# Thesis Summary

## Phylogenetic relationship and population structure of Asian tiger frogs (genus *Hoplobatrachus*) from Bangladesh and neighboring countries elucidated by mtDNA and microsatellite markers

(ミトコンドリア DNA 及び、マイクロサテライトマーカーに基づくバングラデシュとその周辺諸国に産するトラフガエル類の系統関係及び集団構造に関する研究)

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The five frog species (*Hoplobatrachus tigerinus*, *H. litoralis*, *H. crassus*, *H. rugulosus* and *H. occipitalis*) of the genus *Hoplobatrachus* are widely distributed in Asia and Africa, with Asia being considered its origin. Among them, the Indian bullfrog *H. tigerinus* and its three congeneric species are common frog species and distributed throughout South Asia. Due to recent human activity, they are facing a changing environment and reduction in natural population size. However, genetic diversity and fundamental differentiation processes between and within species have not been studied. For effective conservation and molecular ecological studies, initially, we therefore isolated and characterized microsatellite loci for these frogs. For the isolation of microsatellite loci, we obtained genomic data using an Ion Torrent PGM sequencer and designed 54 PCR primer sets amplifying candidate loci. By genotyping these loci in individuals of *H. tigerinus* and its congeneric species, we screened and isolated 27 loci as highly polymorphic microsatellite loci. Eight of these loci were commonly applicable for all species except *H. chinensis*. These novel markers are considered useful for molecular ecological and conservation genetic studies of *Hoplobatrachus* species group across varying scales from inter-population to inter-individual.

Further, the evolutionary relationships of Asian *Hoplobatrachus* species remain ambiguous, and the fundamental knowledges about speciation processes and genetic population structure within

these species had been limited so far. Therefore, we conducted molecular phylogenetic analysis on Asian *Hoplobatrachus* frogs and population genetic analysis on *H. tigerinus* in Bangladesh using the mitochondrial CYTB gene and 21 microsatellite markers out of 27 newly designed primers. The resultant phylogenetic tree based on CYTB gene revealed monophyly in each species; notwithstanding the involvement of cryptic species in *H. chinensis* and *H. tigerinus*, which are evident by the higher genetic divergence between populations. For populations of *H. tigerinus* in Bangladesh, population genetic analyses using both CYTB and microsatellite markers were conducted. Although CYTB data showed moderate difference of haplotype frequency between Northern and Southern region, that pattern was not clear same as a previous study. While genetic distances between populations based on genotypic frequency showed significant correlation with the geographic distances. However, genetic distances were generally low. In addition, Bayesian inference of population structure by using the microsatellite loci revealed genetic divergence between western and eastern divided by two major rivers in Bangladesh, the Jamuna and Meghna rivers. This was also supported by statistical tests for correlation of two matrixes (Mantel test). Therefore, the populations of *H. tigerinus* in Bangladesh were restricted gene flow by the enormous rivers with 8-10 km of wides. However, this pattern was not clear in southern delta region, which should be resulted by the disturbance of seasonal change of river lines and/or frequent flood in this region. In addition, contrary to the barrier effect of the rivers, water flow itself could enhance directional migration from upper to lower regions (north to south) by transporting individuals. The Vola population, which identified as an outlier population in the DPR analysis, could be such a migration case.

Consequently, our findings clearly suggest that the environment specific to this river system has maintained the population structure of *H. tigerinus* in this region. In conclusion, the result of this study gives us a complete picture of the evolutionary forces shaping the genetic structure of the population and effective conservation of *Hoplobatarchus* species group.