TAXONOMIC STUDIES OF ASIAN HORNED FROG GENUS *MEGOPHRYS* KUHL AND VAN HASSELT 1822 FROM SUNDA SHELF

Abstract

The Asian horned frog genus *Megophrys* sensu lato Kuhl and van Hasselt (Megophryinae) inhabited the secondary to primary mountain forests, from eastern China to the eastern and southern Himalayas, throughout mainland Indochina and the islands of the Sunda Shelf in Indonesia to Palawan and Mindanao in the Philippines. Due to the lack of consensus of rank taxonomic definition, there has been a substantial disagreement regarding genus and/or subgenus within Megophryinae. Within the seven subgenera of *Megophrys* sensu lato, each of them has restricted distribution. Among the seven recognized subgenera, only three subgenera occur in Sunda Shelf and Philippines (i.e., *Megophrys*, *Pelobatrachus* and *Xenophrys*, consisting of which 13 species). The diversity of Asian horned frog is considered highly underestimated and contain a large number of undescribed cryptic species not only in China and their surrounding countries as the centre of their diversity, but also on Sunda Shelf.

The Sunda Shelf in the Malay Archipelago is a shallow continental shelf encompassing the island of Thai-Malay Peninsula, Sumatra, Java, Bali, and Borneo. In the past, these areas are linked by less than 200 m shallow sea during the last period of the Pleistocene. Past studies have been found that two historical events acted as the main influence on the speciation in the Sunda shelf. In this study, I conducted molecular phylogenetic, morphological and bioacoustics analyses to pursue the following questions regarding the Sunda Shelf *Megophrys* s.l.: (1) how many species exactly occur?, (2) when is divergence dates of major lineages and how have geological and climatological processes influenced the diversification of *Megophrys* in this region?.

Through a combination of extensive amphibian inventories, multi-scale DNA sequencing efforts, species delimitation analyses, morphological and bioacoustics studies of *Megophrys* s.l. that display a broad range of morphological similarities reveals a striking case of hidden genetic diversity. In this study, I identified support for the presence of eight putative new species in total within the Sunda Shelf *Megophrys* s.l. The second chapter of this thesis reevaluated the taxonomic status of widespread species *M. nasuta*

based on molecular, morphological and acoustic evidence. Analyses revealed that *M. nasuta* is a species complex that consisted of two distinct lineages. Those two lineages easily distinguished each other on the molecular, morphological and bioacoustics data. As a consequence, later I described one of the lineages that endemic to the highland of Southern and Northern Borneo as new species namely *Megophrys* (*Pelobatrachus*) *kalimantanensis.* Although this new species is geographically separated into southernmost and northernmost of Borneo populations, they are conspecific with low genetic variation.

The reassessment of the species diversity within the Sumatran *Megophrys* in Chapter 3 revealed more new species. With the extensive samples from Sumatra, I conducted mtDNA barcoding analyses and found three unnamed lineages only within the Sumatran *Megophrys* s.s. Later, combined with the morphological data we described two of them as a new species, namely *Megophrys acehensis* and *Megophrys selatanensis*. One remaining lineage is not described yet, since it is recognized as a species complex within *M. parallela*. Further species delimitation analyses and comprehensive taxonomic work on the previously detected species complex in Chapter 4 also resulted in more number of new species. Taxonomically, *M. paralella* should be divided into four species in near future; all of those four species is distributed within the central to northern Sumatra.

Multilocus divergence estimation suggested *Megophrys* s.s. diverged from *Pelobatrchus* s.s. in the late Oligocene to early Miocene, later the *in situ* diversification of *Megophrys* s.s. continued to the early Pliocene. The diversification of *Megophrys* s.s. began with the split of southern species and continued with the multiple colonizations in Sumatra, in addition, the southern species also migrated to western Java once time. Like in other amphibian species and other animal taxa in Sunda Shelf, the geographic distribution of Sunda Shelf *Megophrys* s.l. is thought to have been promoted through geographic isolation. The diversification in Sumatran and Javan *Megophrys* s.s. seem to have been separated by the discontinuity of suitable habitats, volcanic activities, and sea formation changes in the past. Our divergence time estimation suggests that Sunda Shelf *Megophrys* species have remained isolated from each other for several million years. The *Megophrys* s.s. shows consistent patterns in distribution and genetic diversity among isolated mountain peaks. *Megophrys* s.s. show high pairwise genetic diversity, lack of sympatry among species, mostly have allopatric distribution.

In the case of the diversification of Sumatran Javan *Megophrys* s.s., the ancestral lineages of these frogs were originally from Borneo. *Megophrys* s.s. seemingly migrated from the south to north in directions after they migrated from Borneo to Sumatra. On other hand, Javanese *Megophrys* were originally from Southern Sumatra that invaded western Java then occupied central and eastern Java. These patterns are different from the previous study, which found cascade frogs were migrated from Borneo to Java first then to Sumatra. Although both cascade frogs and *Megophrys* s.s. were revealed to have south-north diversification routes in Sumatra. Java, on the other hand, has arrived at its present configuration is much newer either than Sumatra and Borneo and was most likely colonized by southern Sumatran species only in the recent past.

Amphibians can serve as forest conservational indicators, because they are sensitive to environmental changes in general. The *Megophrys* frog can be one of the focal species, which is a critical component of conservation planning, along with the representation of ecosystems and ecologically sustainability management with another flagship species. My taxonomic study on *Megophrys* frog, which reveals the true diversity of this frog and also their narrow distribution information will contribute to future conservation activities.