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Software usage testing for studies of genetic variability in marine shrimp aquaculture

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Short Abstract: An application was performed in order to test the GeneMapper® software v3.7 (Applied Biosystems) in the development of a genetic improvement program in Northeastern Brazil marine shrimp farms. Results are interesting for helping to implement rational breeding programs, contributing even for more general population genetic studies.

Long Abstract:
Currently most Internet online resources for investigating genetic information contain both bioinformatics tools and sequence data obtained from software usage. An application was performed in order to test a software on the development of a program of genetic improvement in Northeast Brazil, a region included among the top ten producers in terms of growth in aquaculture production according with FAO statistics. The research group chooses to test this flexible genotyping software package because it provides quality allele calls for all Applied Biosystems electrophoresis-based genotyping systems. The tested version software offers methods for analyzing microsatellite markers that contain di-nucleotide repeats studied in this work, with one of the most used marine shrimp species in world aquaculture. The variability constitutes the basis of all genetic improvement programs, and preliminary studies in commercial herds of peneids have demonstrated the presence of “genetic erosion”. Brazilian mariculture already suffers with the loss of performance due to this erosion that influences, for example, the reduction of growth rates of cultivated shrimp. Nowadays, Brazil holds a leading position in the worldwide marine shrimp production, but it does not make use of organized information about the genetic basis or breeding of animals. Therefore, there is a great need for studies aiming at guidance and directions towards the selection of breeders and the construction of lineages of high performance, which can confer sustainability and guarantee the increase on productivity. The main reasons for developing programs of genetic improvement in shrimp are: increase of the resistance to illnesses, increase of productivity rates and reduction of the dependence of wild broodstock. In this sense, one of the priorities must be the analysis of the genetic variability of wild populations; this can be used in the formation of families, as well as on the breeding in captivity. Regarding this, the molecular biology literature provides polymorphic markers already described for the species Litopenaeus vannamei. The motivation of the present study is to apply the GeneMapper® software v3.7 (Applied Biosystems) to generate strategies of formation of reproductive breeding of the marine shrimp from available germplasms. It aims at preservation of the variability level and at the design of basis for one program of regional genetic improvement,

in direct support to the sustainable development of the marine shrimp production in Northeastern Brazil. For this, the endogamy levels in the breeding will be initially diagnosed, through the use of microsatellite markers. Samples were collected from pleopods of L. vannamei domesticated broodstock; obtained from eight shrimp farms of four states of Northeastern Brazil. Genomic DNA was extracted and used in PCR assays to perform this initial microsatellite analysis for six loci previously reported in literature for this species. Oligonucleotides were previously tested, optimized and the forward primer was labelled with a fluorescent dye (FAM, HEX or NED) according the size of fragment. Primers identification and sequence with respective dye set was (5' to 3'): Lv8.193 [FAM]F: GATGTACACAACTGTACTTCG, R: GAGATGATAAGAGAACGAAAG; Lv8.2 [HEX]F: CCTCCTGTCCATTCAGCAG, R: GGTCAGATATGTATTCGAGTRCGG; Lv5.27 [NED]F: CAGACCCTAAATCTCCGTGC, R: TGGAAAGGTCAGAGGTCACG; Lv5.38 [FAM]F: CCTTTATGACTTCCCCCGAC, R: CCGTACAGAAACGGAACGTC; Lv8.32 [NED]F: TTACCGCCTAAGAGCGAATG, R: TGTCCTTTCGTACCAGTCAAG; and Pv0013 [HEX]F: TGCTCTGGTAACGACAAACG, R: AGACCTGTGGCGAAGTGC. Resulting amplification products were sized by capillary electrophoresis on an automated ABI 3100 Prism® 3100 Genetic Analyzer (Applied Biosystems). Prior to injection, PCR products were diluted 1:10 with ultra-pure water and mixed with GeneScan™-350 ROX™ size standards according to the manufacturer's instructions (Applied Biosystems). Genotypes were scored with the help of marker panel set options implemented in the program GeneMapper® v3.7 as well the visual inspection and manual corrections of allele peak data. Although these are undergoing studies, the resulting data have been very useful in order to provide possibilities of testing new microsatellite regions and more alleles. These will be interesting for mating analysis in rational breeding programs, contributing even for more general population genetic studies.