University of the Philippines Marine Science Institute

SEAFDEC/AQD
Southeast Asian Fisheries Development Center / Aquaculture Department

REPUBLIC OF THE PHILIPPINES
PHILIPPINE COUNCIL FOR AGRICULTURE, AQUATIC AND NATURAL RESOURCES RESEARCH AND DEVELOPMENT (DOST-PCAARRD)
DEPARTMENT OF SCIENCE AND TECHNOLOGY

Genome Sequencing And Genome-wide Copy Number Analyses Reveal Potential Candidate Regions And Genes Linked To WSSV Resistance In The Philippine Black Tiger Shrimp (*Penaeus Monodon*)

Kreighton E. Cadorna[1], Edgar c. Amar[2], Arturo O. Lluisma[1]

[1]Marine Genomics and Molecular Genetics Laboratory, The Marine Science Institute, University of the Philippines, Diliman, Philippines
[2]Southeast Asian Fisheries Development Center/Aquaculture Department, Iloilo, Philippines

Introduction

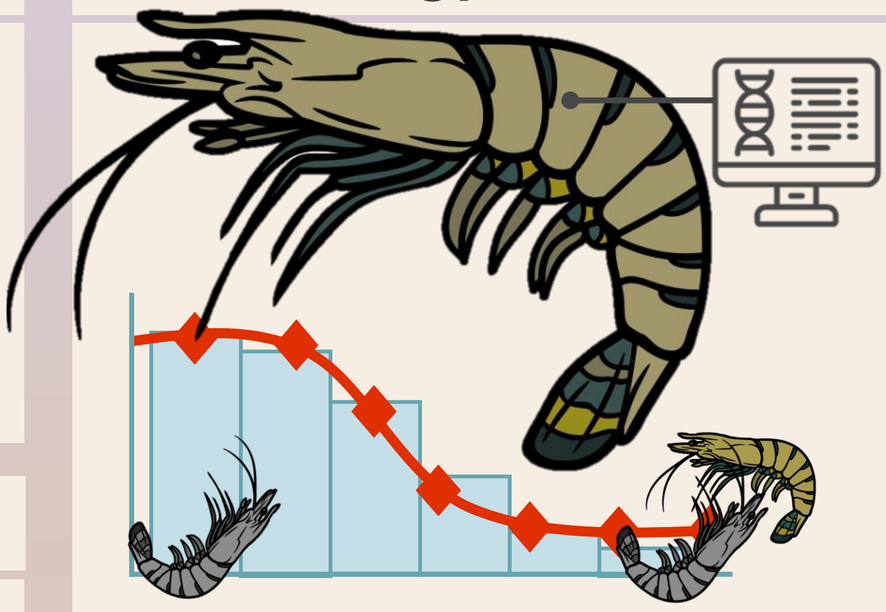
Although draft assemblies of *Penaeus monodon* have been reported in the literature, their quality still requires improvement. High-quality genome assemblies are essential for genetic improvement programs, especially for identifying genetic markers linked to key production-related traits.

Structural variations (SVs) represent substantial differences in genomic structure between two genomes, ranging in size from about 50 base pairs to well over a megabase. SVs have been linked to phenotypic traits, as well as growth, production, and immune-related traits in animals. In this study, SVs were identified in the form of copy number variations (CNVs).

Aims

This study aimed to develop genomic resources specific to a Philippine sample of the black tiger shrimp, thereby advancing genetic improvement efforts for this species. Specifically, the objectives were 1) to assemble and annotate the genome of the black tiger shrimp using various approaches and 2) to identify structural variations associated with WSSV resistance.

Methodology



Genome Assembly
- Illumina, PacBio, Oxford Nanopore Technologies (ONT)
- Meta-Assembly Approach

Structural Variation Discovery
- WSSV resistance (time-to-death)
- Extreme-phenotype (XP) CNV, Pool-based CNV analysis

Scan the QR code for the schematic diagrams of the genome assembly, annotation, and the CNV-trait association analysis

Results

Improved genome assembly of the Philippine black tiger shrimp via assembly merging and linkage-based approaches

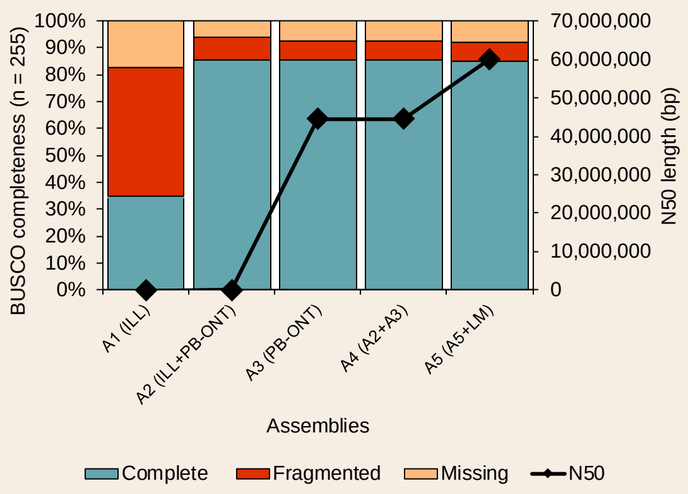


Fig. 1 Assembly quality and characteristics of the Philippine *P. monodon* genome. Contiguity and BUSCO completeness of assemblies. A1 is assembled from Illumina sequqence data using SparseAssembler; A2 is derived from a hybrid assembly of Illumina, PacBio, and ONT sequence data using DBG2OLC; A3 involves reference-assisted assembly utilizing PacBio sequence data with Rebaler; A4 is the outcome of merging A2 and A3 via quickmerge; while A5 anchors scaffolds to a linkage map.

- **2,035 scaffolds** were anchored to **44 pseudochromosomes** spanning a total length of **2,076,493,409 bp**, representing **83.94% of the total assembly.**

Table 1 Assembly statistics of *P. monodon* genomes.

Metrics	Philippines	Thailand	Australia
No. of contigs	533,304	1,149,650	43,387
Largest contig	2,172,420	1,387,722	1,180,159
Total length of contigs	2,195,463,701	2,001,525,299	1,889,120,549
Contig N50	105,155	45,036	97,812
No. of scaffolds	22,537	26,875	31,922
Largest scaffold	131,870,385	65,869,259	21,701,236
Total length of scaffolds	2,473,481,909	2,394,331,783	1,894,853,049
Scaffold N50	54,020,063	44,862,054	496,398
GC(%)	33	31	36
Complete BUSCOs (%)	85	89	87
Complete and single-copy BUSCOs (%)	82	87	86
Complete and duplicates BUSCOs (%)	3	2	1
Fragmented BUSCOs (%)	7	5	5
Missing BUSCOs (%)	8	6	9
No. of protein-coding genes	26,154	30,038	25,809
No. of genes annotated in interproscan	24,297	20,615	17,158

- This assembly has **fewer number of scaffolds and more contiguous** than the Thai (Uengwetwanit et al., 2021) and Australian (Huerlimann et al., 2022) assemblies.

- The improved genome assembly for *P. monodon* was achieved through our assembly merging and linkage-based approaches. The rationale behind employing a linkage-based assembly lies in the ability to utilize the mapping positions of linkage markers for correcting chimeric contigs and scaffolds into chromosomes.

Body weight and source of cultured shrimp may be linked to WSSV resistance

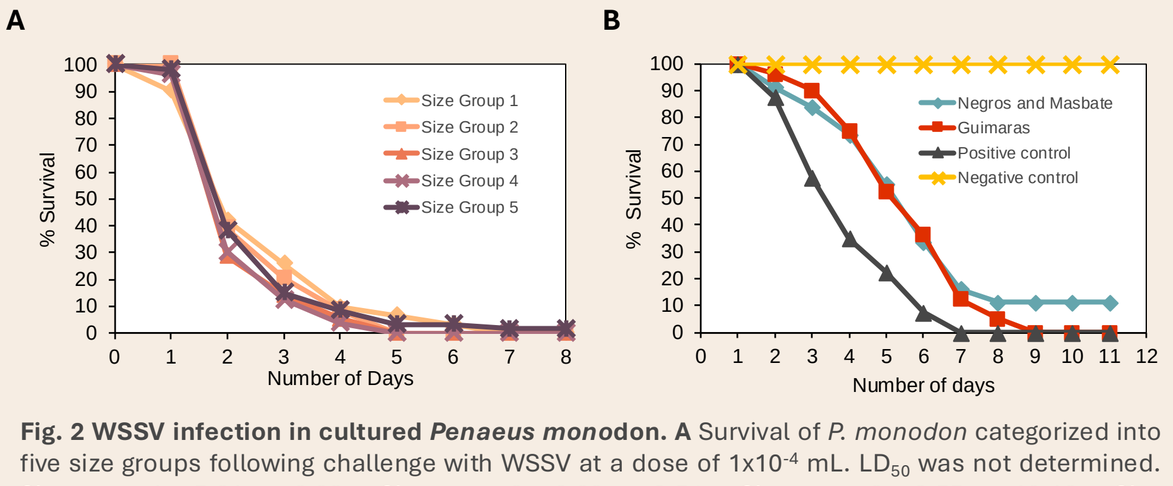


Fig. 2 WSSV infection in cultured *Penaeus monodon*. A Survival of *P. monodon* categorized into five size groups following challenge with WSSV at a dose of $1x10^{-4}$ mL. LD_{50} was not determined. Size group 1 = 5.31 g – 6.92 g; Size group 2 = 6.93 g – 8.54 g; Size group 3 = 8.55 g – 10.16 g; Size group 4 = 10.17 g – 11.78 g; Size group 5 = 11.79 g to 13.55 g. **B** Survival rates of *P. monodon* from various sources when challenged with White Spot Syndrome Virus (WSSV) at a lethal dose (LD_{50} = $1x10^{-5.5}$ mL)

354 genes identified with significant differential read depths between susceptible and resistant sample pools

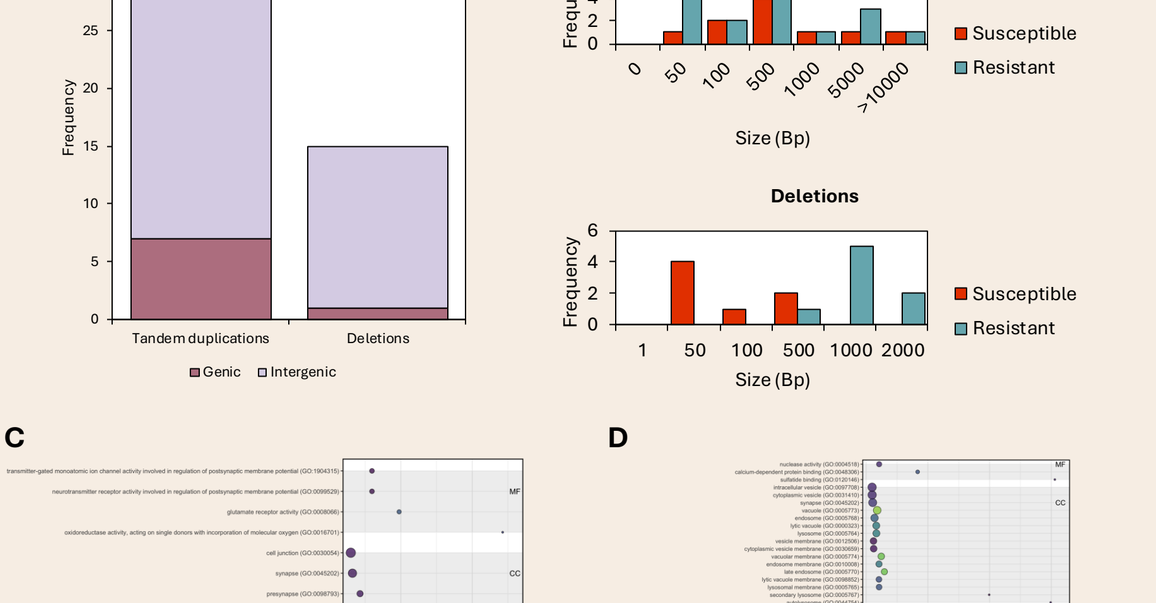


Fig. 3 CNV-trait association analysis for WSSV resistance employing read depth method. A Proportion of Up-CNV and Down-CNV genes in susceptible and resistant pools. **B** Gene Ontology enrichment analysis of candidate CNV genes.

Tandem duplications predominate, while larger deletions are mainly observed in resistant pools

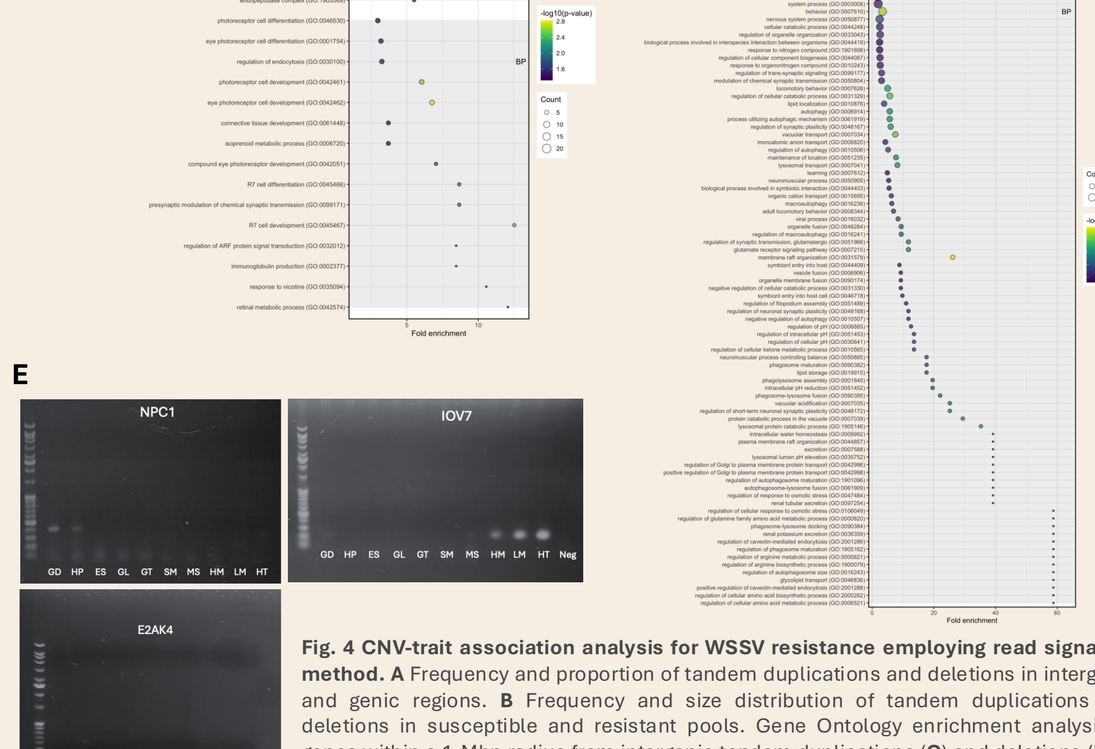


Fig. 4 CNV-trait association analysis for WSSV resistance employing read signature method. A Frequency and proportion of tandem duplications and deletions in intergenic and genic regions. **B** Frequency and size distribution of tandem duplications and deletions in susceptible and resistant pools. Gene Ontology enrichment analysis of genes within a 1-Mbp radius from intergenic tandem duplications (**C**) and deletions (**D**) in the resistant pools. **E** PCR amplification of candidate genes (Genes within 1 Mbp radius of a CNV) using synthesized cDNA from different tissues.

Conclusions

- This study constructed the first genome assembly for *P. monodon* populations in the Philippines, thereby augmenting the available genomic resources for the black tiger shrimp.
- Identified variations serve as a crucial resource for developing genetics-based strategies for improving production of the *P. monodon* in the country.

Acknowledgements
Inland Aquatic Resources Research Division, DOST-PCAARRD
Marine Genomics and Molecular Genetics Laboratory

References
Uengwetwanit, T., et al. (2021). *Molecular ecology resources*, 21(5), 1620-1640.
Huerlimann, R., et al. (2022). *G3*, 12(4), jkac034.

Contacts
kecadorna@up.edu.ph
aolluisma@up.edu.ph
@kreighcadorna