REPTILES & AMPHIBIANS

Interspecific Amplexus of a Long-footed Marsupial Frog, *Gastrotheca longipes* (Boulenger 1882), and a Canelos Treefrog, *Boana appendiculata* (Boulenger 1882), in Yasuní National Park, Ecuador

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Interspecific amplexus in anurans is not uncommon and has been well-documented, particularly in regions with high species richness and overlapping microhabitats (e.g., Ferreira et al. 2019; Serrano et al. 2022; Webb and Zdunek 2023; Ortiz et al. 2024). The exact causes and mechanisms of misdirected amplexus remain unclear, although high abundance of heterospecific males and low abundance of same-species females might be a factor (e.g., Hettyey and Pearman 2003). Such behavior is often interpreted as the result of sensory confusion, either tactile, visual, or chemical, especially during peak reproductive activity (e.g., Narins et al. 2005; Wells 2007). Several documented cases involve hylid males exhibiting mistaken or opportunistic amplexus, typically targeting morphologically similar species (e.g., Duellman and Trueb 1994; Ferreira et al. 2019; Pedro and Nali 2020). We herein present the first direct observation of interspecific amplexus between a Long-footed Marsupial Frog (*Gastrotheca longipes*) and a Canelos Treefrog (*Boana appendiculata*).

Gastrotheca longipes (Hemiphractidae) typically inhabits lowland areas and slopes of primary tropical forests, where it is associated with low vegetation along riverbanks as well as canopy vegetation; it is nocturnal and arboreal, with maximum SVLs of 70.8 mm in males and 88.3 mm in females (Duellman and Trueb 2015). According to the IUCN SSC Amphibian Specialist Group (2018), individuals have not been recorded in disturbed areas. *Boana appendiculata* (Hylidae) is associated with swamps, semi-flooded and flooded forests, and artificially open areas, usually found at night in primary and secondary forests on vegetation 20–600 cm above the ground; average SVLs are 44.3 mm in males and 64.4 mm in females (Caminer and Ron 2020). Both species are of Least Concern (LC) on the IUCN Red List (IUCN SSC Amphibian Specialist Group 2018, 2023). In the Yasuní National Park both species exhibit arboreal and nocturnal habits and are often observed in similar habitats, vocalizing simultaneously on the same substrates (VP, pers. obs.).

Gastrotheca spp. exhibit highly specialized reproductive strategies, such as dorsal brooding and in some cases matrotrophy, where females transfer nutrients to developing

Figure 1. An adult male Canelos Treefrog (*Boana appendiculata*), recognizable by the typical cream/pale brown dorsum with a barely visible "X" on the scapular region and the gray/cream flanks, in amplexus with a female Long-footed Marsupial Frog (*Gastrotheca longipes*), recognizable by the white supralabial stripe, reddish-brown toe-tips, a snout that slopes abruptly in lateral view, and a dorsal marsupium (a trait of females), which is the only species of the genus that inhabits the Amazonian lowlands of Ecuador. Photograph by Vincent Prémel.

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embryos inside the pouch (del Pino and Escobar 1981; Warne and Catenazzi 2016). In contrast, *Boana* spp. generally follow the typical anuran reproductive mode, with external oviposition and an aquatic larval stage (McDiarmid and Altig 1999). A common reproductive behavior in *Boana* is establishing acoustic territories, where males produce advertisement calls to attract females and defend sites from rivals, as observed in *Boana goiana* (Dias et al. 2021).

At 2057 h on 26 November 2023, we observed an adult male *B. appendiculata* in amplexus with an adult female *G. longipes* in Yasuní National Park, Orellana Province, Ecuador (-0.638101, -76.594804; elev. 261 m asl; Fig. 1). The observation took place at the edge of a riparian forest within a mature humid forest typical of the region. In November, the province of Orellana exhibits an equatorial climate characterized by stable temperatures, abundant rainfall, and high humidity (Instituto Nacional de Meteorología e Hidrología 2025). Average 24-hour temperatures are 25.1 °C, monthly precipitation levels are 270–300 mm, and relative humidity remains consistently high, typically between 85% and 90%, reflecting a hot, humid environment with abundant rainfall (Climate Data 2025).

Figure 2. A female Long-footed Marsupial Frog (*Gastrotheca longipes*) with a well-developed clutch embedded in the dorsal pouch characteristic of females in this genus. Photograph by Vincent Prémel.

We experienced frequent and intense rain showers, usually in late afternoon or at night, during the month of our expedition. Both individuals were perched about 1.5 m above the ground on the broad leaf of an unidentified plant above a small forest stream. No temperature data were recorded. Throughout the 20-minute observation, we recorded no movement, no vocalizations, and no attempts to flee as both individuals remained completely motionless on large moss-covered leaves. Upon closer examination, we noticed that the female *G. longipes* was already carrying a developed egg-clutch, which was clearly visible through the dorsal skin, a typical feature of this species (Fig. 2). To confirm this observation and document the female's condition, we carefully removed the male, allowing for a clear view of the female's dorsum and confirming the presence of a well-developed clutch embedded in the dorsal pouch.

Observations of reproductive events of marsupial frogs (*Gastrotheca* spp.) are rare and highly sporadic under natural conditions (Duellman and Chavéz 2010; Gagliardo et al. 2010). Although weather conditions were favorable, amphibian activity remained low during our survey. We detected only a few marsupial frogs and *Boana* spp., despite being common in the area, were present only in low densities and calling activity was limited, suggesting that peak reproductive activity had not yet been reached. In contrast, in some areas, the onset of the rainy season triggers explosive breeding events involving thousands of individuals, which substantially increases the probability of interspecific amplexus, but we did not record any such events during our sampling period. The unique mode of reproduction of marsupial frogs, in which fertilized eggs are deposited and develop in a dorsal pouch, requires very specific environmental triggers and is not readily predictable (Duellman and Chavéz 2010).

The biology and reproductive behavior of the *Boana semilineata* species group remain poorly understood. However, observations from related species suggest that reproduction likely occurs in lotic environments, consistent with field observations at riverine sites. The larvae form dense aggregations that, combined with the tadpoles' dark pigmentation, might serve as an adaptive strategy to reduce predation risk (Caminer and Ron 2020).

Although in situ observations are scarce, the reproductive biology of *Gastrotheca* spp. was relatively well-studied during the 1970s and 1980s (Duellman and Maness 1980). More recently, ex situ conservation programs and captive-breeding centers have made significant contributions to the understanding of reproductive behavior, providing detailed insights into courtship, oviposition, and maternal care that are rarely accessible in the wild (Akmentins et al. 2019). To the best of our knowledge, this is the first documented observation of amplexus in *G. longipes* and the first recorded case of interspecific amplexus involving any species in the genus. This is not the first instance of interspecific amplexus in *Boana* spp. (e.g., Pedro and Nali 2020; Serrano et al. 2022); it does, however, document an additional species exhibiting this behavior.

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