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Observations on amplexus in Congo Spiny Reed Frog *Afrixalus osorioi* Ferreira, 1906 (Anura Hyperoliidae) in the Yoko Forest Reserve, Democratic Republic of the Congo

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ABSTRACT Preliminary data on amplexus process in *Afixalus osorioi* Ferreira, 1906 (Anura Hyperoliidae) from observations conducted in June, July 2015, December 2020, July, September and October 2021 in the Yoko Forest Reserve, Democratic Republic of the Congo (DRC) are provided. Amplexus in this species occurs between 6:30 pm and 10:30 pm on vegetation overhanging permanent water bodies in disturbed habitats. These initial observations conducted in the Kisangani region regarding breeding substrates suitable for *A. osorioi* indicate that mating in this species occurs on vegetation located above permanent water bodies.

KEY WORDS *Afrixalus osorioi*; Amphibians; Amplexus; Vegetation; Yoko Forest Reserve.

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INTRODUCTION

The family Hyperoliidae includes several species of reed frogs or tree frogs (Spawls et al., 2019), among which *Afrixalus osorioi* (Fig. 1). This species is classified as "Least Concern" (Masudi et al., 2019; Musubaho et al., 2024a) and its geographic distribution extends across sub-Saharan Africa, notably in central-western Angola, Uganda, western Kenya, and much of the Congo Basin (Schiøtz, 1975; Dewynter & Frétey, 2019; Masudi et al., 2019; Spawls et al., 2019; Badjedjea et al., 2022; Isingoma et al., 2023; Musubaho et al., 2024a; Frost, 2025). *Afrixalus osorioi* occupies a variety of habitats, including forests, savannas, disturbed areas, clearings, along forest roads, around permanent or temporary watercourses (Lamotte & Lescure, 1977; Spawls et al., 2019; Musubaho et

al., 2024b), along forest streams, in clearings, fallows, and at edges, where individuals reproduce. Given that ecological and biological data on amphibians in Kisangani remain limited (Musubaho et al., 2024a), therefore, this communication provides the first information on amplexus in *A. osorioi* in this region.

MATERIAL AND METHODS

From May to July 2015, and from December 2020 to November 2021, we conducted surveys on amphibian diversity in the Yoko Forest Reserve (between 0°15' and 0°20'N and 25°14' and 25°20'E), located in southeastern Kisangani city, Tshopo Province, Democratic Republic of the Congo - DRC (Boyemba, 2011).

The method used for data collection was nocturnal capture, supported by three techniques, including visual survey, systematic excavation of habitats using a headlamp, and acoustic survey by hearing vocalizations (Musubaho et al., 2021; 2024a; 2024b). During these surveys, we also made six observations (in June, July 2015, December 2020, July, September and October 2021) on amplexus in *A. osorioi*.

RESULTS

Between 6:30 pm and 10:30 pm, mating individuals were recorded on the leaves of plant species, including *Trilepisium madagascariense* DC. (Moraceae), *Pauridiantha callicarpoides* (Hiern) Bremek. (Rubiaceae), *Harungana madagascariensis* Lam. ex. Poiret (Hypericaceae), and *Terminalia superba* Engler & Diels (Combretaceae), located around permanent water bodies. On these leaves, at a height ranging from 0.5 to 1.5 meter, the males emitted calls to attract and guide the females. Upon their approach, the males seized the females (Fig. 2).

The males then clung to the backs of females by passing their forearms under these partners' armpits, thus performing axillary amplexus (Fig. 3). These stages lasted between 30 and 45 minutes across the six observations, after which the males

Figure 1. *Afrixalus osorioi* recorded in June 2015 around water puddles in the Yoko Forest Reserve, Democratic Republic of the Congo (photo by L. Musubaho).

Figures 2, 3. *Afrixalus osorioi* in Yoko Forest Reserve, Democratic Republic of the Congo. Fig. 2: nuptial display (September 2021) at a height of 1 meter above water puddles (photo by L. Musubaho). Fig. 3: axillary amplexus recorded in October 2021 at a height of 0.8 meter above water puddles (photo by L. Musubaho).

released the females after egg laying on leaf surfaces. Before release, when we approached each pair more closely, the bright light from a flashlight did not have effect on the amplexus, as the males remained firmly attached to the backs of their partners.

DISCUSSION AND CONCLUSIONS

The species of the genus *Afrixalus* Laurent, 1944 use vegetation located above water bodies or aquatic plants for reproduction (Schiøtz, 1999; Channing, 2001). Examples include *Ludwigia abyssinica* A. Rich. (Onagraceae) and *Eichhornia crassipes* (Mart.) Solms (Pontederiaceae), aquatic plant species on which two other pairs were observed during the final six and ten minutes of amplexus. Our observations concur with those made in *A. laevis* Ahl, 1930 and *A. lacteus* Perret, 1976, which lay eggs on the surface of leaves without folding them (Amiet, 1991; 2009; 2012; Greenbaum et al., 2022). They contradict observations made in *A. lacustris* Perret, 1960, which folds leaves to build a nest where female lays the eggs (Nečas et al., 2025).

The nest construction and its placements on leaves correspond to reproductive mode 33 recognized in anurans, which helps to reduce the risk of egg predation by aquatic predators (Haddad & Prado, 2005; Kielgast & Lötters, 2009). Other observations indicate that *A. dorsimaculatus* (Ahl, 1930) and *A. uluguruensis* Barbour & Loveridge, 1928 do not wrap their eggs in leaves, unlike *A. laevis* (Greenbaum et al., 2022; Nečas et al., 2025). Also, *A. weidholzi* Mertens, 1938 sticks the edges of the leaves together to form a nest suitable for laying eggs, while this is probably not the case in other species, notably *A. morerei* Dubois, 1986 and *A. orophilus* Laurent, 1947 (Harper et al., 2010; Channing & Rödel, 2019; Dehling et al., 2023; Nečas et al., 2025). At hatching, all these strategies allow the tadpoles to fall directly into the water, where metamorphosis continues. The fact that all our observations were made at night confirms previous research findings that frogs are rarely active during the day (Spawls et al., 2019). Additionally, as predator activity gradually slows after nightfall, amphibian activity intensity increases because air humidity is high and temperature is moderate (Amiet, 1975; 1978b; Pages, 1984; Spawls et al., 2019).

Knowledge of the biology of several species, particularly *A. osorioi*, remains fragmentary. The very limited number of observations (six pairs) restricts the scope of the conclusions drawn in this short communication. Therefore, these initial observations require additional data on many of the couples, the influence of predators on the duration of amplexus, nesting behavior, incubation duration, and egg hatching conditions, as well as the duration of metamorphosis and tadpole survival to better understand the reproductive behavior of this species.

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