

The genome sequence of *Vibrio parahaemolyticus* C5A causing acute hepatopancreatic necrosis disease in shrimps isolated from a Malaysia shrimp culture pond

Md. Ali Amatul-Samahah^{a,b}, Sarmila Muthukrishnan^a, Nurhidayu Al-saari^c,
Natrah Fatin Mohd Ikhsan^a, Mohd Zamri-Saad^d, Mohamad Noor Amal Azmai^e,
Mohd Termizi Yusof^f, Ina-Salwany Md. Yasin^{a,g,*}, Mami Tanaka^h, Sayaka Mino^h, Tomoo Sawabe^h

^a Department of Aquaculture, Faculty of Agriculture, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia

^b Freshwater Fisheries Research Division, Fisheries Research Institute Glami Lemi, Jelebu 71650, Malaysia

^c International Institute For Halal Research And Training, International Islamic University Malaysia (IIUM), 53100 Jalan Gombak, Selangor, Malaysia

^d Department of Veterinary Laboratory Diagnosis, Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

^e Department of Biology, Faculty of Science, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

^f Department of Microbiology, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

^g Aquatic Animal Health and Therapeutics Laboratory, Institute of Bioscience, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia

^h Laboratory of Microbiology, Faculty of Fisheries Sciences, Hokkaido University, 3-1-1 Minato-cho, Hakodate 041-8611, Japan

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ABSTRACT

We report the complete genome sequence of *Vibrio parahaemolyticus* strain C5A causing an acute hepatopancreatic necrosis disease (AHPND) in *Penaeus vannamei* sampled from a culture pond in the east of peninsular Malaysia isolated in 2017.

1. Introduction

Vibrio species can be found ubiquitously in various aquatic environments and are associated with a lot of aquatic animals such as fish, crustaceans and molluscs (Parthasarathy et al., 2016). Among *Vibrio* species, *V. parahaemolyticus* is widely distributed in marine environment and mostly associated with gastrointestinal infection in human after consumption of contaminated seafood (Guin et al., 2019). *V. parahaemolyticus* is also included in one of the known pathogenic *Vibrio* spp. associated with vibriosis in aquaculture (Anjay et al., 2014) causing high economic loss to the industry (Mohamad et al., 2019; Amatul-Samahah et al., 2020).

V. parahaemolyticus has been identified to be the agent of AHPND in shrimp (Tran et al., 2013; Joshi et al., 2014; Kumar et al., 2020). However, only the strains of *V. parahaemolyticus* that produces and releases a certain toxin can cause AHPND. The toxin is homologous to the Pir (Photorhabdus insect-related) binary toxin consisting of two subunits, PirA and PirB (Lee et al., 2015; Han et al., 2015; Sirikharin et al., 2015; Ahmmed et al., 2019). Though *V. parahaemolyticus* was the first *Vibrio* species discovered to carry the plasmid (Sirikharin et al., 2015), other *Vibrio* sp. have also been found to carry the virulent plasmid (Kondo et al., 2015; Liu et al., 2015; Restrepo et al., 2018), and recent findings have clearly demonstrated the occurrence (Dong et al., 2019).

Abbreviations: AHPND, acute hepatopancreatic necrosis disease; BLAST, Basic Local Alignment Search Tool; bp, base pair; CDS, coding sequence; DNA, deoxyribonucleic acid; Gc, guanine-cytosine; m, meter; NaCl, sodium chloride; ORF, open reading frame; ppt, part per thousand; rRNA, ribosomal ribonucleic acid; tRNA, transfer ribonucleic acid; WGS, whole genome sequencing

* Corresponding author at: Aquatic Animal Health and Therapeutics Laboratory, Institute of Bioscience, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.

E-mail address: salwany@upm.edu.my (I.-S.Md.-M. Yasin).

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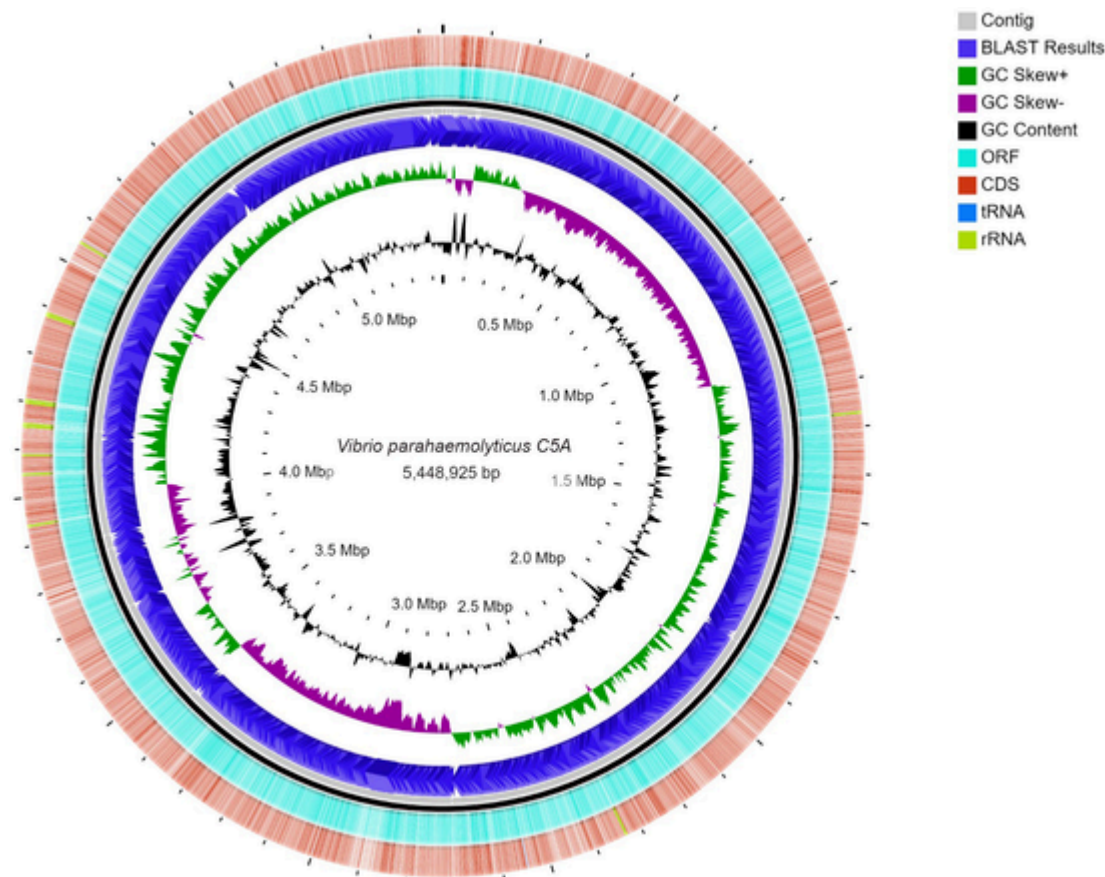


Fig. 1. Circular map of the *V. parahaemolyticus* C5A genome. From the outermost circle to the centre: CDSs on forward strand (including tRNA and rRNA), open reading frame (ORF), BLAST results, GC skew + and GC skew–, GC content and the marker of genome size is the innermost circle.

AHPND is a common threat in shrimp aquaculture industry. This disease imposed huge loss to shrimp aquaculture industry as it could cause huge mortalities up to 100% of post-larvae or juvenile shrimp during the first month after stocking (Tran et al., 2013). Since 2011 until now, there are still reports on the occurrence of this disease, indicating the threat of this disease is still happening (Amatul-Samahah et al., 2020; Muthukrishnan et al., 2019).

This disease normally would affect shrimp postlarvae and juvenile up to the first 30 days upon stocking in culture pond (Tran et al., 2013; Kumar et al., 2020). Based on general clinical observations, among the early signs of this disease are slow growth, loose shells, and discoloration (Kumar et al., 2020). AHPND-causing *V. parahaemolyticus* initially colonize in the stomach of infected shrimp and produce observable symptoms including lethargy, an empty stomach and midgut, and pale to white atrophied hepatopancreas (Lai et al., 2015). This disease causes degeneration of the hepatopancreas (necrosis) by sloughing its tubule epithelial cells, hemocytic infiltration and subsequently causing mortality of post larvae and juvenile shrimp (Tran et al., 2013; Kumar et al., 2020).

Whole-genome sequencing (WGS) allowed a detail and comprehensive study at the molecular level and increasingly becoming an important tool in aquaculture study. Here, we used WGS to identify and characterize the pathogen associated with AHPND outbreak in *P. vannamei* shrimp culture. We sequenced the genome of an acute AHPND-causing *V. parahaemolyticus* strain, C5A, isolated from shrimps obtained from a pond culture located close to the east coast area of peninsular Malaysia.

2. Isolation of the bacterium

The isolate has been screened and confirmed to carry the *pirA* and *pirB* gene which encodes for the AHPND virulence. Screening for AHPND positive strains were carried out using AP4 method. AP4 method is a two-step nested PCR screening of AHPND isolates (Dangtip et al., 2015). The method targeted the tandem genes of *pirA* and *pirB* on pVA plasmid (Lee et al., 2015). *V. parahaemolyticus* C5A was isolated and cultured on marine agar (Oxoid) pre-added with 1.5% NaCl, then incubated overnight at 27 °C for 18–24 h.

3. Genomic DNA isolation, sequencing and data assembly

Genomic DNA of the isolate was extracted using DNA kit (Thermo Fisher Scientific). The sequencing library was produced using the Rapid Barcoding Kit (SQK-RBK001) obtained from Oxford Nanopore Technologies, Oxford, UK for Nanopore sequencing guided by the instructions of the manufacturer's company. The library was later put into the MinION set via a flowcell FLO-MIN106 R9 version (Oxford Nanopore Technologies, Oxford, UK). After that, the sequencing was executed using MinKNOW version 1.7.14. Fast5s from Nanopore sequencing were basecalled using ONT Albacore Sequencing Pipeline Software version 2.0.2. Next, Porechop 0.2.2 (<https://github.com/rrwick/Porechop>) was used for debarcoding and adaptor trimming of the sequences. Nanopore assembly was carried out using Canu 1.6 (Koren et al. 2017) and Nanopolish version 0.8.1 (Fig. 1).

The reads were assembled de novo into 17 contigs of sizes using Canu 1.6 with error and mismatch correction (N50, 729,486 bp). The genome assembly of the isolate has a length of 5,448,925 bp and a GC content of 45.4%. Annotation of the genome with RAST (Rapid Annota-

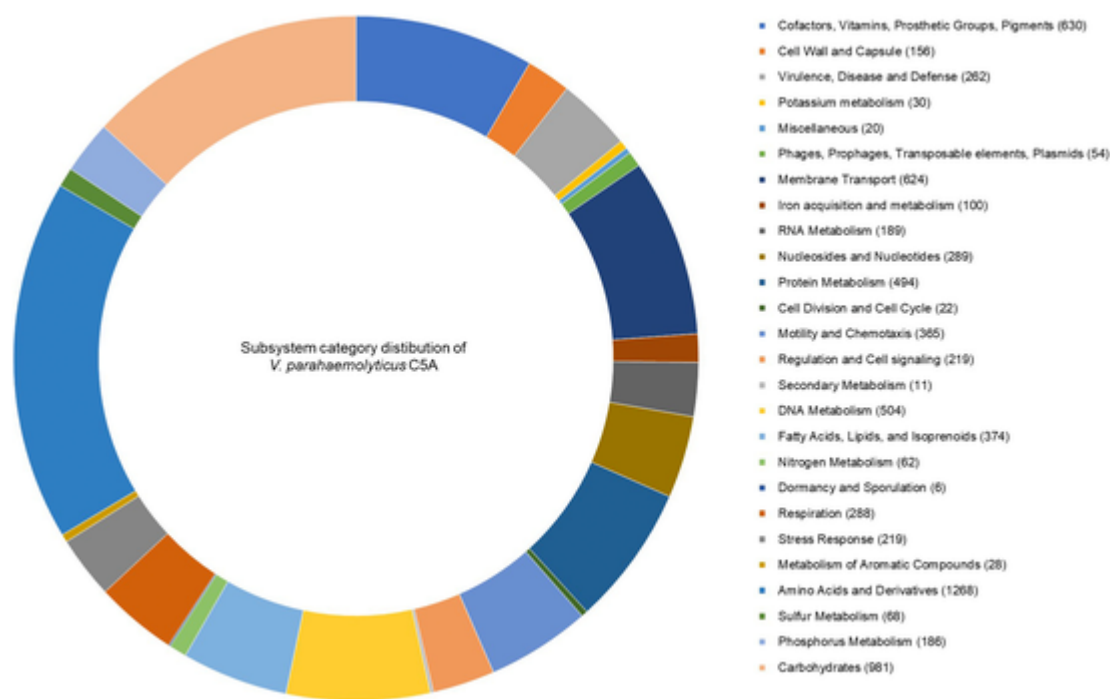


Fig. 2. The subsystem category distribution of *V. parahaemolyticus* C5A based on the SEED viewer.

tion using Subsystem Technology) 2.0 (Overbeek et al., 2014; Brettin et al., 2015) identified 403 subsystems, 13,936 coding sequences (CDS), 166 total RNAs in the genome. Of the total coding sequences obtained, 262 of the coding sequences involved in virulence, disease and defense mechanism of the bacteria. Besides that, 54 of the coding sequences were related to phages, prophages, transposable elements and plasmids (Fig. 2).

This genome sequence of C5A serves as another genome to further validate the occurrence of AHPND in shrimp pond in Malaysia and as an additional genomic information for comparative genomic studies among AHPND strains here in this country (Table 1).

3.1. Genome sequence accession number

This sequencing project has been deposited at DDBJ/ENA/GenBank with the accession number JAAIKJ000000000. It was submitted under the BioProject PRJNA606211 and BioSample, SAMN14087710 with the submission ID SUB6959648. The version described in this paper is the version, JAAIKJ000000000 (Table 2).

CRedit authorship contribution statement

The authors here declare their individual contributions:

Md. Ali Amatul-Samahah: Writing - original draft and editing. Sarmila Muthukrishnan: Investigation. Nurhidayu Al-saari: Investigation. Natrah Fatin Mohd Ikhsan: Validation and reviewing. Mohd Zamri-Saad: Validation and reviewing. Mohamad Noor Amal Azmai: Validation and reviewing. Mohd Termizi Yusof: Validation and reviewing. Ina-Salwany Md.Yasin: Validation, reviewing, editing & supervision. Mami Tanaka: Investigation, data curation, software & validation. Sayaka Mino: Data curation, software, validation & investigation. Tomoo Sawabe: Software, validation, investigation & supervision.

Uncited references

Chu et al., 2016

Table 1

General characteristics and genome sequencing project information of *V. parahaemolyticus* C5A.

Items	Description	
Classification	Domain	Bacteria
	Phylum	Proteobacteria
	Class	Gammaproteobacteria
	Order	Vibrionales
	Family	Vibrionaceae
	Genus	Vibrio
	Species	<i>Vibrio parahaemolyticus</i>
Gram stain	Negative	
Cell shape	Rod-shaped	
Pigmentation	Non-pigmented	
Sporulation	Non-sporulating	
Optimum temperature	25 °C	
Salinity	25 ppt	
Oxygen	Aerobic	
MIGS data		
Submitted to NCBI	GenBank	
Investigation type	Bacteria	
Project name	Genome of <i>Vibrio parahaemolyticus</i> C5A	
Collection date	May 2017	
Longitude and latitude	5° 26' 41.64" N, 102° 49' 55.056" E	
Geographic location name	Setiu, Terengganu, Malaysia	
Environment biome	Coastal area	
Environment feature	Shrimp culture pond	
Environment material	Water	
Depth	0.8–1.0 m	
Biotic relationship	Free living	

Stothard and Wishart, 2005

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Table 2Genome features of *Vibrio parahaemolyticus* C5A.

Items	Description
Genome size (bp)	5,448,925 bp
G + C content (%)	45.5%
CDS (coding sequences)	13,936
rRNA number	37
tRNA number	121

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References

- Ahmed, S., Khan, M.A.A.K., Eshik, M.M.E., et al., 2019. Genomic and evolutionary features of two AHPND positive *Vibrio parahaemolyticus* strains isolated from shrimp (*Penaeus monodon*) of south-west Bangladesh. *BMC Microbiol.* 19, 270. <https://doi.org/10.1186/s12866-019-1655-8>.
- Amatul-Samahah, M.A., Muthukrishnan, S., Omar, W.H.H.W., Ikhsan, N.F.M., Ina-Salwany, M.Y., 2020. *Vibrio* spp. associated with acute hepatopancreatic necrosis disease (AHPND) found in penaeid shrimp pond from east coast of peninsular Malaysia. *J. Environ. Biol.* 41, 1160–1170. [10.22438/jeb/41/5\(SI\)/MS.07](https://doi.org/10.22438/jeb/41/5(SI)/MS.07).
- Anjay, Das, S.C., Kumar, A., Kaushik, P., Kurmi, B., 2014. Occurrence of *V. parahaemolyticus* in marine fish and shellfish. *Indian J. Geo-Mar. Sci.* 43 (5), 887–990.
- Brettin, T., Davis, J.J., Disz, T., Edwards, R.A., Gerdes, S., Olsen, G.J., Olson, R., Overbeek, R., Parrello, B., Pusch, G.D., Shukla, M., Thomason, J.A., Stevens, R., Vonstein, V., Wattam, A.R., Xia, F., 2015. RASTtk: a modular and extensible implementation of the RAST algorithm for building custom annotation pipelines and annotating batches of genomes. *Sci. Rep.* 42, 206–214. <https://doi.org/10.1093/nar/gkt1226>.
- Chu, K.B., Ahmad, I., Siti Zahrah, A., Irene, J., Norazila, J., Nik Haiha, N.Y., Fadzilah, Y., Mohammed, M., Siti Rokhaiya, B., Omar, M., Teoh, N.P., 2016. Current status of acute hepatopancreatic necrosis disease (AHPND) of farmed shrimp in Malaysia. In: Pakingking, Jr., R.V., Acosta, Jr., B.O., de Jesus-Ayson, E.G.T. (Eds.), *Addressing Acute Hepatopancreatic Necrosis Disease (AHPND) and Other Transboundary Disease for Improved Aquatic Animal Health in Southeast Asia, 22-24 February 2016, Makati City Philippines*. Aquaculture Department, Southeast Asian Fisheries Development Center, Tigbauan, Iloilo, Philippines, pp. 55–59.
- Dangtip, S., Sirikharin, R., Sanganrut, P., Thitamadee, S., Sritunyalucksana, K., Taengchaiyaphum, S., Mavichak, R., Proespraiwong, P., Flegel, T.W., 2015. AP4 method for two-tube nested PCR detection of AHPND isolates *V. parahaemolyticus*. *Aquacult. Rep.* 2, 158–162. <https://doi.org/10.1016/j.aqrep.2015.10.002>.
- Dong, X., Chen, J., Song, J., Wang, H., Wang, W., Ren, Y., Guo, C., Wang, X., Tang, K.F.J., Huang, J., 2019. Evidence of the horizontal transfer of pVA1-type plasmid from AHPND causing *V. campbellii* to non-AHPND *V. owensii*. *Aquaculture* 503, 396–402. <https://doi.org/10.1016/j.aquaculture.2019.01.016>.
- Guin, S., Saravanan, M., Anjay, Chowdhury, G., Pazhani, G.P., Ramamurthy, T., Das, S.C., 2019. Pathogenic *V. parahaemolyticus* diarrhoeal patients, fish and aquatic environments and their potential for inter-source transmission. *Heliyon* e01743, 1-6.

<https://doi.org/10.1016/j.heliyon.2019.e01743>.

- Han, J.E., Mohny, L.L., Tang, K.F.J., Pantoja, C.R., Lightner, D.V., 2015. Plasmid mediated tetracycline resistance of *V. parahaemolyticus* associated with acute hepatopancreatic necrosis disease (AHPND) in shrimps. *Aquacult. Rep.* 2, 17–21. <https://doi.org/10.1016/j.aqrep.2015.04.003>.
- Muthukrishnan, S., Defoirdt, T., Ina-Salwany, M.Y., Yusoff, F.M., Shariff, M., Ismail, S.I., Natrah, L., 2019. *V. parahaemolyticus* and *V. harveyi* causing acute hepatopancreatic necrosis disease (AHPND) in *P. vannamei* (Boone, 1931) isolated from Malaysian shrimp ponds. *Aquaculture* 511, 734227. <https://doi.org/10.1016/j.aquaculture.2019.734227>.
- Joshi, J., Srisala, J., Truong, V.H., Chen, I.T., Nuangsang, B., Suthienkul, O., Lo, C.F., Flegel, T.W., Sritunyalucksana, K., Thitamadee, S., 2014. Variation in *V. parahaemolyticus* isolates from a single Thai shrimp farm experiencing an outbreak of acute hepatopancreatic necrosis disease (AHPND). *Aquaculture* 428, 297–302. <https://doi.org/10.1016/j.aquaculture.2014.03.030>.
- Kondo, H., Van, P.T., Dang, L.T., Hirono, I., 2015. Draft genome sequence of non-*V. parahaemolyticus* acute hepatopancreatic necrosis disease strain KC13.17.5, isolated from diseased shrimp in Vietnam. *Genome Announc.* 3, e00978-15. <https://doi.org/10.1128/genomeA.00978-15>.
- Kumar, R., Ng, T.Z., Wang, H.-C., 2020. Acute hepatopancreatic necrosis disease in penaeid shrimp. *Rev. Aquac.* 12 (3), 1867–1880. <https://doi.org/10.1111/raq.12414>.
- Lai, H.C., Ng, T.H., Ando, M., Lee, C.T., Chen, I.T., Chuang, J.C., et al., 2015. Pathogenesis of acute hepatopancreatic necrosis disease (AHPND) in shrimp. *Fish Shellfish Immunol.* 47 (2), 1006–1014. <https://doi.org/10.1016/j.fsi.2015.11.008>.
- Lee, C.T., Chen, I.T., Yang, Y.T., Ko, T.P., Huang, Y.T., Huang, J.Y., Huang, M.F., Lin, S.J., Chen, C.Y., Lin, S.S., Lightner, D.V., Wang, H.C., Wang, A.H.J., Wang, H.C., Hor, L.L., Lo, C.F., 2015. *V. parahaemolyticus*: an opportunistic marine pathogen becomes virulent by acquiring a plasmid that expresses a deadly toxin. *Proc. Natl. Acad. Sci.* 112 (34), 10798–10803. <https://doi.org/10.1073/pnas.1503129112>.
- Liu, L., Xiao, J., Xia, X., Pan, Z., Yan, S., Wang, Y., 2015. Draft genome sequence of *V. owensii* strain SH-14, which causes shrimp acute hepatopancreatic necrosis disease. *Genome Announc.* 3 (6), e01395-15. <https://doi.org/10.1128/genomeA.01395-15>.
- Mohamad, N., Amal, M.N.A., Salwany, M.Y., Saad, M.Z., Nasruddin, N.S., Al-saarif, N., Mino, S., Sawabe, T., 2019. Vibriosis in marine cultured fish: a review. *Aquaculture* 512, 734289. <https://doi.org/10.1016/j.aquaculture.2019.734289>.
- Overbeek, R., Olson, R., Pusch, G.D., Olsen, G.J., Davis, J.J., Disz, T., Edwards, R.A., Gerdes, S., Parrello, B., Shukla, M., Vonstein, V., Wattam, A.R., Xia, F., Stevens, R., 2014. The SEED and the rapid annotation of microbial genomes using subsystems technology (RAST). *Nucleic Acids Res.* 5, 8365. <https://doi.org/10.1038/srep08365>.
- Parthasarathy, S., Das, S.C., Kumar, A., 2016. Occurrence of pathogenic *V. parahaemolyticus* in crustacean shellfishes in coastal parts of Eastern India. *Vet. World* 9 (3), 330–336. [10.14202/vetworld.2016.330-336](https://doi.org/10.14202/vetworld.2016.330-336).
- Restrepo, L., Bayot, B., Arciniegas, S., Bajana, L., Betancourt, I., Panchana, F., Munoz, A.R., 2018. PirVP genes causing AHPND identified in a new *Vibrio* species (*V. punensis*) within the commensal *Orientalis* clade. *Sci. Rep.* 8, 13080. <https://doi.org/10.1038/s41598-018-30903-x>.
- Sirikharin, R., Taengchaiyaphum, S., Sanganrut, P., Chi, T.D., Mavinchak, R., Proespraiwong, P., 2015. Characterization and PCR detection of binary, Pir-like toxins from *V. parahaemolyticus* isolates that cause acute hepatopancreatic necrosis disease (AHPND) in shrimp. *PLoS ONE* 10 (5), e0126987. <https://doi.org/10.1371/journal.pone.0126987>.
- Stothard, P., Wishart, D.S., 2005. Circular genome visualization and exploration using CGView. *Bioinformatics* 21, 537–539. <https://doi.org/10.1093/bioinformatics/bti054>.
- Tran, L., Nunan, L., Redman, R.M., Mohny, L.L., Pantoja, C.R., Fitzsimmons, K., Lightner, D.V., 2013. Determination of the infectious nature of the agent of acute hepatopancreatic necrosis syndrome affecting penaeid shrimp. *Dis. Aquat. Org.* 105, 45–55. <https://doi.org/10.3354/dao02621>.