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Copulation and egg retention in an oviparous Caecilian (Amphibia: Gymnophiona)

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Abstract

Viviparity (i.e., the bearing of live young) has evolved from oviparity (egg laying) independently in various major vertebrate lineages, and several transitional stages have been described. The transition from oviparity to viviparity requires the retention of fertilised eggs in the female reproductive tract. Caecilian amphibians (Gymnophiona) display a considerable diversity of reproductive modes, including oviparity and viviparity. Among amphibians, caecilians have also modified the process of internal fertilisation through a special intromittent organ, or phallus, in males. Here we report the oviposition of "embryonated" eggs ranging from various gastrula-to-neurula stages by female *Ichthyophis* cf. *kohtaoensis* (Ichthyophiidae) from North-eastern Thailand. In addition, we describe a copulation resulting in an oviposition of embryonated eggs. Our findings will have implications for the further understanding of the evolutionary reproductive biology of amphibians.

Keywords: *Ichthyophis* cf. *kohtaoensis*; Egg retention; Reproductive ecology; Oviparity; Embryoparity; Viviparity; Copulation. Gymnophiona

1. Introduction

Oviparity (i.e., the laying of unfertilised or fertilised eggs) and viviparity (i.e., the bearing of life young) are reproductive modes often co-occurring in major vertebrate lineages. Viviparity has independently evolved from oviparity in all three orders of extant Amphibia (e.g., Duellman and Trueb 1986). The distinction between oviparity and viviparity is unclear and controversial (e.g., Wourms and Lombardi 1992; Blackburn 1998; Greven 2002). However, viviparity has been defined as a "phenomenon in which young are carried in the genital tract of the females through part or all of the development" (Wake 1989, p. 236; see, however, the discussion in Packard 1989; Greven 2003). Thus, one of the steps in the transition from oviparity to viviparity is the retention of fertilised eggs in the oviduct. Oviposition of eggs containing embryonic developmental stages can be considered to represent a potential transitional step toward full viviparity.

The limbless, primarily subterranean caecilian amphibians (Gymnophiona) show a high diversity of reproductive modes, associated with parental care (e.g., Wake

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1977; Himstedt 1996; Wilkinson and Nussbaum 1998). It appears that all caecilian species, irrespective of whether they are oviparous or viviparous, practice internal fertilisation with the help of a specialised intromittent organ or phallus (Tonutti 1931; Wake 1972; Gower and Wilkinson 2002). Oviparous caecilians guard their egg clutches in terrestrial chambers (e.g., Sarasin and Sarasin 1887–1890) and either follow the presumed ancestral life cycle with aquatic larvae or develop directly without passing through a larval stage (e.g., Brauer 1897). Females of viviparous caecilians retain fertilised eggs in their oviduct, where embryogenesis is completed (e.g., Peters 1874; Parker 1956; Welsch et al. 1977).

The sequence of events in the reproduction of oviparous caecilians implies a lag time between fertilisation and oviposition of eggs, so that there is the potential for varying degrees of egg retention and development. However, little evidence for this has been reported thus far. Sarasin and Sarasin (1884) found eggs in late cleavage stages in the oviducts of *Ichthyophis glutinosus* (Ichthyophiidae), and Brauer (1897) also observed oviductal eggs in late cleavage stages in caeciliid *Hypogeophis rostratus* and *Grandisonia alternans*.

However, oviposition in caecilians has rarely been documented (Gans 1961). Any empirical data on developmental stages at oviposition would, therefore, significantly improve the understanding of amphibian reproductive biology. It might also inform debates on the oviparity–viviparity transition (e.g., Wourms and Lombardi 1992; Blackburn 1998) and on the evolutionary origin of the amniotic egg (Wilkinson and Nussbaum 1998; Laurin and Girondot 1999; Laurin et al. 2000; Wilkinson et al. 2002; Laurin 2005).

The Ichthyophiidae form the second-largest family of caecilians (Taylor 1968; Wilkinson and Nussbaum 2006). Based on several characters (e.g., the number of body rings, the presence of a tail, skull morphology, presence of larvae), they are believed to retain some morphological and reproductive traits that are regarded as ancestral for caecilians (Wilkinson and Nussbaum in press). Female ichthyophiids lay clutches of terrestrial eggs and guard them until they hatch as larvae that leave the nest to lead a semiaquatic life (Sarasin and Sarasin 1887–1890; Kupfer et al. 2004, 2005).

Here we report on several aspects of the reproductive biology, such as copulation and oviposition, of the South-east Asian *I.* cf. *kohtaoensis* and discuss their implications for our understanding of reproduction in vertebrates in general.

2. Material and methods

We studied the caecilian *I.* cf. *kohtaoensis* in the field in Khemmarat District of Ubon Ratchathani Province in North-eastern Thailand (see also Kupfer et al. (2004, 2005) for further details on the study site and the studied species). Fieldwork was carried out at the beginning of the rainy season, in May and June 2001. With the assistance by native farmers, we searched for specimens at the edges of various types of standing water bodies (i.e., temporary and permanent ponds, large pools, rice paddies) and near (i.e., 0.5–20 m) slow-flowing brooks and one river. Each site was searched for about 15–20 min. Adult *I.* cf. *kohtaoensis* were sexed using methods presented by Kramer et al. (2001).

Twenty-two females were collected and 11 of them kept alive individually in small, lidded plastic boxes ($30 \times 30 \times 25$ cm) containing moist paper towels and leaf litter. Holes were drilled in the lids for air ventilation. Four of these females were kept each with one male to monitor copulations. The boxes were kept at a mean temperature of 26.9 °C ($\pm$1.1 SD, range 24.1–28.7 °C) and were checked daily over 27 days between 06.00 and 09.00, 16.00 and 17.00, and 18.00–21.00 h. The developmental stage of eggs was determined under a stereomicroscope and according to the illustrations of Sarasin and Sarasin (1887–1890).

Some eggs (field numbers AK 01054-1084) were taken from each clutch, preserved in neutral-buffered formaldehyde, and placed into the collection of The Natural History Museum, London. The dimensions of the preserved eggs together with their capsules were measured under a stereomicroscope with a micrometer. The body mass of females was recorded with an electronic balance (Kern). The total length (TL) of adult caecilians was determined by placing animals that had been anaesthetised with MS 222 (3-aminobenzoic acid ethyl ester), 5% on plastic-coated graph paper.

3. Results

3.1. Mating

A single copulation was observed on June 2, 2001 (Fig. 1A). It was already in progress when our observations began (09:00 h). The copulating pair (TL male 357 mm, TL female 335 mm) was not hidden by either leaves or towels throughout the episode. From the start of the observation, copulation continued at least for another 45 min, but the end of the copulation was not observed. The pair was found separated at 10:00 h, by which time the male phallus was completely retracted.

3.2. Oviposition

We observed oviposition (Fig. 1B) in seven females. Some quantitative data are given in Table 1. One female