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Crazy little thing called love: interspecific amplexus and early hybrid embryogenesis in two treefrogs (genus *Dendropsophus*) in Brazil

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During the breeding season, anurans locally aggregate at shared aquatic habitats, where they form dense choruses at breeding sites together with several additional species (Wells, 2007; Bertoluci et al., 2021). Several factors can explain this aggregation behaviour: availability of specific breeding sites, which forces species to breed simultaneously; niche overlap of species with similar breeding habits; reduced individual exposure to predation, as high densities can confuse predators; and increased chances of reproduction, because females are more attracted to large choruses than to single sound sources (Ryan et al., 1981; Murphy, 2003; Hofer et al., 2004; Wells, 2007; Stratman et al., 2021). In large, diverse aggregations, anurans can exhibit interspecific amplexus, which may involve both closely and distantly related species (Serrano et al., 2022). Because these mismatched interactions are often maladaptive, they may generate selective pressures that reinforce reproductive isolation (Pfennig and Simovich, 2002; Pfennig and Rice, 2014; Jaya et al., 2022; Braga et al., 2023; de Sá et al., 2024).

Interspecific amplexus has been documented with some regularity among neotropical frogs, including in several treefrog species (e.g., Sodré et al., 2014; Rocha et al., 2015; Guerra et al., 2018; Lirio et al., 2019; Pedro and Nali, 2020; Amanajás et al., 2024). The hylid genus *Dendropsophus* comprises over 100 species of small treefrogs found throughout the Americas (Frost, 2026). These species are known for their ecological versatility and inhabit a wide range of environments, including forests, open landscapes, and anthropogenically altered habitats (Orrico et al., 2021; Whitcher et al., 2025). Although they have been reported to engage in interspecific amplexus, no confirmed cases of hybridization have been documented (Mozer, 2023). In this note, we report a case of interspecific amplexus between an adult female *D. sanborni* (Schmidt, 1944) and an adult male *D. elianeae* (Napoli and Caramaschi, 1999), two closely related and syntopically occurring treefrogs (Orrico et al., 2021; Whitcher et al., 2025). To our knowledge, these frogs have never been reported engaging in interspecific amplexus or hybridizing with any other frog species.

Materials and Methods

Fieldwork. Fieldwork was conducted on 22 February 2025 at Sítio Cantaclaro, a private rural property in Itapé, Rio Claro Municipality, São Paulo State, southeastern Brazil (22.3268°S, 47.7148°W, elevation 669 m), as part of a study on methodological approaches in small anuran species. An adult female *D. sanborni* (Fig. 1A) and a calling adult male *D. elianeae* (Fig. 1B) were located by visual and auditory searches (Heyer et al., 2014), collected by hand, and placed together in a large (25 x 45 cm) plastic bag. Even though the pair was no longer in its natural microhabitat, they spontaneously engaged in axillary amplexus (Carvajal-Castro et al., 2020; Fig. 2C). To observe the outcome of this unusual behaviour, we chose to maintain the frogs undisturbed in the bag and transporteded them to the Laboratório de Biologia Experimental e Integrativa at the Universidade Estadual Paulista, for observation. The pair remained continuously in amplexus during transport.

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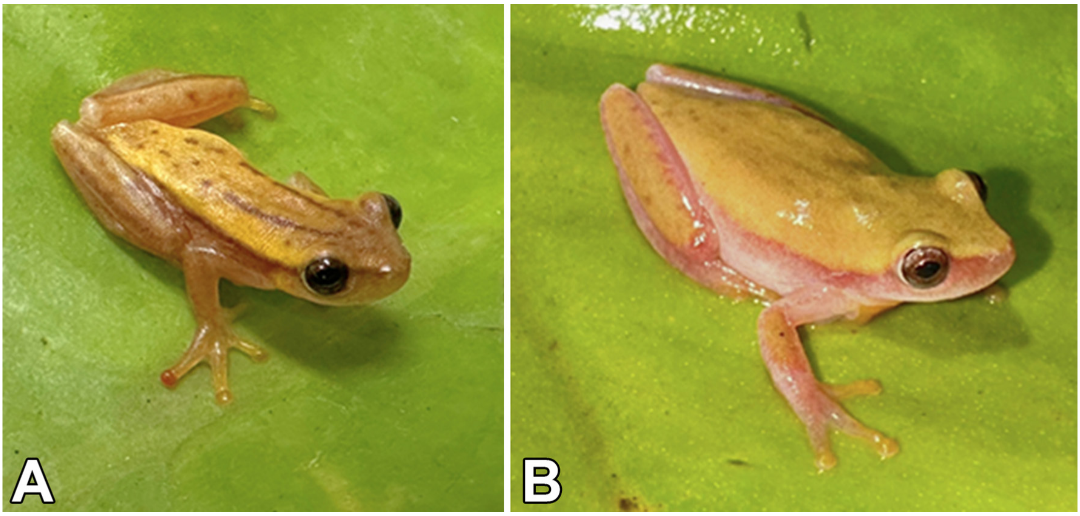

Figure 1. (A) The adult female *Dendropsophus sanborni* (CFBH 48549) and (B) the adult male *Dendropsophus elianeae* (CFBH 48550) observed in interspecific amplexus at Sítio Cantaclaro, Rio Claro Municipality, São Paulo State, southeastern Brazil. Photos by Fábio P. de Sá.

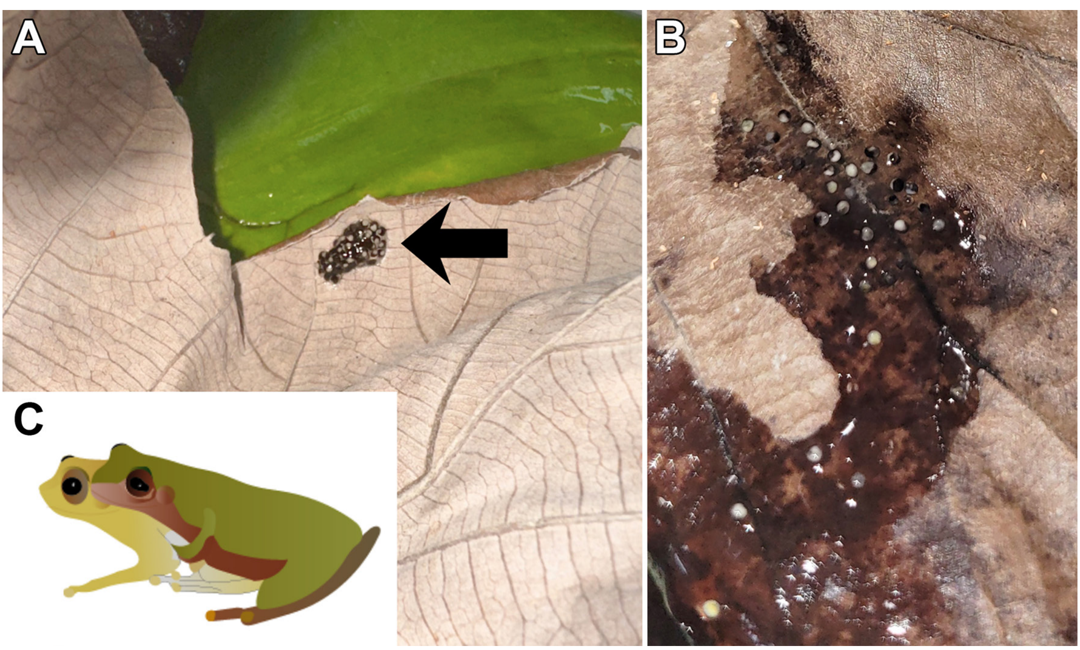

Figure 2. (A) A clutch of hybrid eggs oviposited by a female *Dendropsophus sanborni*, fertilized by a male *D. elianeae*. The clutch is shown as it was first found. (B) After placement into a separate maintenance container, the eggs were observed to liquefy after Day 7 of development. (C) Illustration depicting the observed and filmed interspecific axillary amplexus of the smaller female *D. sanborni* and the larger male *D. elianeae*. Body proportions are based on the actual frog sizes. Photos by Fábio P. de Sá, illustration by Mariana Routh.

Laboratory observations. After arrival in the laboratory, we transferred the amplexed pair to a plastic container (14.5 x 12.0 x 21.5 cm), which was maintained with a 2-cm layer of water, leaves for shelter, controlled temperature (25°C), and a natural photoperiod (~13 h light). By the following day, oviposition had occurred on the leaves, adjacent to and touching the water (Fig. 2A). We carefully transferred the eggs to an aquaterrarium (25 x 12 x 16 cm) with the same 13-h photoperiod. We monitored the clutch daily to verify fertilization and to document developmental progress. To ensure water quality, oxygenation was maintained using an aquarium air pump (SarloBetter Mini-A). Both adults were euthanized, and thigh muscle tissue samples were collected. We deposited voucher specimens and tissues in the scientific Amphibian Collection "Célio F.B. Haddad" (CFBH) at the Universidade Estadual Paulista (*D. sanborni*: CFBH 48549, tissue CFBH-T 24616; *D. elianeae*: CFBH 48550; tissue CFBH-T 24617).

Identification. We first identified the parents based on published descriptions (Boulenger, 1889; Napoli and Caramaschi, 1999; Orrico et al., 2021; Whitcher et al., 2025). The adult female *D. sanborni* measured 18.0 mm in snout–vent length (SVL), whereas the adult male *D. elianeae* was larger, with SVL 21.4 mm. We further confirmed their identities via molecular comparisons. Total genomic DNA was extracted from sampled tissues, and a 618-base pair fragment of the 16S ribosomal RNA gene was amplified using the primers 16sAR and 16sWilk2 and sequenced following the methodology described by de Sá et al. (2015). New sequences and metadata generated in this study have been deposited into GenBank under the accession numbers PX491772 (*D. sanborni*) and PX491773 (*D. elianae*).

Results and Discussion

In the days following oviposition, some eggs rapidly decayed while others remained intact. On Day 7, we observed that the gelatinous coating on the eggs began to show signs of liquefaction (Fig. 2B), indicating the onset of developmental failure. All eggs were preserved in 100% ethanol on Day 7 and examined under a stereomicroscope to assess their developmental stage. Following the guidelines proposed by Gosner (1960) for frog egg development, our observations confirmed that the remnant eggs were fertilized and had reached Stages 8–10, corresponding to the initial stages of embryogenesis (some of these eggs were also preserved in the scientific collection; tissue number CFBH-T 24618). These early developmental stages are characterized by a visible reduction of the light hemisphere and a corresponding expansion of the pigmented area, along with the onset of cell involution at the boundary between hemispheres, which marks the beginning of gastrulation, the final stage of cleavage. Although fertilization and initial embryogenesis occurred, development ultimately did not complete.

Natural selection may favour accurate species recognition mechanisms, especially in areas where closely related species coexist, and the risk of interspecific mating is heightened (Blair, 1964; Toledo et al., 2003; Zina et al., 2007; Braga et al., 2023). Several reproductive isolating mechanisms have evolved to reduce hybridization and its associated fitness costs (Blair, 1964; Coyne, 1974; Singh, 2022). These mechanisms include prezygotic barriers such as seasonal reproductive isolation, species-specific courtship behaviours, distinctive advertisement calls, and mechanical incompatibilities related to body size or morphology that can interfere with amplexus (Blair, 1964; Wells, 1977; Martins and Haddad, 1988; Liao and Lu, 2009; Centeno et al., 2015; de Sá et al., 2016; Nali et al., 2022; Singh, 2022).

Typically, female frogs are larger than conspecific males, which facilitates proper cloacal alignment during amplexus and oviposition, thereby optimizing egg fertilization (Wells, 2007). In our case, the interspecific pair consisted of a smaller female (*D. sanborni*) and a larger male (*D. elianeae*), but this size difference did not impede the reproductive behaviour. When prezygotic isolation does not prevent mating of closely related species, postzygotic barriers may act to avoid successful hybridization (Blair, 1964; Coyne, 1974; Singh, 2022). These barriers can reduce hybrid viability or fertility and may cause developmental incompatibilities that ultimately lead to reproductive failure (Blair, 1964; Coyne, 1974; Malone and Fontenot, 2008; Singh, 2022). In the hybrid *Dendropsophus* clutch we studied, zygotes formed but embryonic development did not proceed past a certain point, resulting in developmental failure (hybrid inviability). In nature, this phenomenon is often attributed to chromosomal or genetic incompatibilities (Blair, 1964; Coyne, 1974; Malone and Fontenot, 2008; Singh, 2022). In our case, we cannot rule out that captivity negatively influenced development. Based on our observation, it is noteworthy that *D. sanborni* and *D. elianeae*, two closely related congeneric and syntopically occurring treefrog species (Orrico et al., 2021; Whitcher et al., 2025), appear not to be prevented from hybridization by prezygotic barriers but require postzygotic barriers to maintain reproductive isolation.

Whereas hybridization between closely related frog species has been documented in laboratory settings (e.g., Anderson and Moler, 1986; Doležálková-Kaštánková et al., 2024), similar events occur in nature when ecological, behavioural, morphological, and biological barriers are overcome (e.g., Green and Pustowka, 1997; Fontenot et al., 2011). Overall, reproductive barriers are not always complete in natural environments, and their effectiveness can fluctuate with ecological and behavioural contexts, reflecting the dynamic nature of species interactions (Wells, 2007; Pfennig and Rice, 2014). Our findings highlight the importance of natural history studies, as mechanisms that reduce interspecific amplexus and hybridization in frogs remain incompletely understood, having either positive or negative consequences for the hybridizing species. Continued field observations, coupled with more focused studies on contact zones between closely related species, are therefore essential for understanding how boundaries are shaped by evolutionary processes, which do not always ensure the maintenance of reproductive isolation.

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