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NOTE

Viral basophilic inclusions in the digestive gland of razor clams *Ensis arcuatus* (Pharidae) in Galicia (NW Spain)

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ABSTRACT: During a histological survey of razor clam *Ensis arcuatus* (Jeffreys, 1865) from Galicia (NW Spain), basophilic inclusion bodies were observed in epithelial cells of the digestive gland. Transmission electron microscopy revealed the intranuclear position of these inclusions containing viral particles with icosahedral symmetry. Size and symmetry of these unenveloped virus particles suggest similarities to the families *Papillomaviridae* and *Polyomaviridae* which have been described as causing a viral gametocytic hypertrophy in oysters *Crassostrea virginica* and *C. gigas*. This is the first report of viral particles in *E. arcuatus*.

KEY WORDS: *Ensis arcuatus* · Histopathology · Ultrastructure · Basophilic inclusion bodies · Viral particles

INTRODUCTION

Viruses that have been described as affecting bivalves belong to the families *Papillomaviridae*, *Polyomaviridae*, *Malacoherpesviridae*, *Togaviridae*, *Retroviridae*, *Reoviridae*, *Birnaviridae*, *Iridoviridae*, *Picornaviridae*, and *Baculoviridae* (Elston 1997, Chang et al. 2002, Renault & Novoa 2004, Davison et al. 2009). A viral gametocytic hypertrophy (VGH) provoked by intranuclear basophilic inclusions in gametes was detected in oysters (Farley 1976, McGladdery & Stephenson 1994, Winstead & Courtney 2003, Choi et al. 2004, Garcia et al. 2006, Cheslett et al. 2009, Meyers et al. 2009). Transmission electron microscopy (TEM), performed by these authors, demonstrated virus-like particles probably belonging to *Papillomaviridae* and *Polyomaviridae* (referred to in previous literature as *Papovaviridae*). Prevalence and intensity were low, and no adverse effect was reported on oyster health. However, mortalities of bivalves associated with the presence of viral particles have been reported (Elston 1997, Renault & Novoa 2004, Maeno et al. 2006).

Basophilic inclusion bodies in epithelial cells of the digestive gland, resembling intranuclear inclusions of the VGH in oysters, were found in histological sections during a pathological study of razor clams *Ensis arcuatus* (Solenidae) from Galicia, Spain (Darriba et al. 2010). The prevalence and intensity of these inclusions were usually low, and no adverse effects were reported in the razor clams. The objective of the present note was to continue with the study of the basophilic inclusion bodies in *E. arcuatus* from Galicia, reported by Darriba et al. (2010).

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MATERIALS AND METHODS

Further analyses were performed on the histological samples used in the study by Darriba et al. (2010). Previously stained (with Harris' hematoxylin and eosin, H&E) slides were re-examined, and new histological sections of the specimens affected by inclusion bodies were made and stained using Feulgen picromethyl blue (Farley 1969). A sample showing high intensity of inclusion bodies in epithelium cells of the digestive gland of *Ensis arcuatus* was selected to perform an ultrastructural analysis. The portion of tissue containing the inclusions was removed from the paraffin block with a scalpel. Paraffin was removed by several rinses in xylene with agitation. Tissue was placed in 2.5% glutaraldehyde, postfixed in 2% OsO_4, and embedded in Epon. Ultra-thin sections were stained with uranyl acetate and lead citrate and examined in a JEOL JEM 1010 transmission electron microscope (TEM) at 80 kV.

RESULTS AND DISCUSSION

The unidentified basophilic inclusions observed, using light microscopy and H&E stain, in epithelial cells of the digestive gland had chromophilic margins (Fig. 1). These inclusions showed a Feulgen-positive reaction, indicating the presence of DNA. TEM examination confirmed the intranuclear position of the basophilic inclusions and the presence of viral particles inside of the inclusions (Fig. 2). The virions were unenveloped, with a rounded appearance suggesting icosahedral symmetry (Fig. 3). The size of virions was 38.27 ± 3.93 nm in diameter (mean ± SD, n = 30). Both empty and full capsids could be observed (Fig. 3). The chromophilic margins, viewed by light microscopy, were identified as peripherally displaced chromatin (Fig. 2). The intranuclear location of the basophilic inclusions of *Ensis arcuatus*, high hypertrophy of the infected cells, and the size and symmetry of unenveloped virions are in concordance with the description of the virus causing VGH in *Crassostrea virginica* (Farley 1976, McGladdery & Stephenson 1994, Winstead & Courtney 2003) and *C. gigas* (Choi et al. 2004, Garcia et al. 2006, Cheslett et al. 2009, Meyers et al. 2009). With regard to the size and symmetry of the unenveloped virions, all the latter authors suggested similarities to the families *Papillomaviridae* and *Polyomaviridae*. Papovavirus-like infection affecting labial palps of *Pinctada maxima* was reported by Norton et al. (1993). In this case, the inclusions observed by light

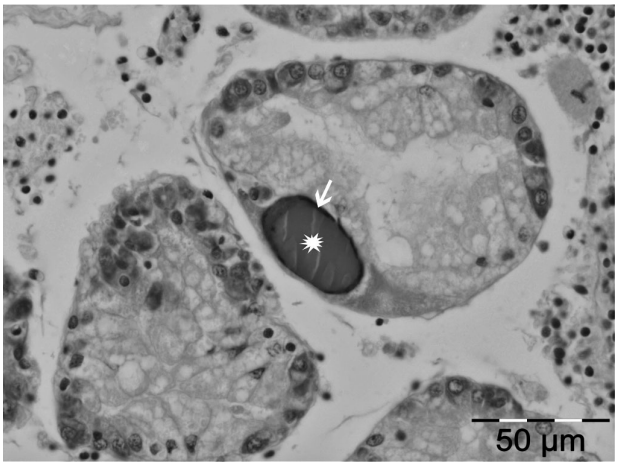

Fig. 1. *Ensis arcuatus*. Light micrograph showing basophilic inclusion body (asterisk) in the epithelial cell of the digestive gland with chromophilic margin (arrow). Hematoxylin and eosin stained

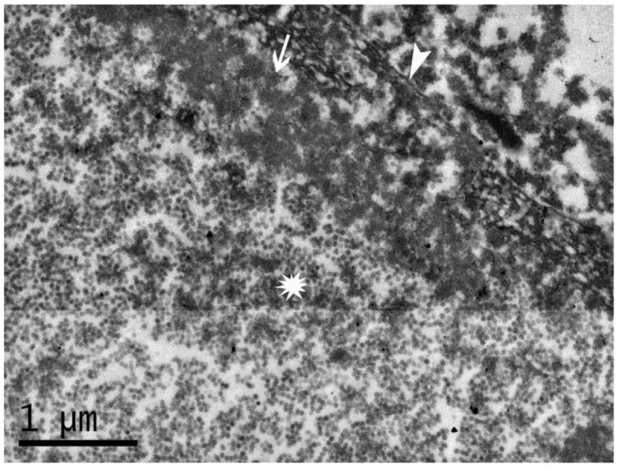

Fig. 2. *Ensis arcuatus*. Ultrathin section detail of an intranuclear inclusion, showing viral particles (asterisk), nuclear membrane (arrowhead) and peripheral chromatin (arrow)

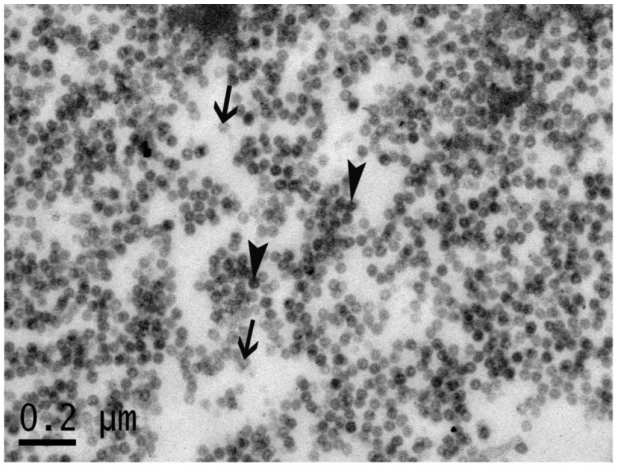

Fig. 3. *Ensis arcuatus*. Ultrathin section showing icosahedral symmetry of the viral particles. Presence of empty (arrows) and full capsids (arrowheads)

microscopy were eosinophilic and usually separated from the peripheral nuclear chromatin by a clear zone, in contrast to the basophilic affinity observed in oysters and razor clams.

Pass et al. (1988) described intranuclear basophilic inclusions in the digestive gland of *Pinctada maxima*, but infected cells showed slight hypertrophy, and virions (about 38 nm in diameter) were inside of an electron-dense amorphous matrix, unlike the viral inclusions of *Ensis arcuatus* in the present study, and of oysters affected by VGH (Farley 1976, McGladdery & Stephenson 1994, Winstead & Courtney 2003, Choi et al. 2004, Garcia et al. 2006, Cheslett et al. 2009, Meyers et al. 2009).

Intracytoplasmatic and unenveloped viruses were detected in the digestive gland of *Perna canaliculus*, *Pecten novaezelandiae*, and *Paphies ventricosum* (Jones et al. 1996, Hine & Wesney 1997) and in gill and kidney of *Atrina pectinata* (Maeno et al. 2006). The presence of these intracytoplasmatic viruses was associated with mortalities. In the case of VGH, absence or limited impact at population levels were reported; however, Garcia et al. (2006) commented that the virus may affect the viability of oyster gametes. In the present study, the low prevalence and intensity detected, and the absence of a host inflammatory response, suggest that these inclusions would not be a serious problem to razor clam populations.

This is the first report of viral particles in *Ensis arcuatus*. Based on ultrastructural morphological characteristics, virions cannot be assigned with certainty to the families *Papillomaviridae* or *Polyomaviridae*. The absence of immunoglobulin production in mollusks, together with the lack of molluskan cell lines, make virus isolation and identification difficult. The further development of molecular techniques will be an important tool in the study of viruses.

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