian coast in 2003 and 2004, VGH
139 had not been detected, as was also the case in France before 2001 (Garcia et al. 2006).
140 In our study we observed between 3 and 14 infected cells per section in 3 female
141 oysters, however the number of oysters examined is too low to establish infection rate
142 or infection intensity.
143 In common with other workers (Choi et al. 2004, Garcia et al. 2006) no haemocytic
144 reaction was observed in our study suggesting limited health implications for the
145 infected oysters. However Garcia et al. (2006) comment that gamete viability and
146 consequently oyster fecundity could be altered by VGH. In our study the stocks
147 examined were experiencing mortalities, but the low number of oysters detected with
148 VGH and the lack of any clinical disease signs would suggest that the observed virus
149 particles were unlikely to be causing the mortalities. Since 1993, oyster mortalities

150 have been repeatedly experienced during the late summer months in many of the Irish
151 *C.gigas* production areas, without the identification of any linked pathogen or
152 pathogens. The mortalities experienced here fit this pattern.
153 So far no serious manifestations are known for this virus but the possibility exists for
154 oncogenic transformation (Farley 1985, Van Regenmortel et al. 2000, Watermann et
155 al. 2008). Potential danger also threatens from cross infection to other species thereby
156 producing disease in other possibly more susceptible hosts. This would have
157 significant implications particularly in the case of the introduction of non-native
158 species (Munn 2006, Watermann et al. 2008).
159 Virus-like particles have been identified in many species of bivalve mollusc (Renault
160 & Novoa, 2004), although proof of aetiology and study of pathogenesis is often
161 lacking (Munn 2006). Viruses may be found in molluscs already debilitated by
162 disease or by other stress factors (Montes et al. 2001). On the other hand viruses may
163 be observed simply due to bioaccumulation and their presence may not necessarily
164 imply disease. Infectious disease is a complex interaction between the agent, the host
165 and the environment. It is also necessary to distinguish between viral infection and
166 actual disease manifestation. By definition a virus is infective for its particular host(s)
167 but may have varying effects on different life stages of the host and may be more
168 virulent for different species (Elston 1997). At present diagnosis of viral disease is by
169 light microscopy followed by confirmation using TEM. The lack of molluscan cell
170 lines has impeded the advancement of bivalve virology but recently the use of
171 molecular tools has become more widespread (Munn 2006).
172 Viral diseases are of concern in intensively reared molluscs because no specific
173 chemotherapies or vaccines are available. A better understanding of the virus and
174 virus-host interaction is required for disease control in aquaculture and for reducing

the transmission of viral diseases between cultured and natural populations of bivalve molluscs. Advancement in the field of molluscan virology will require increased application of physical isolation methods, the development of continuous molluscan cell lines and the use of molecular tools and should be the focus of further studies.

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Figure legends

Fig.1. Location of the infected *Crassostrea gigas* samples in Ireland collected between August and October 2007

Fig. 2. *Crassostrea gigas.* Basophilic intranuclear inclusion in a gonad follicle of the oyster. Light micrograph of oyster gonad follicle (gf), with inclusion (i) and condensed material (arrow) (H&E); (scale bar = 10µm)

Fig.3. *Crassostrea gigas.* Ultrathin section of inclusion body, showing intranuclear viral particles (v) in an ovocyte with a normal nuclear membrane (arrow) and chromatin masses (c); (scale bar = 2µm)

Fig. 4. *Crassostrea gigas.* Ultrathin section of inclusion body showing details of viral particles, which are non-enveloped, icosahedral and 45 to 50nm in diameter; (scale bar 100nm)

Fig 5. Intranuclear 5-sided (white arrow) and 6-sided (black arrow) viral particles (v) in an ovocyte; (scale bar = 100nm)

276 Figure 1

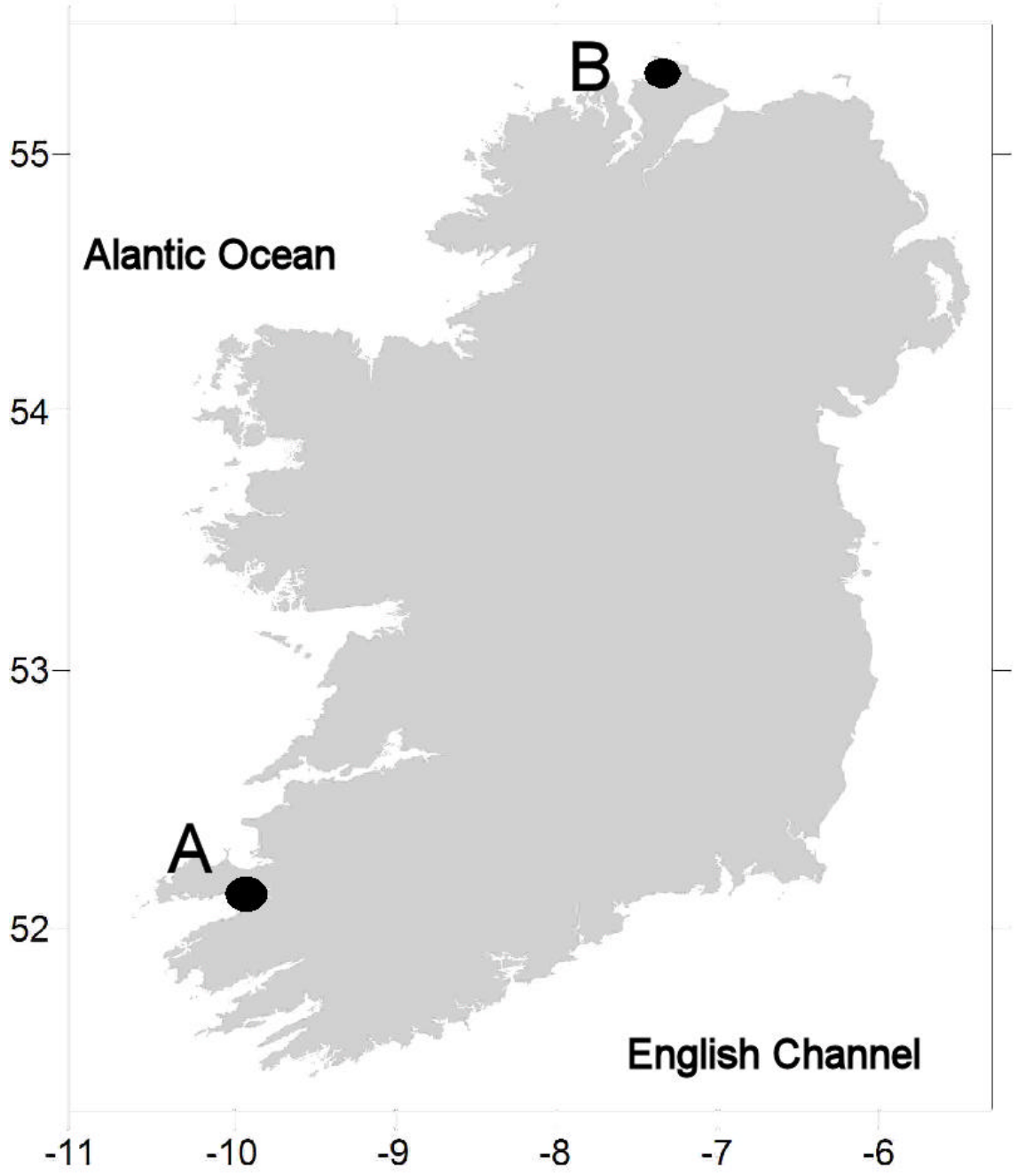

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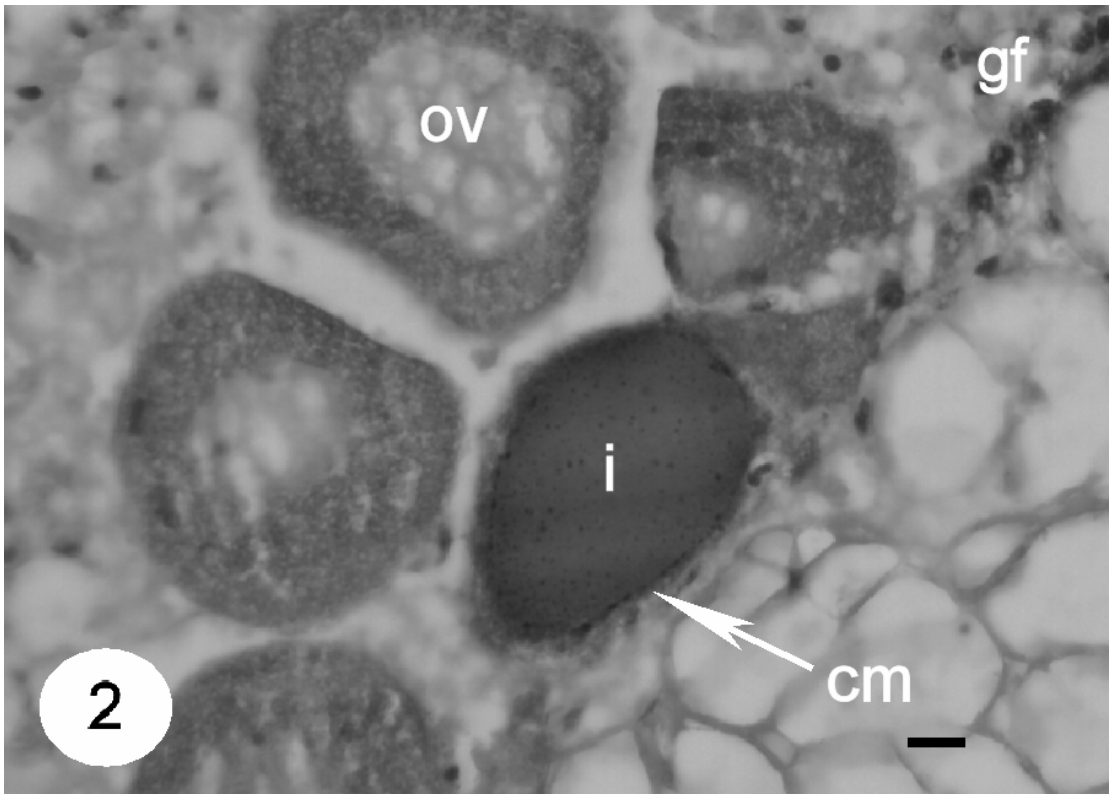
Figure 2



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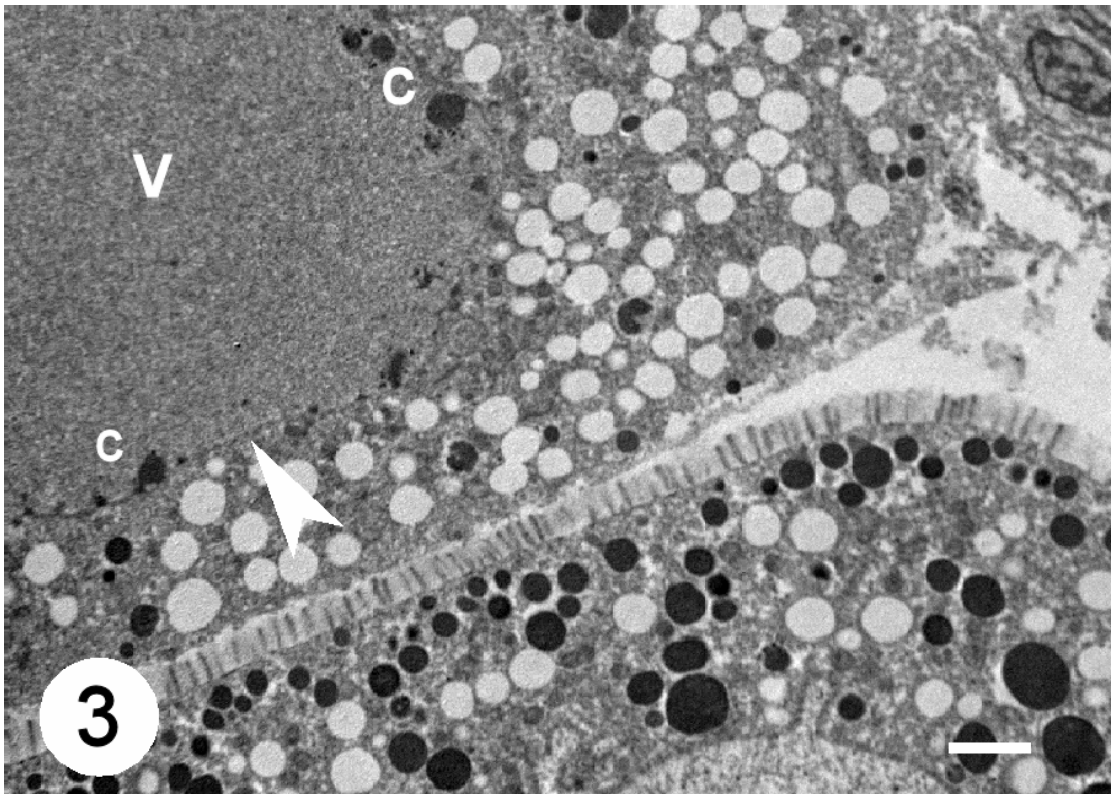

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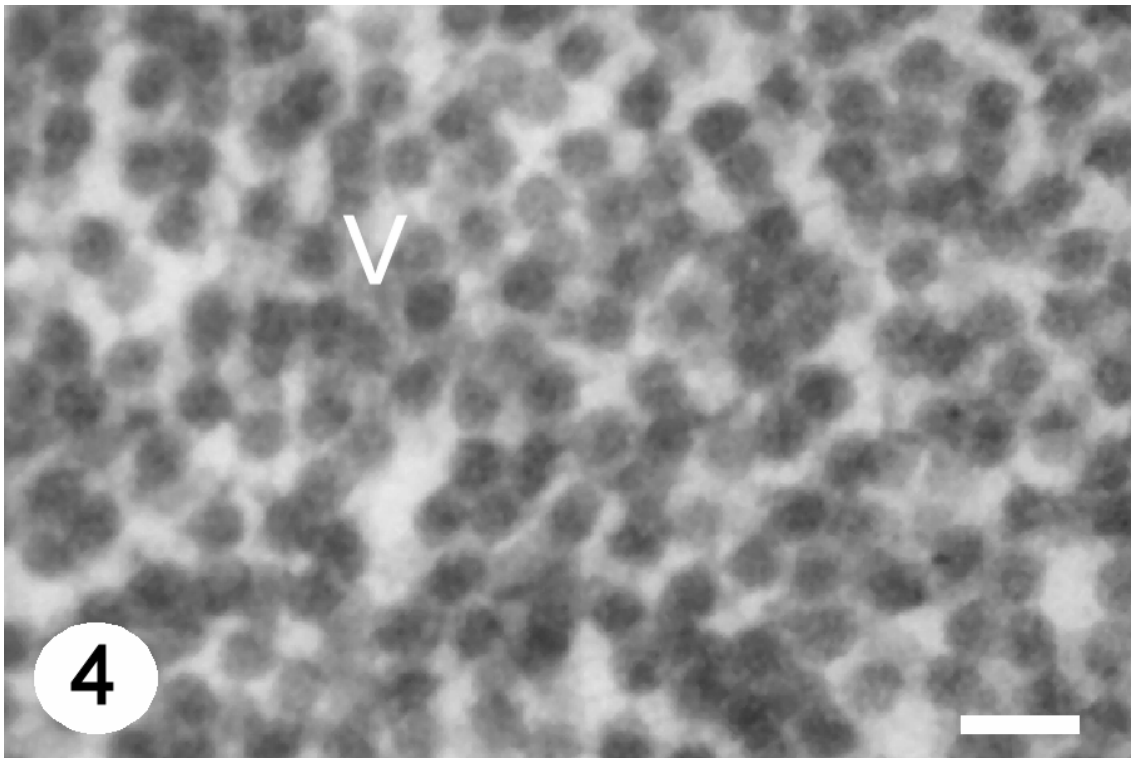

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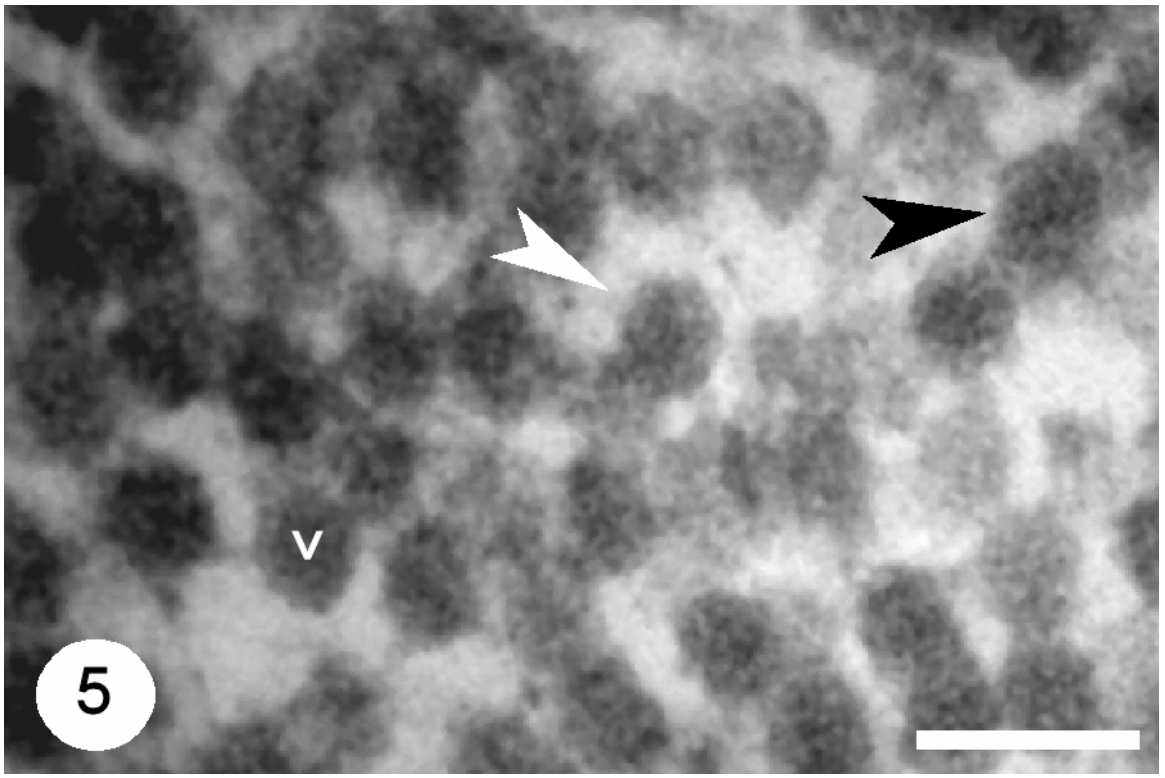
293 Figure 5



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