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Multiple mating and oviposition behavior of *Proceratophrys goyana* (Anura: Odontophrynidae) in the Brazilian Cerrado

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ABSTRACT

Polyandry occurs when a female mate, sequentially or simultaneously, with more than one male. Even though this reproductive strategy has been widely studied for some taxa, reports on amphibians are still scarce. Herein, we report amplexus, egg-laying, aggregative behavior and a case of multiple mating in the frog *Proceratophrys goyana*. Coupling occurs outside water and females, that are noticeably larger than males, move to streams where eggs are released at the stream bottom. Multiple mating was observed in a large chorus context, but males' aggregations seem to be uncommon in the species. Multiple spawning in Neotropical frogs occurs in several lineages and it seems to be related to the behavioral context.

Key Words: Amplexus; Cerrado; Polyandry; Reproduction.

Polyandry occurs when a female mate with more than one male, sequentially or simultaneously (Birkhead, 1995). Even though this reproductive strategy is widely studied in several taxa such as mammals and birds, studies in amphibians are still scarce (Orians, 1969, Stockley, 2003). Polyandry may benefit the female since different males, with different fitness, compete for produce part of the future offspring, increasing allelic diversity of the spawning. This reproductive strategy can also enhance the fertilization rate of the ova, when single males cannot fertilize the ova alone, enhancing female fitness (Byrne and Whiting, 2008). However, sometimes females can be drowned by amplectant males in high male density chorus (Izzo *et al.*, 2012, Trauth *et al.,* 2000), highlighting the complexity of reproductive strategies within amphibians.

Larvae growth and survival are usually larger in clutches produced by multiple spawning when compared to clutches fertilized by only one male (Byrne and Whiting, 2008, 2011). On the other hand, males involved in multiple spawning may either be benefited or harmed by the strategy. For some males, polyandry is one of the alternative strategies that would enable reproduction when the main strategy, calling for females, fails (Pombal Jr. and Haddad, 2007). These males, even competing by egg fertilization with other males, can enhance their fitness in multiple spawning situations (Vieites *et al.*, 2004). However, the male that expended energy calling for females and defending territory may have his fitness reduced as more males aggregate in the spawning (Jennions *et al.*, 1992, Roberts *et al.*, 1999, Vieites *et al.*, 2004). Since very few studies are focused in understanding the ecological role and evolution history of polyandry in amphibians (Zamudio *et al.*, 2016), including the occurrence of multiple spawning in tropical species (Prado and Haddad, 2003), natural history observations are essential for filling gaps and provide basic information about this complex reproductive behavior.

The Goiás Smooth Horned Frog *Proceratophrys goyana* (Miranda-Ribeiro 1937) is a terrestrial species widely distributed in central Brazil (Junior *et al.*, 2012, Martins and Giaretta, 2013), occurring in both open and forested habitats (Santoro and Brandão, 2014). *P. goyana* is a prolonged breeder that shows reproductive mode 2, with eggs and exotrophic tadpoles in lotic water (Bastos *et al.*, 2003; Haddad and Prado, 2005; Santoro and Brandão, 2014).

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frogs deals basically with taxonomic issues (e.g. Brandão *et al.*, 2013; Godinho *et al.*, 2013; Mângia *et al.*, 2014; Mângia *et al.*, 2018; Martins and Giaretta, 2013; Nascimento *et al.*, 2019; Santana *et al.*, 2010). However, very few is known about species natural history, even for common species in the genus, as *Proceratophrys goyana*.

Herein, we report *Proceratophrys goyana* amplexus, egg-laying, aggregative behavior, and a possible multiple spawning event. Amplecting pairs of *Proceratophrys goyana* were recorded at margins of streams and creeks with rocky bed in the Serra do Tombador Private Reserve, in Cavalcante Municipality, state of Goiás (13°42'13.08"S; 47°45'21.64"W, 960 m a.s.l.) in 15 July 2008) (Fig. 1), in São Domingos Village, Cavalcante Municipality (13°35'0.45"S; 47°37'35.23"W, 1020 m a.s.l), in 25 November 2016, in several opportunities in Volta da Serra Farm, Alto Paraíso de Goiás, state of Goiás (14°09'58.2"S; 47°44'25.0"W, 1020 m a.s.l), and in

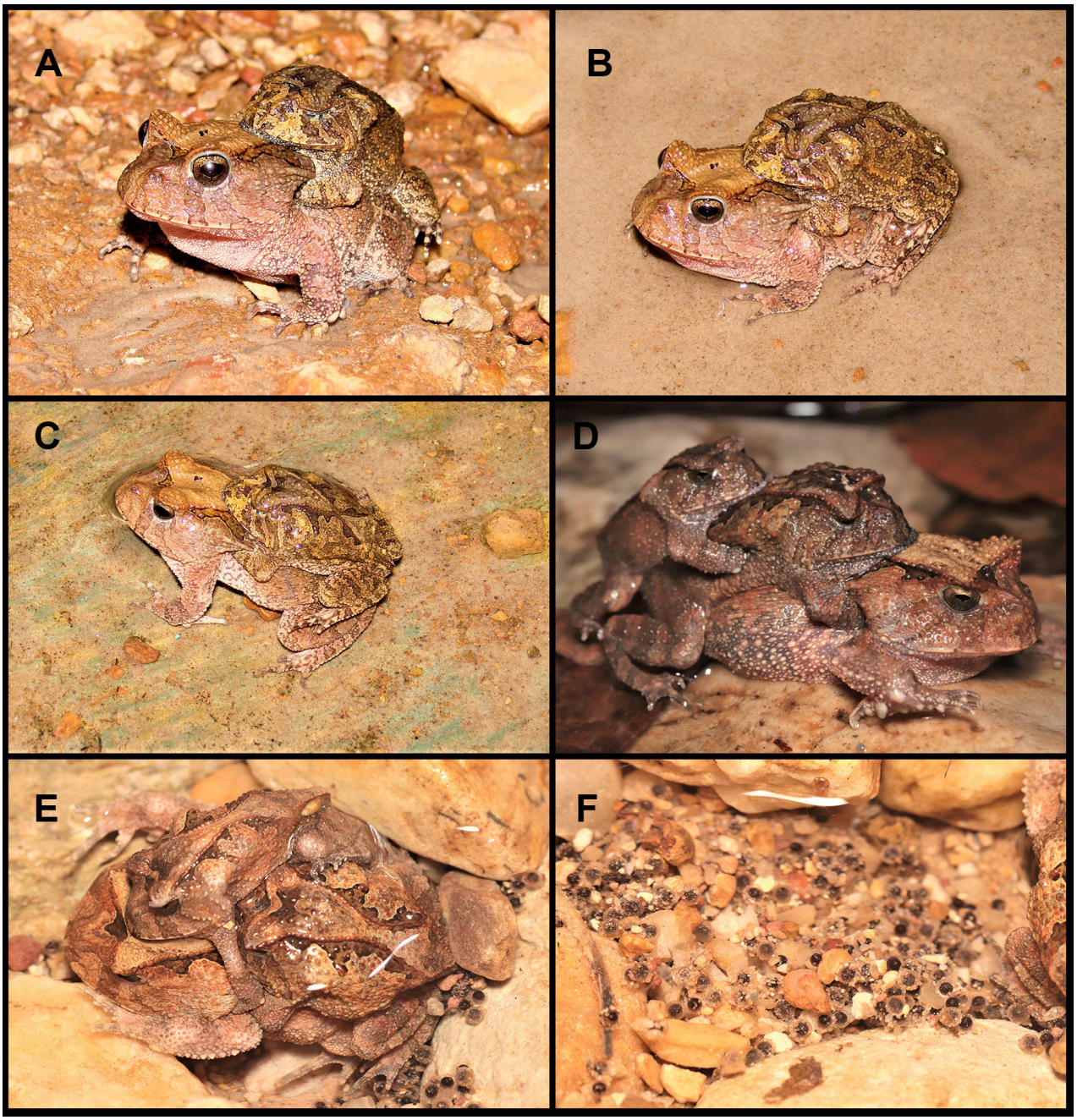

Figure 1. Amplecting pairs of *Proceratophrys goyana*. Amplecting pair in Serra do Tombador Private Reserve formed at stream margin (A), female moving towards the stream (B) and the couple on a rocky bottom stream (C), showing most of the female's body immerged (Photos by RAB). Amplected pair in Volta da Serra Farm before spawning (D), after spawning showing female completely immersed and with some eggs being released (E) and eggs recently released with sand particles adhered (F) (Photos by ASOM).

Chapada dos Veadeiros National Park, Alto Paraíso de Goiás Municipality (about 14'10"S; 47'48"W 1090 m a.s.l). All observed couples moved to the bottom of rocky bed streams or creeks for egg-laying. Eggs were always laid at streams backwaters or in places with slower water flow. The eggs were completely pigmented and presented an outer translucid jelly layer around 4 mm in width including the jelly layer and around 3 mm without the jelly layer (N = 25). Small particles of sand and other debris were quickly adhered to the surface of eggs, turning them confused with the background.

Although reproductive aggregations in *Proceratophrys* species were rarely reported, we recorded a large chorus in Serra do Tombador Reserve on 25 November 2016 and four times in Volta da Serra Farm. These aggregations were recorded at the end of the dry season in the Cerrado or were related to the first heavy rain at the beginning of the rainy season, and are typically composed by dense concentrations of males (up to 44 calling males) surrounding 1 to $2m^2$ pools along perennial or temporary streams. The sex ratio is strongly male-biased, and very few females were observed at these events, suggesting that males arrived earlier to these reproductive sites (see Lodé *et al.*, 2005).

On 06 August 2019 (end of the dry season) in Volta da Serra Farm, we observed at 20:50 hs an amplected pair with a second, noticeable smaller male, grasping the amplected male (Fig. 1). The three individuals were the only ones found on a small pool filled by the streamflow placed between rocks and pebbles at the São Miguel stream margin. We returned to the same place four hours later and found frogs still amplected. At this moment, the smaller male initially placed on top of the first male has moved and grasped the female, and both males were amplexing with the female, who released eggs in the pool (Fig. 1).

Although reproductive aggregations were previously reported for *Proceratophrys vielliardi* (Martins and Giaretta, 2011), this is the first record of multiple mating for the *Proceratophrys* genus. Although the records of polyandry in anurans are scarce (Zamudio *et al.*, 2016) it may be facilitated by some features, as an alternative reproductive strategy (Pombal Jr. and Haddad, 2007). External fertilization is one of the main factors that enable simultaneous polyandry allowing direct access to the ova by the sperm of several males (Byrne, 2004). Multiple mating has been recorded more often in explosive breeders than in species that build hidden nests or are sensitive to outside disturbance, such as territorial species (Chuang *et al.*, 2013, Ovaska and Rand, 2001). High density, as commonly observed in explosive breeders (Wells, 1997), also enables polyandry events, such as the one herein reported, although *Proceratophrys goyana* is a prolonged breeder (Bastos *et al.*, 2003), and male aggregations seem to be uncommon. Populations with high densities, especially for those were sexual ratio is male-biased, often have enhanced competition for females (Mangold *et al.*, 2015), making more suitable the use of alternative reproductive strategies by not amplectant males. It is interesting to note that most males that decide to invest in multiple mating are often smaller than the first male to access females (Vieites *et al.*, 2004; Lodé *et al.*, 2005).

Most of the records of polyandry in frogs are for species with foam nests in water (reproductive modes11 and 13) or in vegetation (reproductive mode 33) (Liao and Lu, 2010; Prado and Haddad, 2003, Ron *et al.*, 2014), because foam nests can allow the retention of sperm and the access of pirate sperms to the ova (Kusano *et al.*, 1991,Vieites *et al.*, 2004, Zamudio *et al.*, 2016). Moreover, polyandry in species with different reproductive modes have also been reported, such as *Afrixalus delicatus*, *Crinia georgiana*, *Chiromantis xerampelina*, *Rana latastei* (Backwell and Passmore, 1990; Byrne, 2002, Jennions *et al.*, 1992; Roberts *et al.*, 1999), and Phyllomedusidae frogs (Dias *et al.*, 2012; Oliveira *et al.*, 2014; Prado *et al.*, 2006, Roberts, 1994).

Our records increase the knowledge about *Proceratophrys* genus natural history, reporting different mating strategies in different lineages that can contribute to studies focusing on the evolution of reproductive strategies in Anura.

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