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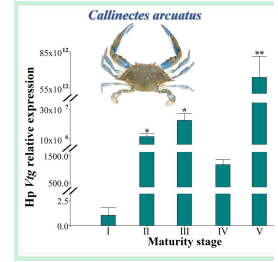
Female transcriptome from the Pacific blue swimming crab *Callinectes arcuatus* and vitellogenin expression at different gonadal maturity stages

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We use this protocol and it's working

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Abstract

The swimming crab is a commercially and nutritionally important resource. In Mexico, the East Pacific has the highest catch volumes. From the Pacific crabs of the genus *Callinectes*, the blue crab *C. arcuatus* has the widest distribution and can be found year-round. Its close resemblance to well-studied species like the Atlantic blue swimming crab *C. sapidus* makes it an excellent model for molecular reproductive evaluation in the Mexican Pacific. Through new generation sequencing, this study aims to characterize the transcriptome from female gonads and in particular the vitellogenin (*Vtg*) and its expression in the ovaries and hepatopancreas from *C. arcuatus*, at different gonadal maturity stages. Reproduction-related genes from *C. arcuatus* transcriptome correspond to 1.81%. Almost 200 proteins are reported for this species, 33 of them never reported before, including the complete *Vtg*. The expression of *Vtg* is tissue-specific, with levels in the hepatopancreas being 13 orders of magnitude higher than in the ovary. In the hepatopancreas, *Vtg* expression shows an exponential increase from stage I to stage V of gonadal maturity, while the pattern is the opposite in the ovaries. The hepatopancreas, with its abundant nutrient reserves, serves as an ideal site for *Vtg* expression and synthesis.

Sample collection

- 1 Samples were obtained with the help of local fishermen, each one with personal collection permit given by CONAPESCA (by its initials in Spanish: Comision nacional de pesca y acuicultura). To obtain mRNA transcription profiles throughout an entire year, five live females of *C. arcuatus* were collected monthly from Mazatlán, Sinaloa, from 2018 to 2019. All animals were handled according to ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines. The organisms were sacrificed by freezing and subsequently dissected to obtain hepatopancreas and ovaries tissues. Gonadal development was identified using morpho-colorimetric methods (Castañeda-Fernández et al. 2015). The tissues were preserved at -20 °C until further use.

RNA isolation, Illumina sequencing and bioinformatic analyses

- 2 In order to found a wide range of reproductive transcripts, total RNA from each organisms and each tissue (ovaries and hepatopancreas) was obtained to make a sample pool for next-generation sequencing (NGS). Total RNA was extracted from 100 mg of each tissue using the PureLink RNA Mini Kit following the manufacturer's instructions (Thermo Fisher Scientific). The integrity of the ribosomal RNAs was verified under native conditions in 1.5% agarose gels in 1X TAE buffer with 0.1% SYBR Safe (Invitrogen, USA), and electrophoresis was conducted using a Mini-Sub Cell GT Systems chamber (Bio-Rad, USA). Degraded samples were discarded, and only intact RNA was used for further analysis. Total RNA was treated with DNase I (Roche, USA; 1U/μg RNA) to remove genomic DNA, following the manufacturer's instructions.
The sample pool was submitted to Genoma Mayor, Universidad Mayor in Chile (Santiago de Chile). The library was constructed with the protocol TruSeq Stranded mRNA (Illumina, San Diego, CA) and sequenced in an Illumina MiSeq equipment following the manufacturer's instructions. First De novo assembly and ontological identification were conducted as previously described (Jimenez-Gutierrez et al. 2019).
A second De novo assembly and ontological identification was performed to confirm each sequence: the adapter sequences from each read and low-quality reads were first eliminated with the software Trimmomatic 0.39. The normalization was done with Trinity version 2.15.1 with the function: `<insilico_read_normalization.pl>`. The de novo assembled was carried out with the software Trinityrnaseq. TransDecoder

version 5.5.0 was used for ORF identification and Coding Sequence Prediction. Finally, to establish the correlation between the sequences assembled and their function, a BLAST (Basic Local Alignment Search Tool) alignment was done with software Diamond version 2.1.7 versus the “Nucleotide collection” database. The sequences of interest were individually verified for a third time through BLAST alignment in NCBI. Subsequently, the sequences of interest with a possible reproduction role were individually verified for a third time using the BLAST algorithm at NCBI.

mRNA expression of *C. arcuatus* Vtg

- 3 Specific primers were designed for β -actin as a housekeeping gene, and the double-verified *C. arcuatus* Vtg sequence using the FastPCR program (Supplementary material 1). For gene expression tests, at least four females were sampled for each stage of maturity. Total RNA was extracted individually as described above. Complementary DNA (cDNA) synthesis was performed on intact and treated RNA using the FirstStrand commercial system (Invitrogen, USA) following the manufacturer's instructions. PCR amplification of each gene was conducted using the TopTaq PCR Master Mix kit (QiagenTM, USA) with the following components: 12.5 μ L of TopTaq mix, 2.5 μ L of Coral dye, 1 μ L of each 20 μ M primer, cDNA template from each tissue, and milliQ water to reach a final volume of 25 μ L. PCR products were analyzed on 1.5% agarose gels and digitized using a Gel DocTM EZ photodocumenter (Bio-Rad, USA). First, Vtg amplification was performed, along with the constitutive β -actin gene of hepatopancreas and ovarian tissues. These samples were purified using Nucleospin columns (Macherey-Nagel, New Zealand) following the manufacturer's instructions. The concentration of the purified products was quantified using nanodrop. For absolute expression quantification, two samples with two replicates were amplified for each stage, resulting in four subreplicates. Along with the Vtg from each maturity stage, purified products were amplified. The absolute expression was quantified based on the optical density using the previously purified product of known concentration as a control, with the assistance of ImageTM Lab 2.0 software (Bio-Rad, USA). Subsequently, relative expression was calculated using the $2^{-\Delta\Delta CT}$ method with modifications according to Montes-Dominguez et al. (2021), with β -actin gene as the first control and stage I as the reference point (time zero) for the second control.

Statistical analysis



- 4 Data normality and homoscedasticity from *Vtg* expression values were checked, and a one-way analysis of variance (ANOVA) was conducted for the independent variable: stage of gonadal development. Subsequently, a post hoc comparison of means was performed using the Tukey-Kramer test ($P < 0.05$). All statistical analyses were carried out using STATISTICA software (StatSoft, EU), version 5.5, in the GLM (General Linear Model) section.