

***Pir*^{VP} genes causing AHPND identified in a new *Vibrio* species (*Vibrio punensis*)
within the commensal *Orientalis* clade**

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Figure S1. Phylogenetic reconstruction based on 16S rRNA gene, confirmed that the BA55 strain did not belong to the *Harveyi* clade. Percentage bootstrap values (1000 replicates) >85% are shown. The reference sequences were described by Sawabe et al.⁵ for *Harveyi* and *Orientalis* clades. Our results show that the BA55 strain grouped together with *V. brasiliensis* which is the clade with the majority of members of the *Orientalis* clade (red lines). Nevertheless, phylogenetic reconstruction based on 16S rRNA does not have sufficient resolution (**Fig. S3**).

Figure S2. Detection of *Pir*^{VP} genes associated with AHPND in *P. vannamei*. Lane 1: Macerates from hepatopancreatic tissue from shrimp belonging to BA55 strain of the challenge assay; lane 2: Macerates from hepatopancreatic tissue from shrimp belonging to negative controls of the challenge assay; lane 3: TSB stock for the negative control used in the challenge assay; lane 4: Macerates from hepatopancreatic tissue from shrimp belonging to *V. parahaemolyticus* BA94C2 strain of the challenge assay; lane M: DL1000 DNA marker.

Table S1. *Vibrio* genomes used in this study.

Figure S3. Phylogenetic reconstruction based only on 16S rRNA. Percentage bootstrap values (one thousand bootstrap replicates). Our results show that the phylogenetic reconstruction based on 16S rRNA does not have sufficient resolution to evaluate the relationship between *Vibrio* species.

Table S2. Genomic islands (GIs) characterized for the strain *V. punensis* BA55.

Figure S4. Phylogenetic reconstruction based on concatenated FtsZ, gapA, mreB, topA, and 16S rRNA sequences to evaluate the relation between species from *Orientalis* clade. The reference sequences were as described by Dubert et al.⁶¹ A Bayesian based

phylogeny on a concatenated multiple sequence alignment using the GTR+G+I evolutionary model is shown. Percentage bootstrap values (1000 replicates) >50% are shown. Bar, 0.02 expected nucleotide substitutions per site.

Table S3. DDH, ANIb and TETRA values.

Table S4. Primers used for amplification.

Figure S5. Phylogenetic reconstruction based only on 16S rRNA gene with all non-redundant sequences for *Vibrio* species. Percentage bootstrap values (one thousand bootstrap replicates).

Table S5. Bacterial count after challenge test. Morphological characteristics of the bacteria: Large size and convex isolated (LCI); Small size and convex isolated (SCI); Large and plain isolated (LPI).

Table S6. GenBank accession numbers of the sequences of five genes (gapA, ftsZ, mreB, topA and gyrB), used for the creation of phylogenetic trees.









