VI. SUMMARY
White spot syndrome virus (WSSV), member of a new virus family called Nimaviridae, is a major scourge in worldwide shrimp cultivation, due to its ability to spread quickly and cause 100% mortality in 3-10 days. Geographical isolates of WSSV identified so far are very similar in morphology and proteome, and show little difference in restriction fragment length polymorphism (RFLP) pattern. A recent study examining genetic variations among the 3 completely sequenced white spot syndrome virus (WSSV) genomes isolated from China, Thailand and Taiwan revealed five major differences among them and revealed an overall nucleotide identity of 99.32%. The major differences included (i) a large deletion of about 13.2 kb in WSSV-TH and about 1.2 kb in WSSV-CN genome relative to WSSV-TW. (ii) a variable region prone to recombination. (iii) a transposase sequence present only in WSSV-TW. (iv) variation in the number of repeat units within homologous repeats (hrs) in the noncoding region and direct repeats in coding region. (v) single nucleotide mutations, including deletion, insertion or single nucleotide polymorphisms (SNPs). Of these the variations the number of repeat units associated with ORF 75, ORF 94 and ORF 125 can be used for genotyping WSSV isolates in epidemiological studies. The deletion region between ORF 23/24 and a variable region of ORF14/15 prone to recombination were of particular evolutionary significance and may be used for reliable monitoring of WSSV infections world wide and for shrimp health certification.

The present study was initiated with the aim of genotyping WSSV prevalent in India using variable number of tandem repeats, associated with ORF 94, ORF 125 and ORF 75 and to compare the utility of the three minisatellites as epidemiological markers. The genomic changes induced by different environmental conditions and host on these minisatellite loci were also studied. The virulence between three different strains of WSSV was also compared. The differences associated with ORF 23/24, ORF 14/15 and transposase region were used to characterize the evolution of WSSV.

It was observed that nearly 20 different genotypes of WSSV are prevalent in India; the samples with the same repeat pattern in one ORF did not always show identical repeat patterns in one or both of the other repeat regions. This suggested that the combined analysis of all three variable loci could be used to differentiate and
characterize specific WSSV strains. For general epidemiological studies, the best marker, with maximum variation is ORF 94, followed by ORF 125 and ORF 75. The 3 repeat regions above were used to compare WSSV genotypes from disease outbreaks on 3 sets of farms from different locations in the state of Andhra Pradesh. The genotypes within each farm set were almost identical but differed between farm sets, suggesting that WSSV transmission occurred directly through virus carriers or water exchange between adjacent farms at each location. These findings show that genotyping can be a useful epidemiological tool in tracing the movement of WSSV within infected populations. Further it was found that the repeat pattern associated with a particular genotype of virus did not change with change in host or induced environmental stress such as change in salinity, temperature, formalin, dissolved oxygen, EDTA, Sodium hypo chlorite and ultraviolet radiation.

There was no difference in virulence between the different genotypes of WSSV used in the study, as all the strains could cause disease in the same time period, but a difference in fitness was observed. It was found the WSSV strain with the smaller genome size was the fittest.

The evolutionary study on WSSV suggested the Indian strains carried a 10,970 bp deletion in the ORF 23/24 region relative to WSSV-TW and WSSV-TH-96-II. Analysis of the ORF 14/15 regions revealed two novel strains of WSSV with unique sequences which could have evolved by recombination. None of the WSSV isolates had a transposase sequence or VP35 gene as reported for Taiwan isolates. Bioinformatics based studies showed the Indian strains were closely related to Thailand strains suggesting movement of a putative ancestor from Thailand to other parts of the world including India.