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Field Observation of Egg-laying Behavior of a Puddle Frog *Occidozyga sumatrana* from Bali, Indonesia (Anura: Dicroglossidae)

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Abstract: *Occidozyga sumatrana* is not uncommon in some parts of Southeast Asia but its reproduction in nature is poorly known. We observed egg-laying behavior of this species in Bali, Indonesia. The amplectic position was inguinal and the oviposition site was out of the water in *O. sumatrana*, both of which are unique given its phylogenetic position and the mainly aquatic habits of adults.

Key words: Behavior; Indonesia; Inguinal amplexus; *Occidozyga sumatrana*; Terrestrial oviposition

INTRODUCTION

The genus *Occidozyga* Kuhl and van Hasselt, 1822 occurs chiefly in Southeast Asia, and consists of a small number of tiny frog species living around still-waters such as puddles, marshes, or swamps. *Occidozyga sumatrana* (Peter, 1877), which is often confused with *O. laevis* (Günther, 1858), occurs in Sundaland (Iskandar, 1998; Frost, 2011). Although this species is very common in some areas, their reproductive ecology in nature has been poorly known. Here, we report a case of egg-laying behavior of *O. sumatrana* observed in a rice field in Bali, Indonesia.

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MATERIALS AND METHODS

Observations were made at a terraced rice field area in Ubud (8°27'34"S, 115°16'21"E), Bali, Indonesia, from the middle of the night to early morning of 4 August 2012. The area is scattered with patches of short trees and bushes. The dry season usually lasts from April to October in Bali, but it rained lightly on the day prior to our observations, and it was relatively cool and humid at night. The air and water temperatures near and in the channel where observations were made were 21.9C and 23.1C, respectively, at the beginning of observation.

We initially observed frogs with a small fluorescent lamp, but because this appeared to alter their behavior, we used a red LED lamp. However, after reproductive behavior began, we again used a fluorescent lamp for detailed observation and video recording (Sony HDW-750). After the observations were completed, we collected the frogs, deeply anesthetized them in saturated chloretone, and fixed in 10% formalin. For specimens later transferred to 70% ethanol, we took measurements of body size and made gross inspection of the condition of oviducts and ovaries of the female by dissection.

OBSERVATIONS

We found an amplectant pair of *O. sumatrana* in a small channel surrounded by rice fields. The channel with a muddy bottom was approximately 8 m in length, 20–70 cm in width, and 50 cm in depth. The water in the channel was shallow (<5 cm) and moving slowly, eventually flowing into the rice field. Both sides of the channel, which were nearly vertical, were formed by muddy soil and continued to the edge of the rice field. Some other frog species (*Microhyla palmipes* Boulenger, 1897, *Fejervarya* cf. *limnocharis*, *F. cancrivora* (Gravenhorst, 1829), and *Hylarana nicobariensis* (Stoliczka, 1870) were calling around the channel and rice field, but the calls of *O. sumatrana* and *M. palmipes* were heard

FIG. 1. An inguinally amplectant pair of *Occidozyga sumatrana* found in a rice field area of Bali.

FIG. 2. Egg-laying behavior of *Occidozyga sumatrana* in a hole above the water.

most frequently.

An amplectant pair of *O. sumatrana* (male SVL=29.5 mm; female SVL=38.0 mm) was found in the channel, where the width was 60 cm, at 0150 h. The male was holding the inguinal region of the female (Fig. 1). The pair stayed in the water close to the left wall, with their heads facing downstream. At this time, the pair was frightened by the light of the lamp and the male released the female.

At 0155 h, after we changed the lamp, the male called twice behind the female, jumped in front of her, and gave three calls. These calls sounded like normal advertisement calls (Matsui and Eto, unpublished data). Subsequently, the female jumped directly in front of the snout of the male, and they resumed amplexus (0200 h). Just after grasping the female, the male slightly shook his body for a short time. The pair started moving at 0242 h. First they jumped forward in a downstream direction several times, and then turned upstream at 0316 h. Then they turned again (0339 h) and moved downstream. At 0354 h, they climbed the left wall approximately 10 cm, and moved upstream on the wall. During this movement, the female occasionally pressed her head to the wall and dug the soil with her forelimbs. At this time we turned the fluorescent lamp on again for detailed observation and video recording. The pair moved down the wall at 0413 h, moved upstream, then turned their heads towards the left wall.

There was a small hollow (2.5 cm in maximum diameter) on the wall, 10 cm above the water. The female first put her head into the hollow, then turned her body. The posterior half of their bodies were put inside, with the rest of their bodies largely exposed. At 0419 h they started laying the first egg mass. The female inclined her head and elevated her pelvic region by standing on fully stretched hindlimbs, and then put the egg mass on the ceiling of the hollow. During oviposition, the body of the male was shifted anteriorly (downwards) so that the cloacae of both frogs were positioned closely together, although the forelimbs of the male still held the female's waist (Fig. 2). Oviposition was short, lasting approximately three sec. Egg diameter was about 1.2 mm and the number of eggs laid was eight.

The pair started moving again at 0423 h. They moved up and down the wall several times in an upstream direction. At 0431 h, they finally went into a small hole on the left wall head first. The hole was located at approximately 90 cm upstream from the first hollow, and was 10 cm above the bottom of the wall and 20 cm distant from the water. The maximum diameter of the hole was 4 cm but its depth was undetermined because it was winding inside. The hole might have been made by other animals (e.g., crabs or small mammals).

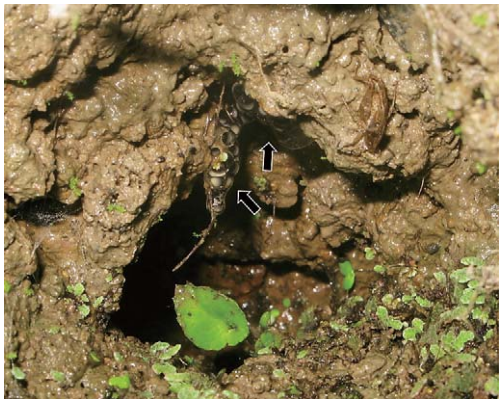

FIG. 3. Two egg masses (shown by arrows) that were successively attached to upper part of a hole by *Occidozyga sumatrana*.

The pair turned around in the hole, facing out of the hole, and started laying the second egg mass at 0440 h. The egg mass was laid on a grass root emerging from the ceiling of the hole entrance. At 0444 h the third egg mass was laid next to the second one, again on the ceiling of the hole (Fig. 3). There were 12 and 10 eggs in the second and third egg masses, respectively. At 0448 h, when the female stretched her hindlimbs and assumed an egg-laying posture, the male jumped to leave her. The female maintained the posture for a few sec, but then jumped into the water.

We found four more egg masses, each containing 10 to 14 eggs, around the channel and surrounding rice fields, and all of them were laid directly on muddy slopes or in holes close to water. No egg masses were found under the water despite our intensive search there.

The female had no eggs in her oviducts after the above breeding activity, but she did have a large number of eggs of various size in her ovaries. We also observed another pair of *O. sumatrana* (male SVL=26.5 mm; female SVL= 37.0 mm), which laid their eggs in a plastic bag after collection. A total number of 40 eggs in at least three separate masses were obtained, and no egg was found in the oviduct of the female.

DISCUSSION

From these observations, *O. sumatrana* in Bali is thought to lay a small number of eggs (about 30–40 at one breeding activity) on the wet ground close to the water in multiple small egg masses (each containing 8–14 eggs). However, it is possible that the two pairs we studied had already laid some egg masses before they were found and that the true clutch size of the female is larger. This is because we found many eggs of various sizes still left in ovaries in the females. The species may actually lay multiple clutches intermittently during a prolonged breeding season like some other anuran species (e.g., *Pelophylax porosa brevipoda* [Ito, 1941] [Matsui and Kokuryo, 1984]; *Fejervarya kawamurai* Djong, Matsui, Kuramoto, Nishioka and Sumida, 2011 [Sichi et al., 1988]).

The present observation, in which *O. sumatrana* adopts an inguinal amplexus, is interesting because this is not common in neobatrachians. Inguinal amplexus is universally seen in primitive frogs (archaeobatrachians or mesobatrachians), whereas most derived frogs adopt axillary amplexus with a few exceptions (Duellman and Trueb, 1994). Thus inguinal amplexus in *O. sumatrana* is thought to be a secondary modification among neobatrachians. Because a congeneric species *O. martensii* (Peters, 1867) also exhibits inguinal amplexus (Ziegler, 2002; Chanard, 2003), this amplectic style is probably common in this genus. However, little information about amplectic postures is available for the family Dicroglossidae Anderson, 1871 and whether or not inguinal amplexus is limited to the genus *Occidozyga* is unknown.

Another interesting issue is the terrestrial oviposition of *O. sumatrana* the adult of which is often found in or around the water and known to prefer aquatic habitats like its congeners (Iskandar, 1998). The functions of inguinal amplexus and terrestrial oviposition in *O. sumatrana* are not clear, but some suggestions about the amplectic position are provided by our observation. When the female

of *O. sumatrana* inclines her head and elevates the pelvic region by standing on fully stretched hindlimbs for oviposition, the male's body is forced to slide anteriorly resulting in his cloaca being located at nearly the same or little bit anterior position of the female's cloaca despite keeping an inguinal amplectic posture. This situation seems to be attained by the male's smaller body size than the female. If amplexus was axial, the cloaca of the male would be positioned too far forward of the female's cloaca, resulting in less efficient fertilization of eggs, which are apparently attached to the ceilings of holes or hollows. Thus, inguinal amplexus of *O. sumatrana* may be related to its sexual size dimorphism and terrestrial oviposition.

One reason for terrestrial oviposition might be to protect eggs from carnivorous conspecific tadpoles (Iskandar, 1998), although larvae of this species in Bali did not seem to favor frog eggs as far as we could determine (Eto, unpublished data). For more detailed discussion, it is necessary to assess the amplectic positions and oviposition sites of other species of *Occidozyga* and of dicroglossids in general.

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