SUMMARY

Osteological study of Setouchi salamander *Hynobius setouchi*

Sotaro Hara

This study aimed to unravel the of morphological diversification of the family Hynobiidae by surveying the skeletal characteristics and variation of a representative species of the genus *Hynobius* and comparing them with those of other genera. Present taxonomic information is often based on comparisons of a few characters observed by past techniques and most of the character states, especially osteological ones, were not revised until now. This situation had not been a great problem when the number of species in a given taxa was small. However, the family Hynobiidae is one of the most rapidly species-increasing taxa in Asian amphibians, and just come to the time to revise and renew the taxonomy inside the family thoroughly (Chapter 1 and 2). Therefore, I described all the skeletal elements found in the family Hynobiidae in detail using the Setouchi salamander *Hynobius setouchi* (Chapter 3). The genus *Hynobius* is the most species in the family and the species has a general morphology of the genus. The samples used in this study were introduced in an artificial pond located in the forest of Kyoto University, and the population has been breeding stably under semi-natural conditions until now and its collection will not affect the decreasing number of salamanders in the wild. Then, variation in external and skeletal morphology related to growth, maturation, and sex were examined (Chapter 4). Finally, I revealed the details of intraspecific variation in skull elements, which are particularly important for taxonomy (Chapter 5), and re-evaluated the overall skeletal characters used in the taxonomy of the genus *Hynobius* (Chapter 6).

In Chapter 3, I mainly conducted qualitative observations, confirming what bones were present in the salamanders and also examining their variations. The results of this Chapter showed that the cranium was composed of 11 dermal investing bone elements and six endochondral bones, the mandible of three dermal investing bone elements and single Meckel's cartilaginous elements, the hyobranchial apparatus of six bone elements and cartilage, the vertebrae of five regions (single atlas, 15–16 trunk vertebrae, single sacrum, 2–3 caudo-sacral vertebrae, and 26–28 caudal vertebrae), the pectoral girdle of two bone plates and cartilage, the forelimbs of five regions, the pelvic girdle of one bone plate and a bone element, and the hindlimbs of five regions. These bone elements were well ossified than other genera of hynobiid salamander except the genus *Pachyhynobius*, and the posterior centrum keel of the trunk vertebra and the radial processes, which have never been observed in the genus *Hynobius*, were newly revealed. Most of the bone elements are ossified in all individuals, while the articular,

pubis, carpus, and tarsus were remained cartilaginous in smaller sized individuals. In addition, the part of the cartilaginous hypobranchial I and the cartilaginous coracoid of the pectoral girdle exhibited ossification in large individuals. This qualitative observation revealed few differences between the sexes, but the process on the flexor side of the radius in males was noticeably larger than in females.

Therefore, in order to clarify the differences between juvenile and adult skeletons and the differences between the sexes in Chapter 4, I mainly used the linear measurement analysis to evaluate them. There were no differences between sexes in juveniles. The head characters and atlas are already well developed in juveniles and tend to grow bradymetrically after maturation. The limbs of females grew bradymetrically compared with body size after maturation, compared with males. In addition, the radial and the posterior position of vertebrae of males grew tachymetrically compared with that of females. These sexual differences in growth formed a sexual dimorphism that adult males had larger and/or longer head and limbs than females. Especially, the process on the flexor side of the humerus of the forelimb was larger, which suggested that it might be associated with flexion movement in males. As for the tail, males had longer caudal vertebrae than females and a more flattened haemal arch. In contrast, females had larger numbers of caudo-sacral vertebrae and more posteriorly directed first haemal arch than males. These differences were suggested that might be related to reproductive behavior and these skeletal characters can be referred to as cryptic sexual dimorphisms hidden by the external morphology. Because the humerus and haemal arch differ significantly between the sexes, it is possible to sex the specimens in question, including fossils, using these skeletal characters.

Finally, the qualitative observations and the geometric morphometric analysis of the skull elements revealed the differences between juveniles and adults and the sexual differences in Chapter 5. Most of the juveniles showed the anterodorsal fontanelle (cavum internasale) and the skull roof fontanelle, the exposed lacrimal, and absent the articular. The occipital bone regions of the cranium were already developed after metamorphosis, and the maxilla of the upper jaw and the frontal region became larger toward adults. In addition, the squamosal and quadrate were located from anterior to posterior positions towards the adult, while the pterygoid extended anteriorly. The length of the inner branch relative to the outer branch of the vomer tooth series in the juveniles was shorter than in the adults and the number of teeth also increased from juveniles to adults. In adults, qualitative observations (Chapter 3) showed no difference between males and females. However, the nasal region was larger in adult males than adult females in this quantitative study. The nasal region may be a secondary sexual characteristic and associated with reproductive behavior. In contrast to the cranium, the shape of the mandible did not change from juvenile to adult, but the number of teeth increased from juvenile to adult.

Most of the taxonomic characteristics of the family Hynobiidae are based on skull elements, but only a few characters, such as the cavum internasale, skull roof fontanelle, lacrimal, and vomerine tooth series are used for actual identification, and some characters are still not validated for taxonomic utility. Hence, it is necessary to describe the skeletal characters in detail and compare them with other taxa of the family. From the above chapters, all of these taxonomic characters changed from juveniles to adults, and it was suggested that it is necessary to be careful when using them as taxonomic characters. Other than revising the traditional taxonomic characteristics mentioned above, I found several new characters that will probably be used as taxonomic characters, such as the stapes and the trunk vertebral process. These results indicate that some previously ignored characters are quite useful for species or genera diagnosis.

Especially in recent years, molecular information is often used to describe new species and higher taxa such as new genera or subgenera. It is worth to testify the taxonomic validity of such new taxa by investigating the newly found osteological characters. The skeletal morphology of the Setouchi salamander showed great intraspecific variation, and the developmental degree of each skeletal element caused intraspecific variation. These characters with great intraspecific variation have been used as discriminatory characters for other genera in the family Hynobiidae. This probably causes the diversity of genera in the family by diversifying the variable skeletal morphology that is related to feeding and reproductive behavior. Recently, we can obtain plenty of information of target gene by a high-performance next generation sequencer for understanding morphological evolution related to the gene. However, only genetic and developmental surveys cannot answer the evolutional question of why a character was evolved and/or a lineage of the target animal was divaricated into many species. In order to answer these questions, we need to integrate multiple research fields including osteology, development, genomics, ecology, and behavior, in the future study.