Reproductive ecology of *Adelphobates quinquevittatus* (Steindachner, 1864) in the southwestern Brazilian Amazon (Anura, Dendrobatidae)

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The Rio Madeira Poison Frog, *Adelphobates quinquevittatus* (Steindachner, 1864), is an amphibian belonging to the family Dendrobatidae. It occurs in restricted areas in Brazil, in the southern part of the Amazon rainforest in the Brazilian state of Rondônia and adjacent Amazonas, along the Madeira River drainage, and can possibly be found in adjacent localities in Bolivia (Caldwell & Myers, 1990; Medeiros et al., 2021; Frost, 2022). Like other dendrobatids, they possibly feed on small and abundant prey, such as ants and termites (Parmelee, 1999). *Ad. quinquevittatus* produces toxic alkaloids of the histrionicotoxin and pumiliotoxin-A class (Caldwell & Myers, 1990). which can possibly be related to such a specific diet, as it was found to commonly occur in other dentrobatids (Mebs et al., 2010).

Many amphibians breed during rainy periods in bodies of water with the presence of vegetation, which decreases the amount of sunlight. Consequently, evaporation is reduced, and a constant temperature and humidity are maintained (Caldwell & Myers, 1990; Camargo & Kapos, 1995; Chen et al., 1999; Egan & Paton, 2004; Williams et al., 2008). *Adelphobates* spp. use empty fruit shells for egg-laying, have large tadpoles, and feed on any invertebrate or tadpole of another species that is deposited in the same place (Caldwell & Myers, 1990). The use of vegetation is linked to the reproductive mode of the

species, which require various climatic conditions to achieve reproductive success, such as rainfall, drought, and humidity of the rainforest (Pombal & Haddad, 2005).

This study reports on the reproductive behavior of *Ad. quinquevittatus*, an amphibian of the family Dendrobatidae endemic to the Madeira River basin, in the Brazilian Amazon. Any new information about the species is extremely important for preservation and its reproductive behavior is still insufficiently known. We also report the use of *Attalea speciosa* (Mart. ex Spreng.) spatix as a reproductive reservoir.

We conducted field observations at the "Base de Treinamento da Polícia Militar" (Military Jungle Police Base) in the municipality of Porto Velho, state of Rondônia, Brazil, in the southwestern Brazilian Amazon. During a herpetofaunal survey, specimens of *Adelphobates quinquevittatus* were found in a typical terra firme Amazon rainforest substrate. Identification was done by approaching diagnostic morphological characteristics such as orange background color with black spots on the limbs, and dorsal black coloration with five longitudinal and generally white stripes, typical of the species (Caldwell & Myers, 1990). On November 13, 2020, at 04:32 AM a specimen was observed (-9.1014°, -64.0259° WGS84) on a spadix (structure protecting the inflorescence) of *Attalea speciosa* (Mart. ex Spreng.) (Fig. 1A). Upon careful analysis, it was found that there were tadpoles of *Ad. quinquevittatus* deposited on the spadix of the *Attalea speciosa* palm tree, a thick and highly resistant structure (Linhares, 2016). It was observed that in this one location there were five tadpoles of *Ad. quinquevittatus* (Fig. 1B), three of which were relatively larger in size than the others, about approximately 1,5 cm (e.g., Fig. 1C). The total size of the *At. speciosa* spadix used as a nest was approximately 77 cm long and was filled with water and leaves.

In another field trip to the Military Jungle Police Base, on January 6, 2021, at 08:57 AM, several Brazil nut fruits *Bertholletia excelsa* (Humb. & Bonpl.) were found near a Brazil Nut Tree (It has a hard, woody shell) being used as nests for *Ad. quinquevittatus* tadpoles (Fig. 1D). One adult was spotted, but not photographed due to its agility in hiding under leaves on the forest floor. There were 22 brazil nut fruits on the ground, and they could be grouped in six distinct points. Figure 2 shows the representative map of B. excelsa fruits present at the breeding site of the dendrobatid *Ad. quinquevittatus*, where the configuration of each point in the breeding area is described.

Point 1: it could be observed that at Point 1 there was one nut open at the top with the presence of water.

Point 2: distant 6,73 m from Point 1. There were seven nuts, and only four of them contained water. Among them, three had an upper opening and one had a lateral opening. There was only one nut of the first type containing a tadpole, with leaves forming its substrate. In the case of nuts without water, one had a lateral opening and two had an upper opening.

Point 3: 1,36 m distant from Point 2. Four nuts were located, all of them with an upper opening, and three of them had water inside.

Point 4: 1,90 m distant from Point 3. Three nuts were located, two without water, one with a lateral opening and two with an upper opening. Of those with an upper opening, one contained a tadpole and there were leaves forming its substrate.

Point 5: distant 0,66 m from Point 4. Three nuts were located, where one had a lateral opening and contained water, one had an upper opening and contained water, and the other had a lateral opening and contained no water.

Point 6: distant 1,23 m from Point 5. Four nuts were located, two containing water and two without water, all with upper opening. One tadpole was found, with the nut substrate being formed mainly by leaves.

At the same site, a Trap Camera, model HC900A, was installed to observe which animals were visiting the breeding site of *Ad. quinquevittatus* (Fig. 3A). On January 9, 2021, one individual of *Dasyprocta fuliginosa* (Wagler, 1832), commonly locally known as "Cutia", was recorded at the site (Fig. 3B). Cutias are one of the main dispersers of *Bertholletia excelsa*, being responsible for opening and removing the fruits, moving them away from the mother tree (Haugaasen et al., 2012; Oliveira Wadt et al., 2018). This rodent species is a hunting target in the state of Rondônia (Belfort et al., 2020; Ramos et al., 2020) and his overhunting can directly impact fruit dispersal and consequently, the availability of local breeding sites for *Ad. quinquevittatus*.

Little is known about the reproductive behavior of *Ad. quinquevittatus*, although its family, Dendrobatidae, usually lays two to six eggs and during the development period the male of the species stays close to the nest (Caldwell & Myers, 1990). Tadpoles, in general, develop in denser vegetation, which creates an effective barrier against solar radiation and the action of predators, thus increasing the chances of survival (Heyer, 1969; Toft, 1980; Babbit & Tanner, 1998; Hartel et al., 2007). Habitat loss due to human actions is a problem for amphibians since many species depend on specific climatic conditions and plants for their reproduction (Verdade, 2010).

From what was exposed in this study, it can be pointed out that frogs of the species *Ad. quinquevittatus* places a larger number of tadpoles on substrates such as *Attalea speciosa* spadix in the study area, possibly because they have larger size, water storage capacity compared to the previously reported *B. excelsa* nut. However, the observed distribution of tadpoles, as illustrated on the descriptive map (Fig. 2), directly impacts the reproductive success of the species. If any problems occur in a spadix of *At. speciosa*, all offspring will be lost, and other problems such as interspecific predation and cannibalism can also occur if tadpoles are distributed on a single substrate. This decreases the probability of tadpoles reaching adulthood, but with the distribution in several nuts of *B. excelsa*, the species can reduce the risk of total loss of the offspring and annul the chances of intraspecific predation, thus increasing the chances of more tadpoles reaching adulthood. Hence, it is plausible to infer that Brazil nut reservoirs may have an important role in *Ad. quinquevittatus* reproductive ecology on the study site. Consequently, threats such as deforestation and overhunting of its dispersal vectors in the area can probably affect this species population dynamics and possibly its conservation.

Finally, this study reinforces the importance of preserving Brazil nut trees *B. excelsa* because their nuts may directly impact the reproductive success of anuran dendrobatids, such as *Ad. quinquevittatus*. Our observations increase the knowledge of the reproduction of this species in a little-studied area of the Brazilian Amazon, but we reinforce that it is still necessary that future research give more focus on this subject as very little is currently known.

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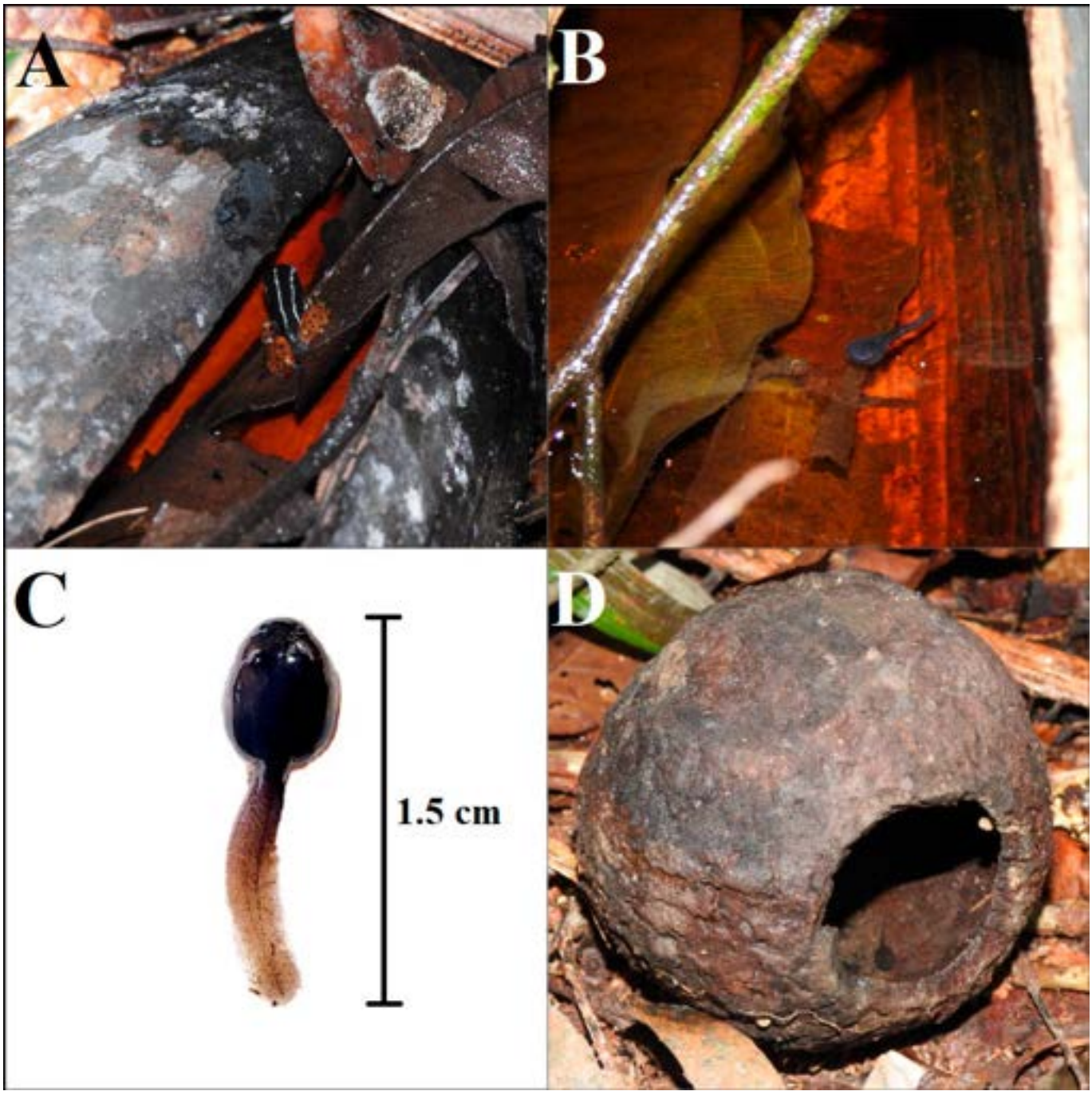

Figure 1. Photographic record. A) Individual of *Adelphobates quinquevittatus*; B) Tadpole of *Adelphobates quinquevittatus* on *Attalea speciosa* spadix; C) Tadpole of *Adelphobates quinquevittatus*; D) Tadpole of *Adelphobates quinquevittatus* in the nut of *Bertholletia excelsa*.

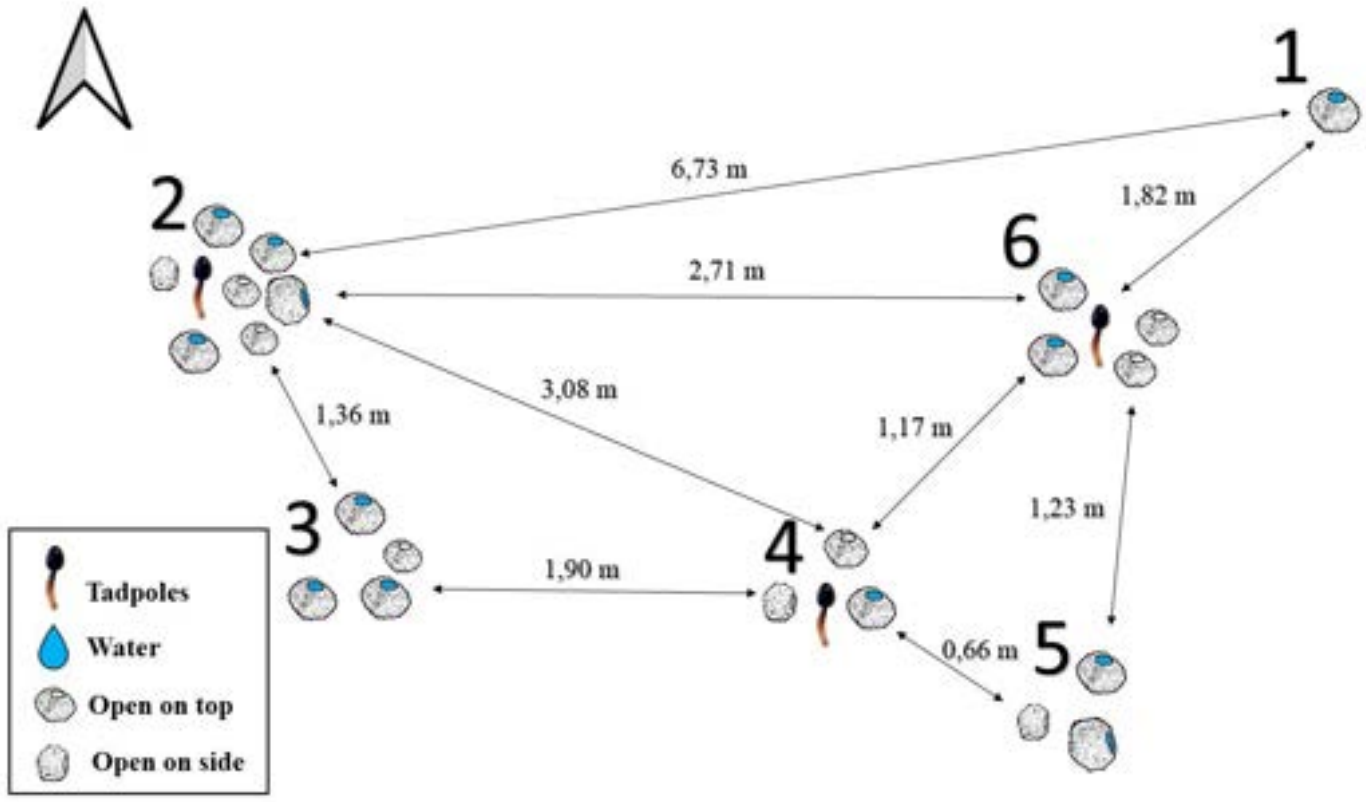

Figure 2. Map of the breeding site of *Adelphobates quinquevittatus* in *Bertholletia excelsa* nuts.



Figure 3. A) *Adelphobates quinquevittatus* at the breeding site;
B) *Dasyprocta fuliginosa* individual.