Species diversity of subfossorial reptiles and amphibians in Sundaland

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Summary

Sundaland is one of the biodiversity hotspots in Southeast Asia, and is known for its extremely high biodiversity. In Sundaland, complex tectonic and climatic changes are thought to have played a major role in the formation of its current diversity in biota. Recently, molecular phylogenetic studies of amphibians and reptiles in Sundaland have revealed that many cryptic species exist and the taxonomic and genetic diversities of these groups are still underestimated. Also, these studies have showed that the species diversity of subfossorial reptiles and amphibians in leaf litter layer of Sundaland is higher than previously thought, thus it is becoming clear that even in ubiquitous and easily accessible environments such as leaf litter layer, herpetofaunal diversity is high, with species diversification continuing to occur. In general, thickness of the leaf litter layer positively correlates with the density of vertebrates therein, though leaf litter layer in tropical forests is much thinner than in temperate forests. This suggests that factors other than environmental diversity also contribute to the creation of tropical species diversity. Understanding the diversity of animals in the leaf litter layer in Sundaland may provide new insights into the formation of biodiversity in the tropics, but taxonomic studies on these subfossorial amphibians and reptiles are still substantially insufficient, probably due to the difficulty in collecting enough samples and in recognizing boundaries among many morphologically poorly differentiated lineages. Thus, I hypothesized species diversity of the relevant taxa is still underestimated. In this study, I conducted taxonomic studies of litter dwelling amphibians and reptiles to elucidate cryptic but actual diversity and their speciation pattern in Sundaland.

First, in Chapter 2, I conducted a taxonomic revision of *Calliophis intestinalis.* This is a small subfossorial snake, which exhibits high color variations depending on populations, resulting in a number of synonyms. On the other hand, the species is thought to be a species complex. I examined the external morphological characters of specimens of *C. intestinalis* and information on morphological characters was also taken from the previous studies. I sequenced mitochondrial ND4 gene of samples of the species and conducted phylogenetic analyses. Morphological examination and literature survey revealed *C. nigrotaeniatus*, which is treated as a synonym of *C. intestinalis* so far, was morphologically distinct from *C. intestinalis* and other congeneric species, and several synonyms described for *C. nigrotaeniatus*. Molecular phylogenetic analyses indicated that the Sundaic and Philippine *Calliophis* form a highly supported monophyletic group and that at least three undescribed species are included therein. *Calliophis nigrotaeniatus* was phylogenetically diverged from *C. intestinalis* and other congeneric species. Thus, I resurrected and redescribed *C. nigrotaeniatus*.

Next, in Chapter 3, a taxonomic study of *Larutia* was conducted. *Larutia* is a small to medium skink, highly adapted to fossorial or subfossorial life style and have no or extremely reduced limbs with no to two digits. Because of its secretive nature, it is difficult to find individuals of the

genus and several of its species are known only from one or a few specimens. I examined the morphology of specimens of *Larutia* species and also collected data for morphological characters. Comparative data were also obtained from relevant literature. I sequenced ND1 gene of samples of the genus and conducted phylogenetic analyses. Morphological comparisons of the genus revealed that the newly obtained specimen from Mt. Penrissen, Borneo was distinguished from all other species of the genus by having a unique combination of morphological characters. Molecular phylogenetic analyses revealed that the specimen was diverged from other species of the genus. Thus, I described the Mt. Penrissen specimen as a new species.

Next, in Chapter 4, a taxonomic reassessment of *Kalophrynus nubicola* was conducted. *Kalophrynus* is a small to medium-sized terrestrial or subfossorial frog. *Kalophrynus nubicola* is one of the smallest species of the genus distributed only in a highland on Mt. Mulu, Borneo and a previous study showed there were three phenotypes for the species, which were morphologically and acoustically distinguished from each other. However, no subsequent studies have determined the taxonomic status of each phenotype, although several herpetological surveys have been conducted at high elevations on Mt. Mulu, where the species occur. I examined the morphology of specimens representing all three phenotypes of the species and compared to each other as well as to other species of the genus. I sequenced mitochondrial DNA of the 16S rRNA gene and nuclear DNA of RAG1 gene of samples of the genus and conducted phylogenetic analyses. Also, I recorded advertisement calls of the three phenotypes from the three habitat ranges and compared them to each other and to calls of other species of the genus. Morphological analyses revealed the three phenotypes clearly distinguished from each other by snout-to-vent length. The three phenotypes were also clearly differentiated from other congeneric species. Molecular analyses indicated that the genus *Kalophrynus* was a highly supported monophyletic group and that it was divided into two highly diverged clades, the *K. nubicola* clade and a clade formed by all remaining species. The *K. nubicola* clade were composed of three subclades that correspond to the three morphologically distinguished phenotypes. Recorded calls were clearly distinct among the three phenotypes, and matched the three call types reported by the previous study. The three phenotypes were clearly morphologically, phylogenetically and acoustically distinct and two of them remain undescribed. I thus described them as new species, and redefined *K. nubicola* accordingly.

These results indicate that the diversity in at least several taxa of subfossorial reptiles and amphibians in Sundaland has been underestimated from species level to lineage level. In Sundaland, 26 new species of amphibians and 42 new species of reptiles have been described during the last five years, but among these new species, there are only nine species of fossorial or subfossorial reptiles and no fossorial or subfossorial species of amphibians. This indicates that the taxonomic study of reptiles and amphibians in the habitat like leaf litter layer in Sundaland is lagging. In addition, all of the undescribed species found in this study were distributed in montane areas, and it is confirmed that the species diversity is particularly high in the highlands of this region as was predicted by some previous studies. In montane areas of Malay Peninsula and Sumatra, so-called sky-islands, there are

examples of mountain-specific speciation in reptiles and amphibians, that has been caused through interruption of gene flows between populations by intervening lowlands. The montane area from Mt. Kinabalu in northern Borneo to the Sarawak-Kalimantan border in Central Borneo is likely to have acted as another group of sky-islands, similar to the Malay Peninsula and Sumatra. Furthermore, several reptile taxa such as *C. nigrotaeniatus* and *Lartia* showed a unique distribution pattern of being confined to the highlands of large land masses and on small adjacent islands. In glacial periods, these small islands are thought to have been connected to nearby landmasses by dropped sea level and acted as montane areas. Thus, during the period these taxa were widely distributed in montane areas including present islands. These taxa were then trapped by the islands and likely to show the present distribution pattern, due to the subsequent rise in sea level. No amphibian species have been found with this distribution pattern, possibly because amphibians are more vulnerable to environmental changes, such as aridity, than reptiles, and are more likely to disappear when left on islands with limited environmental diversity. In some cases, such as *K. nubicola*, speciation by elevation within a single mountain was observed. Such distribution patterns may have been constructed by past climate changes and sea level changes, and differences of distribution patterns probably reflect the differences in ecological requirements among the taxa. In addition, there were many examples of multiple congeneric fossorial or subfossorial species distributed sympatrically in several taxa, including the three taxa treated in this study. These sympatric species generally showed body size differentiation, ecomorphological differentiation, or acoustic divergences. Thus, most of the subfossorial reptiles and amphibians distributed sympatrically in Sundaland are thought to be able to coexist through ecological and reproductive differentiation.