# CHAPTER V

# CONCLUSIONS

1. SNPs in *PmCnn1*$_{530}$, *PmCnn1*$_{425}$, *PmCyC* and *PmCdc25* gene segments of BUM03, SNP3A and PM05 samples of *P. monodon* juveniles were examined using SSCP and DNA sequencing.

2. Relationships between SSCP patterns of *PmCnn1*$_{530}$ and average BW, TL, and HPW of SNP3A juveniles were found where shrimp carrying pattern I and II exhibited a greater average BW, TL, and HPW than those of shrimp carrying pattern III ($N$ = 156; $P < 0.05$). The relationships were not significant in BUM03 and PM05 samples ($P > 0.05$). Six intronic SNPS were identified in *PmCnn1*$_{530}$. Relationships between genotypes of each SNP of *PmCnn1*$_{F/R}$ and growth parameter indicated that the SNP3A shrimp with G/G$_{209}$ and (G/A)$_{209}$, T/T$_{210}$ and (T/A)$_{210}$, -/-$_{212}$ and (-/G)$_{212}$, -/-$_{213}$ and (-/T)$_{213}$, C/C$_{218}$ and (C/T)$_{218}$ and G/G$_{240}$ and (G/A)$_{240}$ had a greater average BW, TL and HPW than those with A/A$_{209}$, A/A$_{210}$, G/G$_{212}$, T/T$_{213}$, T/T$_{218}$ and A/A$_{240}$ ($P < 0.05$).

3. For *PmCnn1*$_{425}$, shrimp exhibiting SSCP pattern I showed a greater average BW and TL compared with those carrying other patterns (SNP3A, $N$ = 151; $P < 0.05$). Six intronic SNPS were identified in the *PmCnn1*$_{425}$ gene segment and can be categorized into 3 SNP diplotypes: D1$_{425}$, -/-$_{291}$-/-$_{292}$-/-$_{293}$A/A$_{294}$T/T$_{298}$-/-$_{315}$; D2$_{425}$, G/G$_{291}$T/T$_{292}$G/G$_{293}$C/C$_{294}$G/G$_{298}$G/G$_{315}$ and D3$_{425}$; (-/G)$_{291}$(-/T)$_{292}$(-/G)$_{293}$(A/C)$_{294}$ (T/G)$_{298}$ (-/G)$_{315}$. These diplotypes corresponded to shrimp exhibiting SSCP patterns I+V, II+IV and III, respectively. Juveniles exhibiting D1 showed a greater average BW and HP than those carrying D3$_{425}$ ($P < 0.05$). Moreover, those with diplotype D1$_{425}$ showed a greater average TL than those carrying diplotype D2$_{425}$ and D3$_{425}$ ($P < 0.05$).

4. The full-length cDNA of *PmCyC* was successfully identified by RACE-PCR and it was 1443 bp in length containing an ORF of 804 bp corresponding to 267 amino acids.

5. For *PmCyC*, the average BW and HPW of the SNP3A shrimp carrying SSCP pattern II was significantly greater than that of the shrimp carrying pattern I and III ($P < 0.05$). In addition, shrimp exhibiting this SSCP pattern also showed a greater average TL than those with pattern I ($P < 0.05$) but not with pattern III ($P > 0.05$). Nevertheless, the BU03 and PM05 samples exhibiting different SSCP patterns did not showed different growth parameter. Three exonic ($A/G_{31}$, $G/A_{379}$, and $T/C_{382}$) and two intronic ($T/C_{134}$ and $T/C_{188}$) SNPs corresponding to SSCP pattern I, II and III were observed in the *PmCyC* gene segment, respectively. Each SNP of the SNP3A shrimp with SSCP pattern II: $G/G_{31}(C/T)_{134}C/C_{188}A/A_{379}C/C_{382}$ had a significantly greater average growth parameters (except HSI) than those with each SNP of shrimp found in SSCP pattern I: $A/A_{31}C/C_{134}T/T_{188}G/G_{379}T/T_{382}$ and III: $(A/G)_{31}$ $(C/T)_{134}(T/C)_{188}(G/A)_{379}(T/C)_{382}$.

6. A total of 2, 1 and 2 SSCP pattern of *PmCdc25* were identified in SNP3A, BUM03 and PM05 samples, respectively. In the SNP3A sample, *P. monodon* juvenile with SSCP pattern I had a greater average BW, TL and HPW than those with pattern II ($P < 0.05$). The PM05 shrimp carrying different SSCP patterns did not reveal different BW and TL neither sexes of shrimp were regarded nor disregarded ($P > 0.05$). One SNP were found in *PmCdc25* of the SNP3A shrimp and those with $A/C_{243}$ genotype had significantly greater average BW, TL and HPW than those carrying $C/C_{243}$ genotype ($P < 0.05$).

7. PCR-RFLP was successfully applied for simple detection of SNPs in $PmCnn1_{530}$ and *PmCyC* of the SNP3A sample in this study.

8. The expression level of *PmCnn1* in hepatopancreas of 3-month-old *P. monodon* (SNP3A) exhibiting SSCP pattern III was significantly greater than those exhibiting SSCP patterns I and II ($P < 0.05$). For *PmCdc25*, the expression level of this gene in shrimp exhibiting SSCP pattern I was significantly greater than those exhibiting SSCP pattern II ($P < 0.05$).

9. Recombinant PmCnn1 protein was successfully expressed as the soluble protein in *E. coli*. The polyclonal antibodies against this recombinant protein was successfully produced in rabbit. Western blot analysis did not reveal a different level of PmCnn1 protein in hepatopancreas of SNP3A shrimp exhibiting different growing rates.

10. SSCP patterns and SNP genotypes of *PmCnn1*, *PmCyC* and *PmCdc25* was significantly related with growth parameters in at least one examined sample set. Accordingly, polymorphism of these genes can be applied as molecular markers to assist the genetic selection of the growth lines in *P. monodon*.