# White Spot Disease

## Agent Description

White Spot Disease (WSD) is considered to be infection with the virus White Spot Syndrome Virus-1 (WSSV). Virus particles appear rod-shaped to elliptical and measure 80-120 x 250-380 nm. WSSV belongs to the *Whispovirus* genus in the *Nimaviridae* Family. WSSV is a dsDNA virus with a 293kb genome.

WSD is the most serious threat facing the shrimp farming industry. The economic impact of the disease in value of lost production and trade has been reported to approach 10 billion USD since 1993.

### Stability
Agent is inactivated in <120 minutes at 50ºC and <1 minute at 60ºC. Viable for at least 30 days at 30ºC in seawater under laboratory conditions and is viable in ponds for at least 3-4 days.

### Replication
Replication cycle is approximately 20 hours at 25ºC.

### Transmission
Vertical (trans-ovum), horizontal (cannibalism, predation etc.) and water-borne routes likely. Transmission can occur from apparently healthy animals in the absence of disease. Dead and moribund animals may be a source of disease transmission.

### Prevalence
Highly variable, from <1 % in infected wild populations to up to 100 % in captive populations. High prevalence associated with catastrophic crop losses.

### Geographical distribution
Throughout East, South-East and South Asia, North, South and Central America. WSD free zones are known within these regions. Reported outbreaks in European shrimp production regions.

### Vectors
Rotifers, bivalves, polychaete worms and non-decapod crustacean hosts including *Artemia salina* and copepods, non-crustacean aquatic arthropods and insect larvae. All these species can accumulate high concentrations of viable WSSV but there is no evidence of virus replication. Likely that any animal in contact with pond water and/or feeding/in contact with infected hosts may act as a vector for WSSV.

### Mortality
All farmed penaeid shrimp species are highly susceptible to infection often resulting in high mortality. Crabs, crayfish, freshwater prawns, spiny lobsters and clawed lobsters are susceptible to infection but mortality and morbidity is highly variable. Disease outbreaks may be induced by stressors (for example a rapid change in salinity). Water temperature has a profound effect on disease expression with average water temperatures of below ~30ºC being conducive to WSD outbreaks. Lower temperature limits of WSD expression and transfer of hosts from ‘carrier’ to ‘diseased’ status have not been well studied, particularly for temperate water species.

### Control and Prevention

No effective vaccines for WSSV are available. Use of specific pathogen free (SPF) or PCR-negative seed stocks and use of biosecure water and culture systems. General husbandry practices have been used to successfully manage WSD e.g. avoid stocking in cold season.

## Gross Pathology

### Penaeid shrimp

Presence of white spots under the cuticle and a high degree of colour variation with a predominance of reddish and pinkish discoloured shrimp, reduction in feed intake, increased lethargy, movement of moribund shrimp to the water surface and pond/ tank edges and consequent attraction of shrimp-eating birds. Note: all of these signs are generally considered non-specific and as such cannot be used as definitive for a diagnosis of WSD.

### Crabs, crayfish, freshwater prawns, spiny lobsters and clawed lobsters

Gross symptoms of WSD in non-penaeid crustacean hosts are not well documented but are likely to include at least a reduction in feed intake, an onset of lethargy and other behavioural changes. White spots beneath the cuticle may be unlikely due to the thickness of the carapace of most species.

## Clinical Pathology

### Histology

H&E staining reveals intranuclear inclusion bodies as prominent eosinophilic to pale basophilic in hypertrophied nuclei. Most commonly seen in the cuticular epithelial cells and connective tissue cells, and, less frequently, the antennal gland epithelium, lymphoid organ sheath cells, haematopoietic cells and fixed phagocytes of the heart. Feulgen staining reveals the intranuclear inclusion bodies to be Feulgen positive. Intranuclear occlusion bodies are absent (Annex 1).

### Transmission Electron Microscopy (TEM)

WSSV particles can be seen within the intranuclear inclusion bodies of infected cells. Virions are rod-shaped to elliptical, non-occluded and measure between 80 - 120 nm in width and 250 - 380 nm in length (Annex 2).

## Susceptible Species

A comprehensive assessment of host susceptibility to WSD has recently been completed by EFSA (EFSA 2008). The assessment takes in to account the key susceptibility criteria of pathogen replication, bioassay, characteristic pathology and anatomical location of pathogen and has critically assessed the available literature on host-pathogen interaction with respect to WSD. Currently, all decapods are listed as susceptible to WSD virus in the Directive 2006/88/EC. However, the EFSA assessment identified a total of 98 potential host species or genera from the scientific literature. Detailed reviews of each host is presented in EFSA (2008). The report suggests that scientific data are available to support susceptibility of 67 of these species but for the other 20 species, information was considered insufficient to scientifically assess susceptibility with regards to the criteria stated above.

The Decapoda comprise over 20,000 species across 2 suborders (Dendrobranchiata and Pleocyemata). Members of both suborders Dendrobranchiata and Pleocymata have been shown to be susceptible to WSD. This higher-level taxonomic diversity in

WSD susceptibility demonstrated by representation across these two suborders is likely the basis for the statement in Directive 2006/88/EC that 'all decapods' are susceptible to WSSV. However, it should be taken into account that most of the Families within the two suborders have not been tested. Only 3 families (Penaeidae, Solenoceridae, Sergestidae) of the seven families in the Suborder Dendrobranchiata have been studied in this context. Similarly, of the approximately 94 families that comprise the various Infraorders and Superfamilies of the Suborder Pleocyemata, only 24 have been demonstrated to be naturally or experimentally susceptible (or to act as carrier/vector). Furthermore, within the Suborder Pleocyemata, of the 8 Infraorders (Anomura, Astacidea, Brachyura, Caridea, Palinura, Palinuridea, Stenopodidea and Thalassinidea), only 5 have been demonstrated to contain susceptible or vector species (exceptions being the Infraorders Palinuridea, Stenopodidea and Thalassinidea). Nevertheless, WSD appears to have a wide host range compared to TS and YHD. In addition, all decapod crustaceans from marine and brackish or freshwater sources that have been subjected to experimental infection trials have been successfully infected (EFSA 2008).

## OIE Recommended Techniques for Surveillance and Confirmation

The methods listed in the table below are the OIE recommended techniques for surveillance and confirmation testing:

| Pathogen | Surveillance (Juveniles and Adults only) | Confirmatory Techniques |
|---|---|---|
| White Spot Syndrome Virus | Polymerase Chain Reaction (PCR) | Histology, Transmission Electron Microscopy (TEM), DNA probes *in situ*, PCR and Sequencing |

## Surveillance Testing

### Polymerase Chain Reaction (PCR)

The suggested protocol is that described by Lo *et al.* (1997 & 1996), and is recommended for all situations where WSSV diagnosis is required. A positive result in the first step of this standard protocol implies an advanced WSSV infection; when a positive result is obtained in the second amplification step only, a latent or carrier-state infection is indicated.

Commercial PCR diagnostic kits are available and have been very useful in the standardization and harmonisation of the technique. It is recommended that the most recent OIE Diagnostic Manual be consulted for up-to-date developments in molecular diagnostics for WSD.

## Confirmatory Testing

### Histology

Anaesthetise by immersing in ice until immobilised. Small animals (e.g. shrimp) can be fixed and prepared whole by injection of Davidson's seawater fixative (for marine species) or neutral buffered formalin (for freshwater species), followed by transfer to a larger volume of the same fixative for 24-48 hrs. Fixed specimens should be transferred to 70% industrial methylated spirit (IMS) for storage or shipping prior to

histological preparation. For larger animals (e.g. crab, crayfish, lobster) dissect the sub-cuticular epidermis, hepatopancreas, gut, gill, heart, gonad, nervous tissue and body musculature and place immediately into Davidson's seawater fixative for 24-48hrs followed by transfer to 70% IMS. Samples can then be infiltrated with paraffin under vacuum according to standard histology protocols and sections cut at a thickness of 3-5 µm, mounted onto glass slides, and stained with haematoxylin and eosin (H&E) or Feulgen stain.

**Transmission Electron Microscopy (TEM)**

Anaesthetise by immersing in ice until immobilised. Dissect small blocks (2 $mm^3$) of target tissue (e.g. sub-cuticular epidermis, lymphoid organ) and fix for electron microscopy in 2.5 % glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.4) for at least 2 h at room temperature. Rinse fixed tissue samples in 0.1 M sodium cacodylate buffer (pH 7.4) before post-fixation for 1 h in 1 % osmium tetroxide in 0.1 M sodium cacodylate buffer. Rinse samples in 0.1 M sodium cacodylate buffer before dehydrating through a graded acetone series. Embed samples in an epoxy resin and polymerise according to manufacturers guidelines. Semi-thin (1-2 µm) sections are stained with Toluidine Blue for viewing with a light microscope to identify the suitable target areas. Ultra thin sections (70-90 nm) of target areas are mounted on uncoated copper grids and stained with 2% aqueous uranyl acetate and Reynolds' lead citrate (Reynolds 1963).

***In situ hybridisation* (ISH)**

WSSV infected nuclei can be intensely marked by a DIG-labelled DNA probe for WSSV with *in situ* hybridisation assays. The suggested protocol is that developed by Nunan & Lightner (1997).

**Polymerase Chain Reaction (PCR)**

As for surveillance. Follow protocol of Lo *et al.* (1996, 1997) or most recent OIE Diagnostic Manual.

**Sequencing**

For confirmation of suspected WSSV, the DNA fragment amplified from the two-step nested diagnostic PCR should be sequenced. The suggested cloning and sequencing protocols are those described by Claydon *et al.* (2004). It is acceptable to sequence the PCR amplicon directly. If a positive result is obtained, compare the sequences to available databases using the Basic Local Alignment Search Tool (BLAST) to determine approximate phylogenetic affiliations. If a negative result is obtained the sample should be tested again.

**EU-legislation**

White Spot Disease is listed as a non-exotic pathogen in EC Directive 2006/88.

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## References

Claydon, K., Cullen, B. and Owens, L. (2004) OIE white spot syndrome virus PCR gives false-positive results in *Cherax quadricarinatus*. *Dis. Aquat. Organ.*, **62**, (3), 265-268.

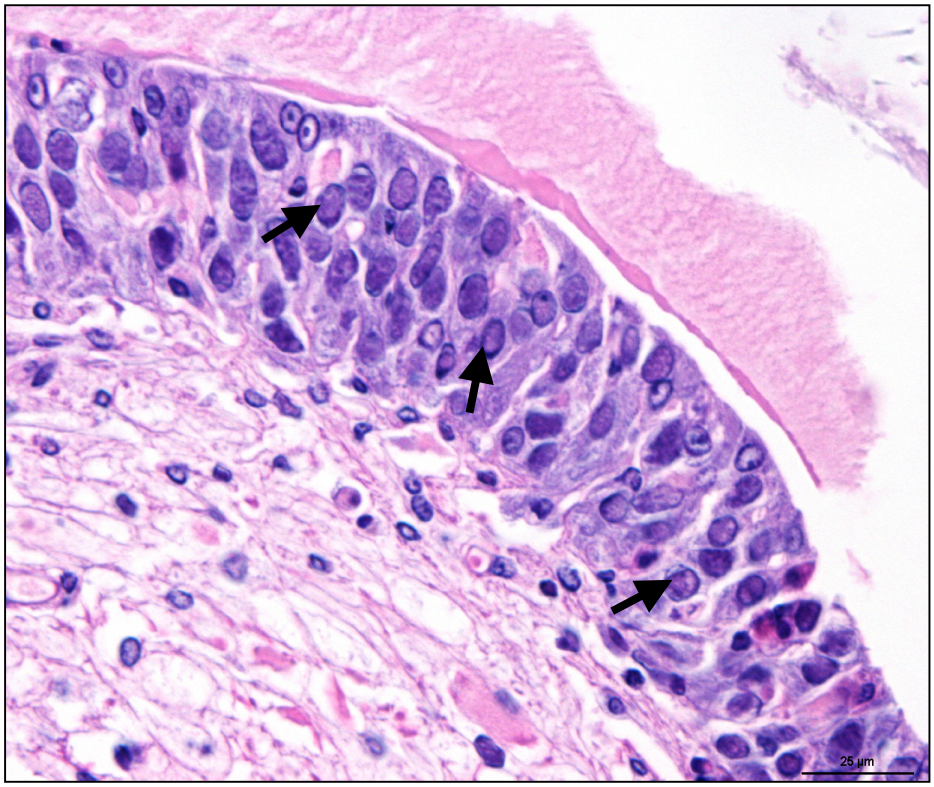
Lo C.F., Ho, C.H., Chen, C.H., Liu, K.F., Chiu, Y.L., Yeh, P.Y., Peng, S.E., Hsu, H.C., Liu, H.C., Chang, C.F., Su, M.S., Wang, C.H. and Kou, G.H. (1997) Detection and tissue tropism of white spot syndrome baculovirus (WSBV) in captured brooders of *Penaeus monodon* with a special emphasis on reproductive organs. *Dis. Aquat. Organ.*, **30**, 53-72.

Lo, C.F., Leu, J.H., Ho, C.H., Chen, C.H., Peng, S.E., Chen, Y.T., Chou, C.M., Yeh, P.Y., Huang, C.J., Chou, H.Y., Wang, C.H. and Kou, G.H. (1996) Detection of baculoviruses associated with white spot syndrome (WSBV) in penaeid shrimps using polymerase chain reaction. *Dis. Aquat. Organ.*, **27**, 215-225.

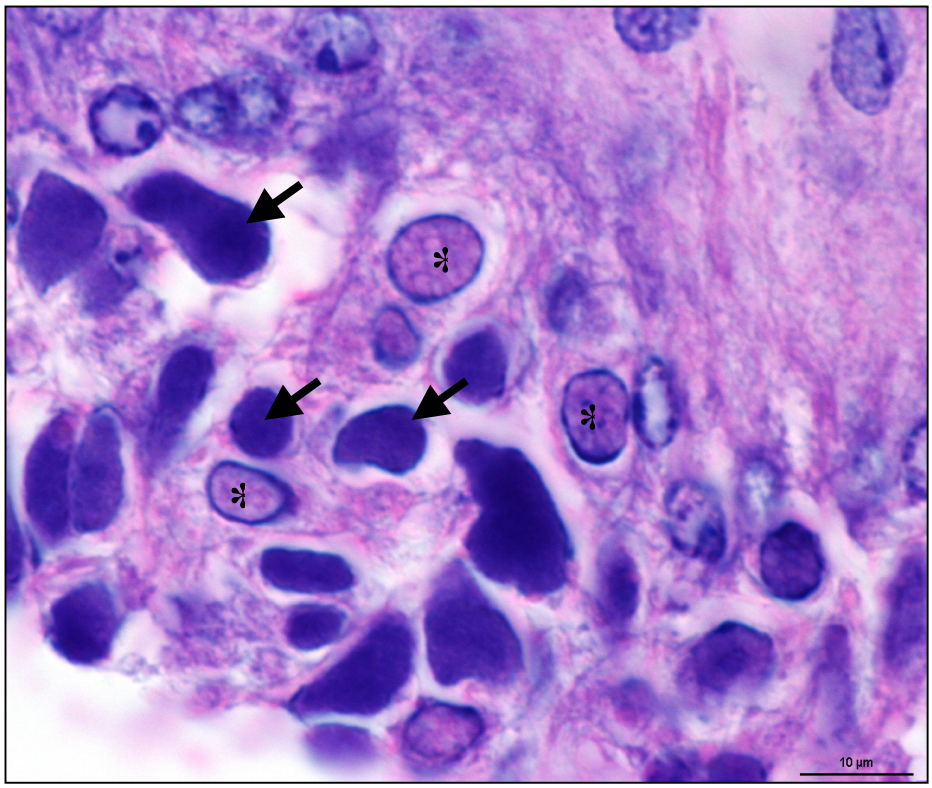
Nunan, L.M. and Lightner, D.V. (1997) Development of a non-radioactive gene probe by PCR for detection of white spot syndrome virus (WSSV). *J. Virol. Methods*, **63**, 193-201.

Reynolds, E.S. (1963) The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *J. Cell Biol.,* **17**, 208-212

## ANNEX 1 WSD Histology

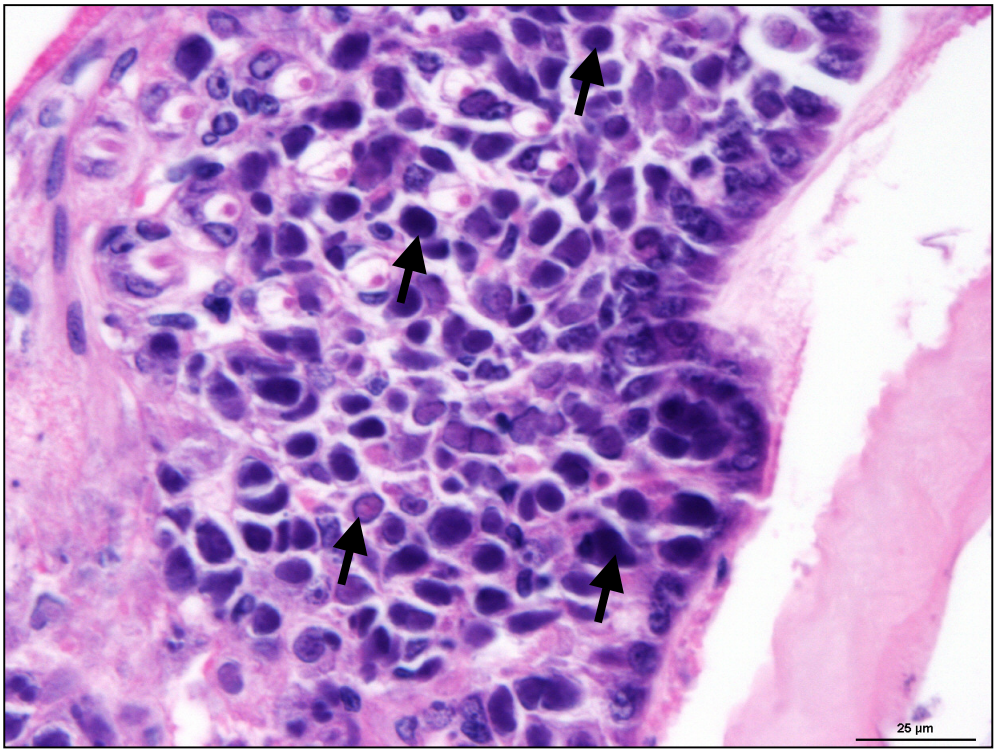



**White Spot Disease (WSD)** in *Penaeus vannamei*. Hypertrophied nuclei (arrows) are evident throughout the sub-cuticular epithelial cells. H&E stain. Scale = 25µm.

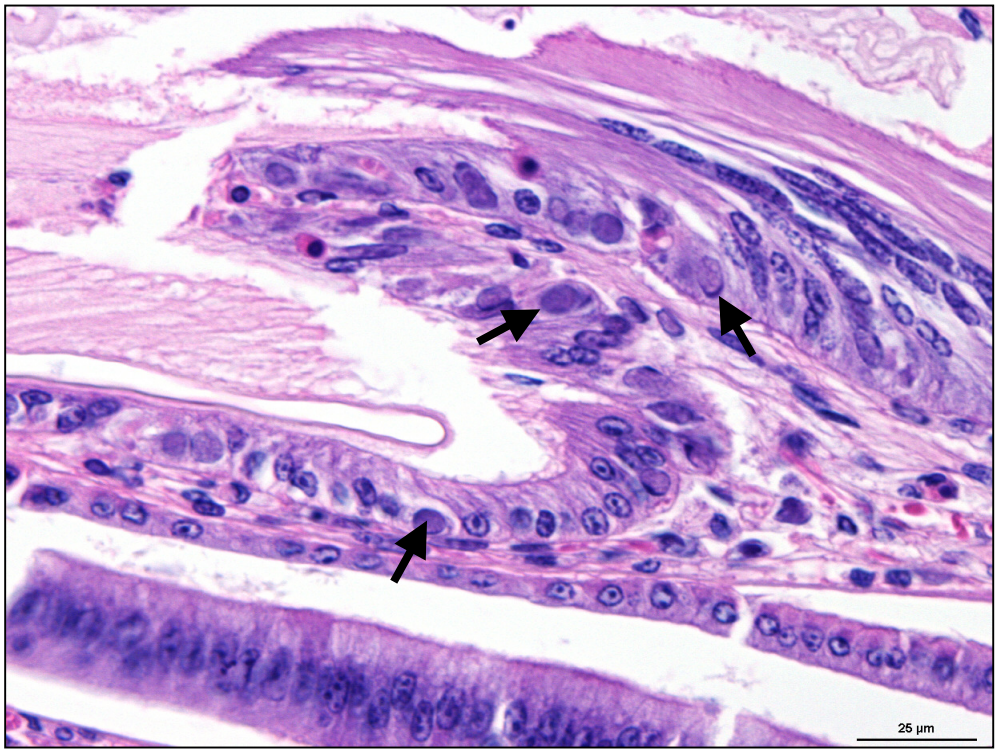



**White Spot Disease (WSD)** in *Penaeus vannamei*. Hypertrophied nuclei within sub-cuticular epithelium. Note granular eosinophillic staining (*) within some nuclei and dense haemotoxylin staining in others (arrows). H&E stain. Scale = 10µm.

## ANNEX 1 WSD Histology

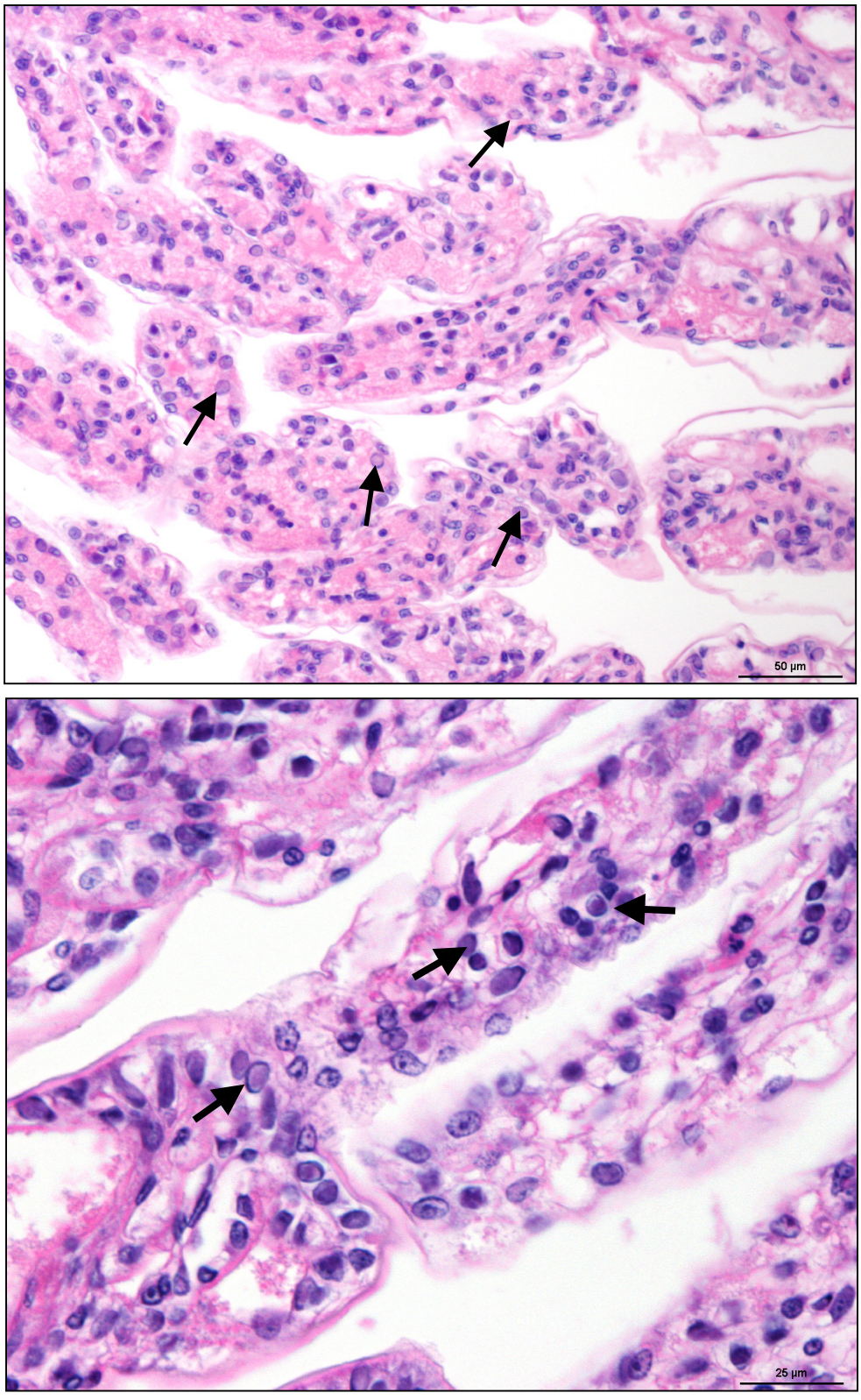



**White Spot Disease (WSD)** in *Penaeus vannamei*. Infected sub-cuticular epithelial cells. Hypertrophied nuclei are evident in majority of cells (arrows). H&E stain. Scale = 25µm.



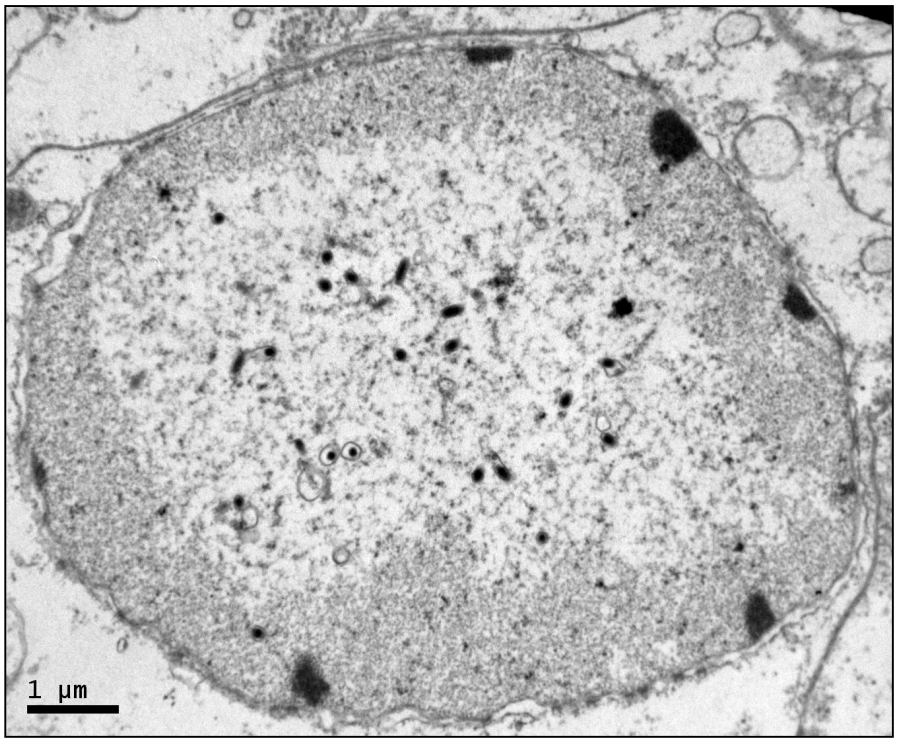

**White Spot Disease (WSD)** in *Penaeus vannamei*. Virus infected cells dispersed throughout the sub-cuticular epithelium. H&E stain. Scale = 25 µm.

## ANNEX 1 WSD Histology

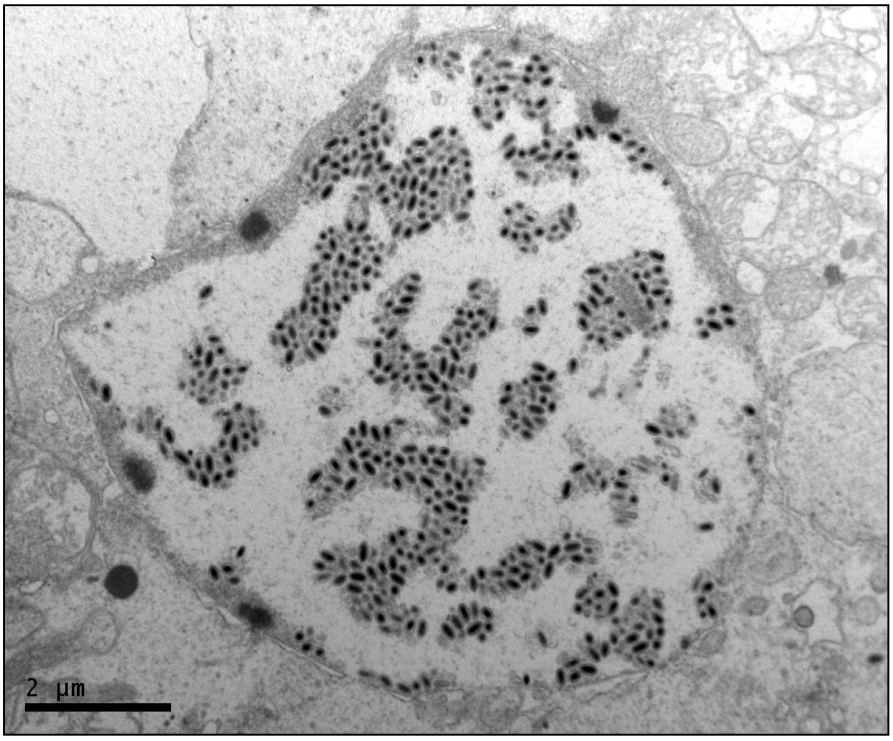



**White Spot Disease (WSD)** in *Penaeus vannamei*. Virus-infected gill epithelial cells dispersed throughout the gills. Hypertrophied nuclei identify infected cells (arrows). H&E stain. Scale = 50 µm (top) and 25 µm (bottom).

## ANNEX 2 WSD ultrastructure

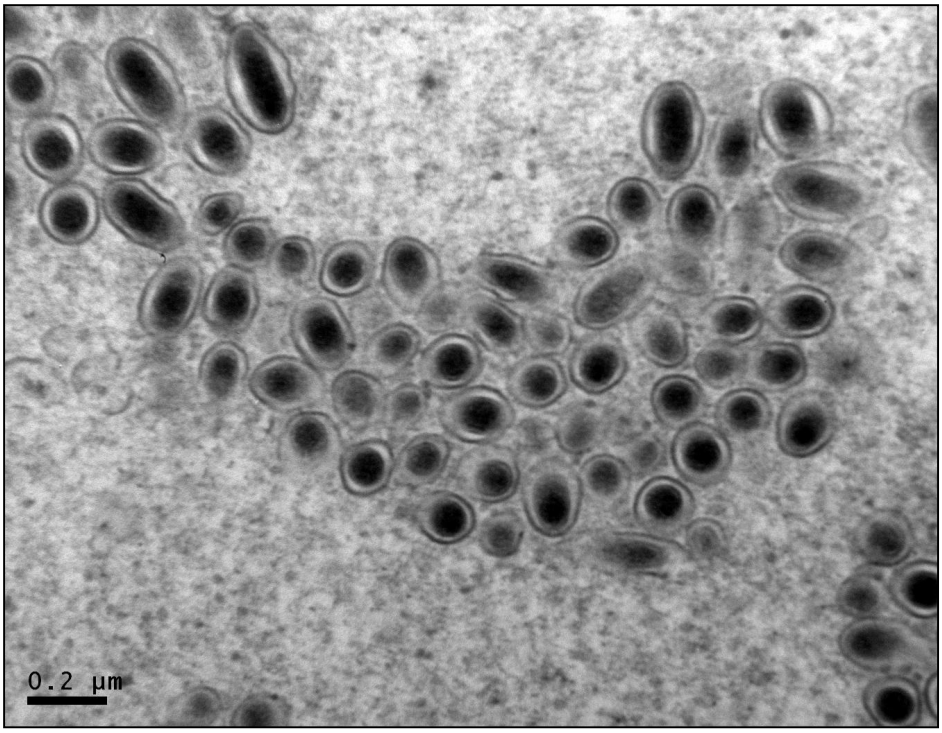



**White Spot Disease (WSD)** in *Penaeus vannamei*. WSSV particles developing within nucleus of infected cuticular epithelial cell. TEM. Scale = 1µm.

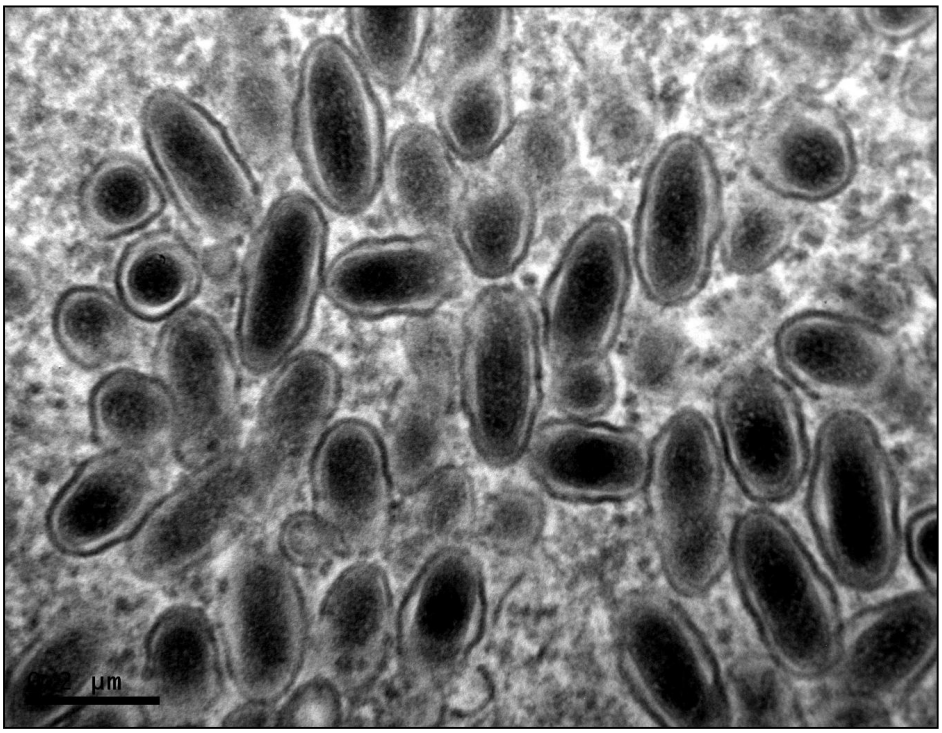



**White Spot Disease (WSD)** in *Penaeus vannamei*. Cuticular epithelial cell nucleus containing clusters of WSSV particles. TEM. Scale = 2µm.

## ANNEX 2 WSD ultrastructure




**White Spot Disease (WSD)** in *Penaeus vannamei*. WSSV particles in longitudinal and cross section within the nucleus. TEM. Scale = 0.2µm.




**White Spot Disease (WSD)** in *Penaeus vannamei*. WSSV particles in longitudinal and cross section within the nucleus. TEM. Scale = 0.2µm.