

Reproduction of *Chirixalus eiffingeri* (BOETTGER)

By

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(With 3 Text-figures)

INTRODUCTION

Chirixalus eiffingeri (BOETTGER) is a small arboreal species distributed on Ishigaki and Iriomote Islands located at the west end of the Ryukyu Archipelago as well as widely in Taiwan. This frog was first named by BOETTGER (1895) as *Rana eiffingeri*. Thereafter, the genus was changed from *Rana* into *Polypedates* by STEJNEGER (1907) and into *Rhacophorus* by NAKAMURA and UENO (1963). Seven years later, LIEM (1970) changed the genus of this species into *Chirixalus*.

While *Chirixalus eiffingeri* was considered to be distributed on the Ryukyu Archipelago at large by BOETTGER (1895), STEJNEGER (1907) and OKADA (1931), NAKAMURA and UENO (1963) has described that this species occurs on Okinawa Island, Ishigaki Island, Iriomote Island, etc. of the Ryukyu Archipelago and in Taiwan. OOKOCHI (1979) has confirmed that this frog is distributed on Ishigaki and Iriomote Islands of the Ryukyu Archipelago and in Taiwan. According to LUE and CHEN (1982), this species is abundant in northern, central and southern parts of Taiwan and lives in mountains lower than 2,000 m above sea level.

KURAMOTO (1972) has discovered that *Chirixalus eiffingeri* on Iriomote Island lays eggs in hollows of trees. OOKOCHI (1979) has also found that this frog lays eggs in water-containing joints of leaves in addition to hollows of trees. The same was described by LUE and CHEN (1982) in *Chirixalus eiffingeri* of Taiwan. The present author observed the spawning behavior of this species on Ishigaki Island and the development of embryos and tadpoles in the laboratory in order to elucidate the means by which this frog can reproduce in narrow hollows of trees. The results of these observations will be reported here.

MATERIALS AND METHODS

In December, 1976 and February, 1978, two female and nine male *Chirixalus eiffingeri* were collected by the present author in forests at 200~400 m above sea level of Mt. Omoto of Ishigaki Island. They were brought back to our laboratory and reared in a plastic container, 60 cm × 39 cm × 15 cm in size, equipped with a water pipe and a drainpipe. The bottom of the container was covered with sand at the height of about 2 cm, except about one-eighth of the bottom near the

drainpipe. A small amount of water always flowed into the container.

Tadpoles were reared in Cl-free tap-water of glass dishes 18 cm in diameter, a concrete tank 83 cm × 54 cm × 17 cm in size and laid outdoors, and the above plastic container together with adult frogs. Some tadpoles were preserved in NAVASHIN's fluid and cut into serial sections at 12 μ . The preparations were double stained with SMITH's hematoxylin and eosin.

OBSERVATION

I. Eggs

1. Egg-laying in the field

Eggs were laid in hollows formed on the trunks or at the forks of branches of trees, such as *Syzygium buxifolium*, *Castanopsis cuspidata* and *Ficus erecta*. A total of seven hollows including eggs of *Chirixalus eiffingeri* were discovered in 1976 and 1978 by the present author. These hollows were found at the height of 50~150 cm and contained a considerable amount of water. On the bottom of each hollow, there was an abundant deposit of fallen leaves and eroded substances. The water temperature was about 20°C in December, 1976 and about 16°C in February, 1978.

Most of the eggs were attached to the inner wall of the hollow above the water surface. The eggs attached to dry and wet positions of the hollow were similarly enveloped with their gelatinous membranes which contained sufficient water in store. In about half of the number of hollows, there were embryos or tadpoles which differed from each other in developmental stage. This indicated that egg-laying had been repeatedly performed during a short time in each hollow. For example, a mass of 34 morulae and a mass of 41 tail-bud embryos were found together with several tadpoles in a hollow at the height of 130 cm on the trunk of *Castanopsis cuspidata*. This hollow was about 10 cm in diameter, 32 cm in depth and contained water up to the height of about 20 cm. By examining 11 spawns attached to the walls of the hollows, it was found that each female laid 22~41 eggs, 32.5 eggs on the average, at the same time. The fertilization rate of eggs and viability during the embryonic stage seemed to be very high. In all the egg masses except one, no dead embryos were found. In the hollow including the exceptional egg mass, there were extremely abundant nematodes.

2. Egg-laying in the laboratory

Two mature females and nine mature males of *Chirixalus eiffingeri* were reared in a plastic container left indoors since February, 1978 (Fig. 1). Although this species is arboreal, both females and males hid themselves under broken pieces of unglazed wares. Occasionally, they climbed up the inner wall of the container. In this case, females often laid eggs one or two days later. Males gave mating calls from the wall of the container or from above or under the pieces of unglazed wares.



Fig. 1. A mature female
Chirixalus eiffingeri. $\times 1.2$



Fig. 2. Eggs attached to the wall of the plastic container.
 $\times 1.2$

The first egg-laying occurred after natural amplexus without injecting pituitary hormones. The two females, thereafter, laid eggs 11 times in total during 17 months. Amplexus is axillary. They laid eggs eight times on the inner wall of the container above the water surface and three times on the wet sand under the pieces of unglazed wares (Fig. 2). The eggs of a female (No. 1) collected in 1976 were 1.7 or 1.8 mm in diameter. The animal half of each egg was dark gray and the vegetal half was median gray. The eggs of another female (No. 2) collected in 1978 were 1.6 or 1.7 mm in diameter. While the animal half of each egg was dark gray, being similar to that of female No. 1, the vegetal half was remarkably darker than that of female No. 1.

Female No. 1 laid 42, 26, 43, 48, 16, 61 and 31 eggs on June 12, September 2, 1978, March 3, May 11, May 23, June 30, and October 30, 1979, respectively. Female No. 2 laid 26, 54, 55 and 36 eggs on August 1, 1978, February 25, May 11 and May 18, 1979, respectively. The eggs of 11 spawns were 438 in total and 39.8 on the average. Six spawns of female No. 1 and two spawns of female No. 2 were laid on the wall of the container, while one spawn of female No. 1 and two spawns of female No. 2 were laid on the wet sand. Of the eggs included in the eight spawns in total attached to the wall, 71.4~81.3%, 75.8% on the average, were found above the water surface, while the others were immersed in water. The gelatinous membrane of each egg laid above the water surface always had sufficient water in store. After egg-laying, a male was always found near the spawn. This male climbed up the wall at times and covered the eggs with his wet ventral surface. Such behavior of the male seemed to be a means of moistening the gelatinous membranes of eggs.

All the eggs of the two females began to develop regardless of the season and place of egg-laying. Of each spawn, 94.0~100% cleaved normally, and 92.5~

100% hatched normally.

II. Tadpoles

1. Food

As 34 eggs of *Chirixalus eiffingeri* carried back from Ishigaki Island in 1976 hatched normally in our laboratory, the newly hatched tadpoles were given boiled spinach, but they did not eat this kind of food in contrast to the tadpoles of a large majority of anuran species. Then, the present author gave them leaf mold, a piece of decayed wood, *Daphnia*, mosquito larvae, yeast, etc. However, the tadpoles ate none of these kinds of food. In spite of fasting, they lived for one

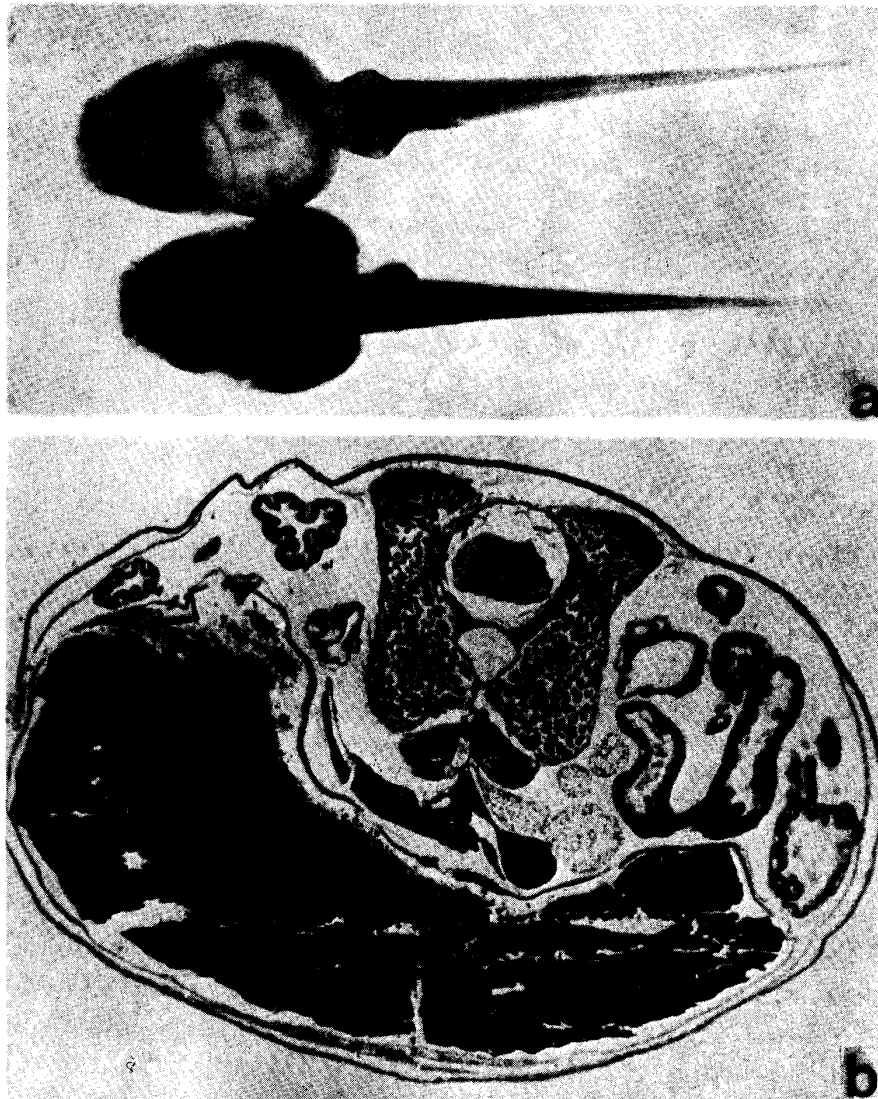


Fig. 3. Tadpoles of *Chirixalus eiffingeri*.

- a. Two tadpoles showing the dorsal and ventral surfaces. × 2.6
 b. A cross-section of a tadpole having a stomach dilated with a large amount of yolk. × 15

month or more, because they did not move. The tadpoles which hatched on June 12 and August 1, 1978 could not be reared with these food, too.

It was astonishing that 10 tadpoles left in the plastic container together with their parents and some other mature frogs were normally growing. When these tadpoles attained stage V (TAYLOR and KOLLROS, 1946), the contents of their stomachs and intestines were examined (Fig. 3a). The results showed that the stomachs were swelled up with some eggs and a large amount of yolk granules (Fig. 3b). In each stomach, no trace of rotifers, ciliates and minute nematodes was found, although these micro-organisms were abundantly contained in the water of the container together with the excretions of frogs. Thus, the tadpoles of *Chirixalus eiffingeri* appeared to feed on the eggs of the same species alone.

It became evident that tadpoles ate eggs obtained from females by the following way. When a female ready for spawning entered the pool, the tadpoles crowded at once around the female and vigorously poked at her skin surrounding the cloaca with their mouth situated at the anterior end of the head. A few tadpoles inserted their head ends into the cloaca as they were shaking the heads. Then, the female began to lay eggs little by little without amplexus by a male. The tadpoles made a rush for the laid eggs to eat them. The spawning of the female continued for about one hour, during which she did not change her position.

In order to confirm whether such a stimulating behavior of tadpoles is performed only at a female of the own species, the present author put a male of the same species and a female of each of *Rana nigromaculata*, *Rana japonica* and *Hyla arborea japonica* in a pool containing tadpoles of *Chirixalus eiffingeri*. The results showed that the tadpoles poked all the frogs. However, the reactions of the tadpoles remarkably differed with the frog species. The tadpoles reacted most vigorously to the male of the own species. A few tadpoles inserted their head ends into the cloaca of the male frog, although the latter ran away as soon as he was poked by the tadpoles. The reaction of the tadpoles to a female of foreign species was so weak that only a few tadpoles which were accidentally near the female gently poked the skin of the latter. All the females of the foreign species ran away at once when tadpoles poked their skin.

As a next trial, the reaction of *Chirixalus eiffingeri* tadpoles to the eggs of foreign species was examined. Some uterian eggs of *Rana nigromaculata*, *Rana rugosa* or *Hyla arborea japonica* were put in a dish which contained tadpoles. The results showed that tadpoles attacked and ate only the eggs which were accidentally near the tadpoles. When the gelatinous membrane of each egg was fully swollen, a tadpole could not eat the egg. When the swelling was at the beginning stage, the tadpole ate the egg, leaving the gelatinous membrane behind. From these experiments, it was evident that the tadpoles of *Chirixalus eiffingeri* could eat eggs of foreign species if the eggs were very fresh.

Owing to food shortage, it was considerably difficult to rear many tadpoles in our laboratory. While all the 26 eggs laid on September 2, 1978 hatched nor-

mally by September 10, 12 tadpoles began to grow normally and protruded their forelegs and climbed up after 56~70 days, 64 days on the average, after hatching. Of 54 eggs laid on February 25, 1979, 53 hatched normally by March 4. Only 22 began to grow normally and 10 protruded their forelegs and climbed up 62~85 days, 73 days on the average, after hatching. Of 55 eggs laid on May 11, 1979, 52 hatched normally by May 19, and thereafter only 11 tadpoles protruded their forelegs and climbed up 45~62 days, 60.5 days on the average, after hatching. The froglets immediately after metamorphosis were 9.5~10.2 mm, 10.0 mm on the average, in body length.

2. Digestive organs

The digestive organs of nine tadpoles at stage XIII (TAYLOR and KOLLROS, 1946) were examined in detail. These tadpoles were 8.5~9.8 mm, 9.2 mm on the average, in body length, while they were 27.0~32.2 mm, 30.5 mm on the average, in total length. The mouth was open at the anterior end of the head in contrast to those of the other Japanese rhacophorids. The tadpoles were $\frac{2}{1,1+1}$ in dental formula. Both the upper and lower tooth-rows were short and had sparsely arranged teeth. However, no degeneration was found in the jaws.

The stomach was sack in shape in contrast to tube in the tadpoles of other Japanese anuran species. The stomach was usually expanded with swallowed eggs and occupied the most part of the body cavity. Such a stomach was 8.1~9.6 mm, 9.0 mm on the average, in length and 4.8~7.0 mm, 6.2 mm on the average, in the largest width. While the yolk granules in the main part of the stomach were mostly in an unchanged state, those in the pylorus were at various stages of digestion. It is evident that the stomach of the tadpole of *Chirixalus eiffingeri* mainly functions as a food storing organ.

The intestine of the tadpole was also characteristic in being remarkably short and not spiral. It was 27.0~30.0 mm, 29.3 mm on the average, in length and arranged as that of the adult frog. The anterior half of the intestine was comparatively broad, being 0.6~0.7 mm in thickness, while the posterior half was slender, being 0.4~0.5 mm thick. The rectum was spindle-shaped as that of the adult frog. The ratio of the total length of the digestive tract to the body length was only 4.8 in contrast to 14.9 in *Rana nigromaculata* tadpoles, 10.1 in *Rana rugosa* tadpoles and 12.5 in *Hyla arborea japonica* tadpoles at the same stage.

The feces seemed to be small in amount. The rectum was filled with masses of bacteria. Many flagellates were found in the gut. These flagellates appeared to be species of the genus *Octmitus*, as they had eight flagella.

DISCUSSION

The existence of an anuran species which lays eggs in hollows of trees was first described by BARBOUR and LOVERIDGE (1928) and confirmed by NOBLE (1929). According to NOBLE, *Hoplophryne uluguruensis* which is a small East African brevi-

cipitid frog lays its eggs in old bamboo stems or between leaf and stalk of banana plants. The tadpoles raised from these eggs were probably exposed to air or possibly crowded into small basins of water. NOBLE has described that the tadpoles of *Hoplophryne* agree with those of some Jamaican hylas in feeding on frog eggs as well as insects and vegetable matters.

It has been reported by KURAMOTO (1972), OOKOCHI (1979) and LUE and CHEN (1982) that *Chirixalus eiffingeri*, a rhacophorid frog, distributed on Ishigaki and Iriomote Islands of Japan and widely in Taiwan, lays its eggs in hollows of trees and bamboos or between leaf and stalk of some plants. Although tadpoles of this species were found in the small basins of the hollows, their diets have not yet been elucidated.

By rearing the adults and tadpoles of this species, the present author could confirm the facts that the tadpoles feed on eggs of their own species alone, that the male displays an interesting behavior of protecting its embryos and, moreover, that the female and tadpoles collaborate to produce eggs as food for tadpoles. *Chirixalus eiffingeri* is one of the rare species which lays eggs in hollows of tree and bamboo or between leaf and stalk of some plants. In contrast to the tadpoles of some East African brevicipitids and Jamaican hylas which feed on frog eggs as well as insects and vegetable matters, those of *Chirixalus eiffingeri* seem to be specialized to eat no other than the eggs of their own species, as far as the specimens from Ishigaki Island are concerned.

Most of the fertilized eggs laid in hollows of trees attached to the inner wall above the water surface, while the remaining are immersed in water. Although both eggs above the water surface and those immersed in water can develop normally, the latter will soon or late become food for tadpoles contained in the hollow. The eggs and embryos developing on the inner wall above the water surface seemed often to be moistened by a male which climbed up from the water surface. Eggs as food for tadpoles seem to be supplied by a female which lays eggs according to stimulation from tadpoles. The site of the mouth at the anterior end of the head seems to be an adaptation of the tadpoles in poking around the cloaca of the female. The underdevelopment of the tooth-rows, short digestive tract and expansive stomach seem also to be adaptive structures for eating eggs. Similar characteristics of egg-eating tadpoles have been reported in African *Hoplophryne* and Jamaican *Hyla* (NOBLE, 1929).

SUMMARY

1. The reproduction of *Chirixalus eiffingeri* distributed on Ishigaki Island was observed in the field as well as in the laboratory.
2. This species lays eggs in hollows of trees which contain a considerable amount of water. The eggs are mostly attached to the inner wall of a hollow above the water surface. The eggs seem to be moistened with the wet ventral surface of a male which climbs up at times from the water surface.
3. The tadpoles of *Chirixalus eiffingeri* feed on eggs of their own species alone.

Eggs for food are laid by a female with stimulation from tadpoles which poke the skin surrounding the cloaca of the female with their mouths open at the anterior end of the head.

4. In the tadpoles, the tooth-rows are remarkably underdeveloped, the digestive tract is very short, and the stomach is sack in shape and usually expanded largely with swallowed eggs.

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