Urgent warning: Positive PCR detection results for infectious myonecrosis virus (IMNV) and decapod iridescent virus 1 (DIV1) in captured *Penaeus monodon* from the Indian Ocean

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Infectious myonecrosis virus (IMNV) was first described from the Americas as a lethal pathogen of the whiteleg shrimp *Penaeus vannamei* (Poulos & Lightner, 2006; Poulos, et al., 2006). However, it was also infectious but not lethal for *P. stylirostris* and *P. monodon* (Poulos Lightner, 2006; Poulos, et al., 2006; Tang, et al., 2005). It was subsequently introduced to Indonesia around 2006 (Senapin, et al., 2007) but has been slow to spread to other Asian countries (Sahul Hameed, et al., 2017; Senapin, et al., 2011). Decapod iridescent virus (DIV1)(Chen, et al., 2019) in *Exopalaemon carinicauda* was first described from China as *Cherax quadricarinatus* iridovirus/CQIV infectious for *C. quaricarinatus*, *Procambarus clarkii* and *P. vannamei* (Li, et al., 2017; Xu, et al., 2016) or as shrimp hemocyte iridescent virus (SHIV)(Qiu, et al., 2017; Qiu, et al., 2018) infectious for *P. vannamei*, *P. chinensis* and *Macrobrachium rosenbergii*. Thus, DIV1 has a wider known host range and is associated with disease in several economically important cultured species.

From a survey of wild, adult *Penaeus monodon* of potential broodstock size from the Indian Ocean in April 2018, we obtained positive nested RT-PCR test results for infectious myonecrosis virus (IMNV) (2/26 shrimp in one specimen lot) and positive nested PCR test results for DIV1 (5/26 shrimp in a different lot). The test results were obtained using nucleic acid extracted from pleopods (swimming legs) and the PCR protocols used were those previously published for SHIV (Qiu, et al., 2017) and IMNV (Poulos Lightner, 2006; Poulos, et

al., 2006); (Senapin, et al., 2007). The amplicon sequences from these tests were 99-100% identical to the matching regions published for the two viruses. As a confirmatory step, a second round of nested PCR tests was carried out using new, in-house primers designed from regions of the respective viral genomes distant from the target regions used in the first round of tests. These new tests had never been used previously in our laboratory and the positive and negative results for both viruses corresponded with those for the same individual specimens from the first round of testing for the partner target gene. Again, the amplicon sequences were 99-100% identical to the matching regions published for the two viruses.

These results suggested the possibility that the grossly normal, PCR-positive captured *P. monodon* specimens might be infected with the respective viruses at the carrier level. If so, they might serve as potential vehicles for introduction of IMNV and/or DIV1 into crustacean culture systems, especially if they were used in hatcheries for production of PL for distribution to shrimp farmers without proper precautions in place. It is already known that *P. monodon* may be infected with IMNV without showing gross signs of disease (Tang, et al., 2005) and our results suggest that the long presence of IMNV in Indonesia after its introduction around 2007 (Senapin, et al., 2007) may have resulted in its transfer from shrimp farms to grossly normal wild stocks of *P. monodon*. If this is so and if infectious IMNV is present in a significant portion of *P. monodon* in the Indian Ocean, it is possible that the recent outbreak of IMNV at a *P. vannamei* farm in Malaysia in June 2018 (WAHID, OIE) may have occurred as a result of this transmission pathway.

Although the presence of IMNV in wild *P. monodon* may be proposed to have arisen because of its long presence in Indonesia after introduction there around 2007, it is more difficult to hypothesize the pathway for occurrence of DIV1-positive specimens because the virus was first described from China less than 4 years ago (Qiu, et al., 2017; Xu, et al., 2016). If DIV1 is a newly emerging pathogen from China it seems unlikely that it could have spread to the Indian Ocean and reached a significant presence in the wild *P. monodon* population there in 3 years simply by movement of wild, infected shrimp. It also seems unlikely that DIV1 could have been endemic in *P. monodon* but been overlooked or not have caused any mortality in exotic *P. vannamei* since it became the dominant cultivated species from the early 2000's onward. This is especially so when one considers that *P. vannamei* went through several years of cultivation near and together with *P. monodon* without DIV1 disease outbreaks before and even after it grew to dominance.

Because we did not try to amplify and sequence the whole viral genome from the specimens, and because we did not do bioassays, we cannot confirm that the shrimp were actually infected with IMNV or DIV1. For example, we could have simply detected endogenous viral elements. However, we believe that our PCR results justify a precautionary warning regarding the possibility of introducing IMNV and DIV1 into aquaculture facilities via use of wild, captured *P. monodon* from the Indian Ocean. To avoid this possibility, we recommend that wild, captured *P. monodon* from the Indian Ocean intended for use as broodstock be subjected to PCR testing before use in a hatchery and that they be discarded, if they are found to be positive. If not positive, their larvae and post-larvae (PL) should be monitored for

presence of these 2 viruses periodically during production and again before they are sold to users. We also strongly recommend that industry practitioners using wild, captured *P. monodon* be discouraged from handling it together with broodstock of other crustaceans listed above in common maturation or hatchery facilities. We also recommend that shrimp farmers be discouraged from cultivating those species together with *P. monodon* in the same pond or on the same farm, especially if the latter originated from wild, captured broodstock that have not been tested for freedom from IMNV and/or DIV1 as applicable based on susceptibility of the specific species. Indeed, since domesticated stocks of *P. monodon* SPF for IMNV and DIV1 are available, we do not recommend the use of captured wild *P. monodon* broodstock for PL production at all. One reason is to prevent not only transmission of these two viruses, but also monodon baculovirus (MBV), hepatopancreatic parvovirus (HPV), white spot syndrome virus (WSSV) and yellow head virus (YHV). In addition, reducing the fishing pressure on shrimp broodstock should help to promote a more sustainable natural shrimp fishery.

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