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The journey of life of the Tiger-striped Leaf Frog *Callimedusa tomopterna* (Cope, 1868): Notes of sexual behaviour, nesting and reproduction in the Brazilian Amazon

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The Tiger-striped Leaf Frog *Callimedusa tomopterna* belongs to the family Phyllomedusidae, which is constituted by 63 described species distributed in eight genera, *Agalychnis, Callimedusa, Cruziohyla, Hylomantis, Phasmahyla, Phrynomedusa, Phyllomedusa,* and *Pithecopus* (Duellman, 2016; Frost, 2017). The reproductive aspects reported for the species of this family are marked by the uniqueness of egg deposition, placed on green leaves hanging under standing water, where the tadpoles will complete their development (Haddad & Sazima, 1992; Pombal & Haddad, 1992; Haddad & Prado, 2005). However, exist exceptions, some species in the genus *Cruziohyla*, *Phasmahylas* and *Prhynomedusa*, besides the species of the genus *Agalychnis* and *Pithecopus* of clade megacephalus that lay their eggs in lotic environments (Haddad & Prado, 2005; Faivovich et al. 2010; Haddad et al. 2013). Several species of the family Phyllomedusidae has aspects of the reproduction described, as *Pithecopus hipocondrialis, Pithecopus rohdei, Phyllomedusa bicolor, Phyllomedusa iheringii, Phyllomedusa trinitatis* (Matos et al. 2000; Wogel et al. 2004; Faivovich et al. 2010; Venâncio & Melo-Sampaio, 2010; Downie et al, 2013; Dias et al. 2017). Despite the many works on reproduction within the family Phyllomedusidae, most of them cover isolated characteristics such as amplexus, spawning or development of tadpoles, but do not describe the sequencing of these events (Matos et al. 2000; Venâncio & Melo-Sampaio, 2010; Downie et al, 2013; Dias et al. 2017).

In 1975, Lescure described the nests and development of tadpoles to *C. tomopterna*, based only on spawns that he had found around the permanent ponds in the French Guiana. However, the author mentions a variation in the number of eggs for some spawns and the use of more than one leaf for confection in some nests (Lescure, 1975). The nests described by Lescure in 1975 are probably from *Phyllomedusa vailantii* as reported by Lescure et al. (1995). The number of eggs in the spawns reported by Lescure (1975) diverge from that described by other authors such as Neckel-Oliveira & Wachlevski, (2004) and Lima et al. (2012). In addition, the use of more than one leaf for confection in the nest mentioned by Lescure (1975), are characteristic of other species belonging to the same family that occur in sympatry both in Brazil and in French Guiana, these being *Phyllomedusa bicolor* and *Phyllomedusa vailantii* (Lescure et al.,

Figure 1. Female of *Callimedusa tomopterna* in the centre of a leaf, shortly before being intercepted by a male. (Photo: L. Ferrante).

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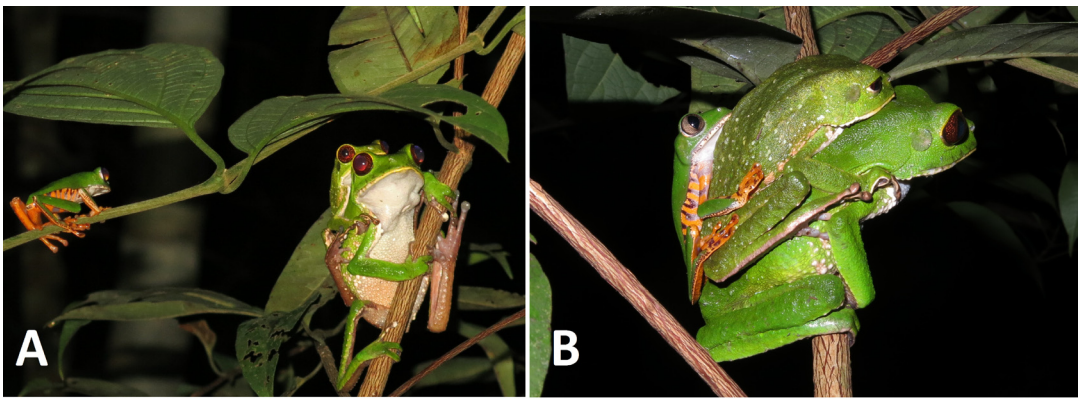

Figure 2. A: Intruder male of *C. tomopterna* moving towards the amplectant pair of *Phyllomedusa tarsius;* **B:** Intruder male of *C. tomopterna* in amplexus with a couple of *Phyllomedusa tarsius.* (Photos: Lucas Ferrante).

1995, Lima et al., 2012). For *C. tomopterna*, Neckel-Oliveria & Wachlevski (2004) investigated the action of predators on eggs. However, no information is available about aspects of nest confection or pre-embryonic development of tadpoles. In this note, we reported various aspects of reproduction for *C. tomopterna* in sequence, such as sexual behaviour, amplexus, nest construction and embryology of tadpoles.

The reproductive events were observed at the Experimental Farm of the Federal University of Amazonas (UFAM), located in the north of the Manaus municipality, Amazonas state in Brazil (2°39'0.79 "S, 60° 3'3.02" W). Our observations were made between June 17 and October 6, 2017. The observations of the first amplexus (amplexus 1) began at 21:00 h on 17 June 2017 with the sighting of the male and female in amplexus. The observations lasted until 05:40 h of 18 June with the preparation of the nest and egg laying. In the same puddle, on 6 October 2017 we observed the behaviour of three females positioning themselves in well-exposed leaves and being intersected by males, this way forming other three amplexus. We also accompany the displacement of these pairs to the edge of the puddle, verifying where they built the nests. For each spawning (N = 4), we observed the positioning and confection of the nest. For three of the spawns we observed the development of the pre-embryonic stage until hatching. All observations were visualized in a plateau area near permanent puddle in the interior of the forest, about 500 m away from the edge.

Figure 3. Male and Female of *Callimedusa tomopterna* in axillary amplexus. (Photo: L. Ferrante).

The aspects of sexual behaviour.—These following observations were made also on October 6, 2017. The forest was humid and the puddle was full, due the rains in the day before. Several species of anurans were heard vocalizing in this puddle, like *Scinax ruber* and *Rhinella merianae*. We visualized 2 females of *C. tomopterna* between 20 and 21 h that descended from the top of the trees and positioned themselves in broad leaves between 1.5 and 2 m on the ground (Fig. 1). Males of *C. tomopterna* did not vocalize around the puddle, but in less than 10 min in which the females positioned themselves on the leaves, they were intercepted by a male forming the amplexus. No vocalization of *C. tomopterna* was heard throughout the night, however we found others four amplexus in the same puddle,

Figure 4. Intruder male of *Callimedusa tomopterna* trying to intercept an amplectant pair of *Callimedusa tomopterna*. (Photo: L. Ferrante).

totalling six amplexus at night.

The males of *C. tomopterna* were visualized at least 1.5 m from other. These males, upon seeing an individual of the same species or of another species, attempted to enter amplexus, regardless of the sex of the individual or if they were individuals already in amplexus (Fig. 2). Sexual interference is reported by Arnold (1976, 1977) for when a male attempt to interfere in a mating situation for the sake of its reproduction. In this way, we denominate of intruding males any male that has moved towards an amplexus, causing it to have to evade the area to avoid interference. We believe, that the aggressive behaviour of males to forage the partners in addition to the fact of females expose themselves in the vegetation favour the reproduction of this species. In this way, the reproduction of *C. tomopterna*, is not limited to the males attracting the females through vocalization.

The journey of the amplexus until the nest construction and egg laying.—The reproduction of *Callimedusa tomopterna* is related only to permanent ponds, not occur in other water bodies (Rodrigues et al. 2010), characterizing as reproductive mode 24 according to Haddad & Prado (2005). All amplexus that we observed to *C. tomopterna* are axillary (Fig. 3). In the first amplexus observed (amplexus 1), the couple was perched on a tree about three metres away from a permanent puddle. The couple remained hugged in the initial tree for approximately 2 hours, until around 23:00 h when the female and male in amplexus began to move under the branches towards the water body. With the male in the back, the female walked among the tree branches until it reached another tree that was 2.5 m distant from the puddle. One hour after the first movement, the female began to walk for another tree about 2 m even to the puddle. At 01:33 h the couple was in a tree about 1 meter of distance away from the puddle and 60 cm of height from the surface of the water. At 02:18 h., we saw the approach of an intruder male (Fig. 4). The couple was perched in a leaf, when the intruder male began to walk towards the amplexus and reached about 50 cm away. The female with the approach of the intruder male, moved to another tree walking through the branches. The intruder male did not pursue the couple in amplexus.

At 03:20 h the couple began a shift to higher branches, where the female began to inspect the leaves to build the nest (Fig. 5a). After 20 min of the female inspect the leaves, it moved towards the treetops. At 03:57 h the couple were about 4 meters off the water film. The displacement continued and at 4:00 h when the couple was reached 7 meters in height on the tree well above the puddle. Finally, at 4:13 h, the female inspected the leaves and positioned herself in the leaf to build the nest. At 04:18 h, the female began oviposition in the leaf with breaks, depositing the eggs along the leaf midrib (Fig 5b). As the female climbed, the male used his hind legs to fold the sheet around the eggs (Fig. 5c). The male's help in making the nest is also mentioned by Lima et al. (2012) for *Phyllomedusa tarsius*, a species that occurs in sympatry with *C. tomopterna*. The oviposition event lasted 1 hour, at 05:18 h we registered the exit of the male (Fig. 5d). Soon afterwards, the female left the place of oviposition (Fig. 5e).

In addition of this nest positioned at 7 meters above the water in 06 October 2017, we observed another three nests in respective 1.5 m, 1.20 m and 12 cm above the water. According to Neckel-Oliveia & Wachlevski (2004), the nests of *C. tomopterna* has an average height of 1.20 m of the water film, range up to 60 cm. According to our observations, we can conclude that the distance of the nest on the puddle varied according

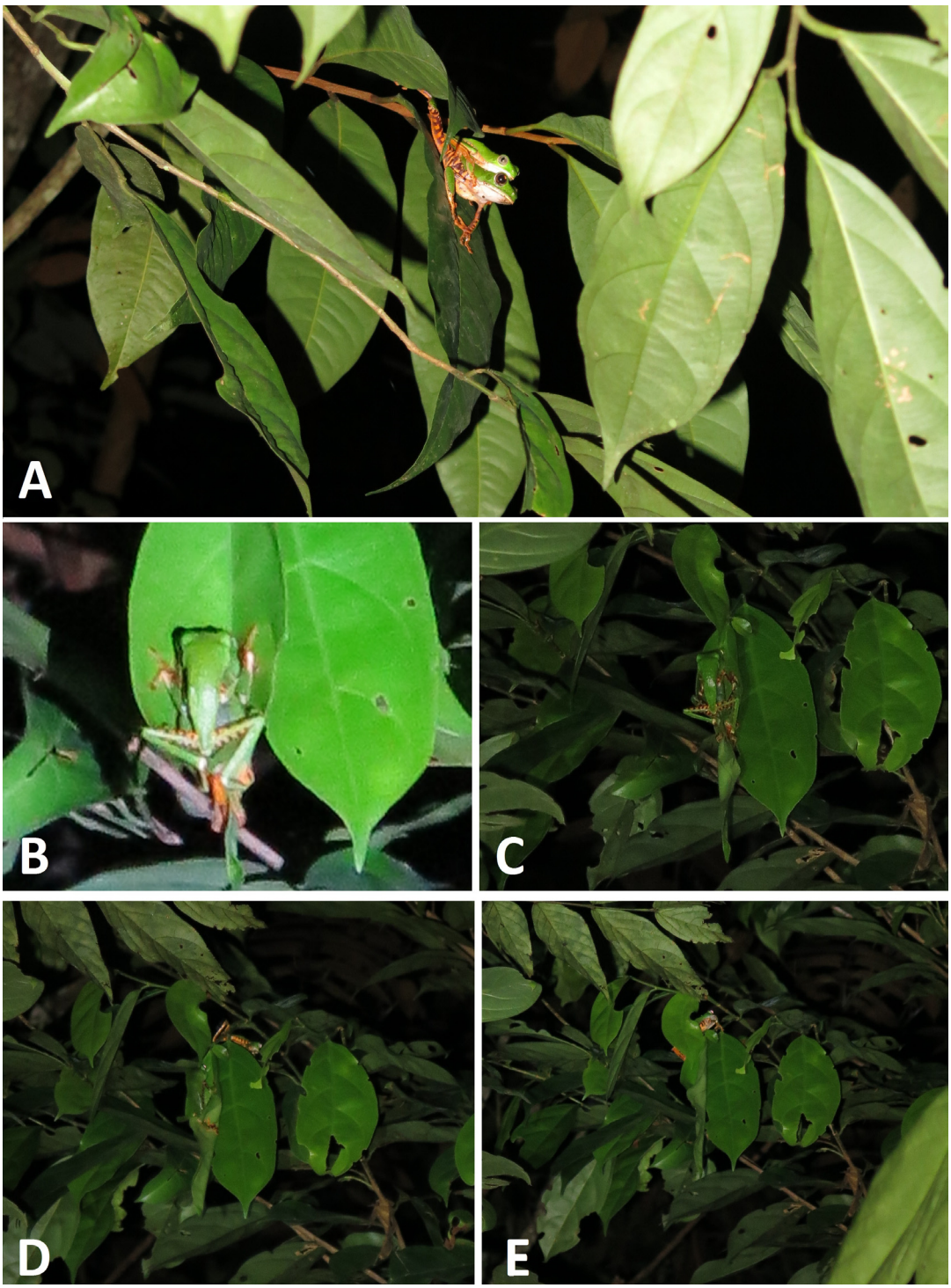

Figure 5. A: Female of *Callimedusa tomopterna* began to inspect the leaves to build the nest; **B:** Female began oviposition in the leaf; **C:** The male used his hind limbs to fold the sheet around the eggs; **D:** Exit of the male; **E:** Exit of the female. (Photos: Lucas Ferrante).

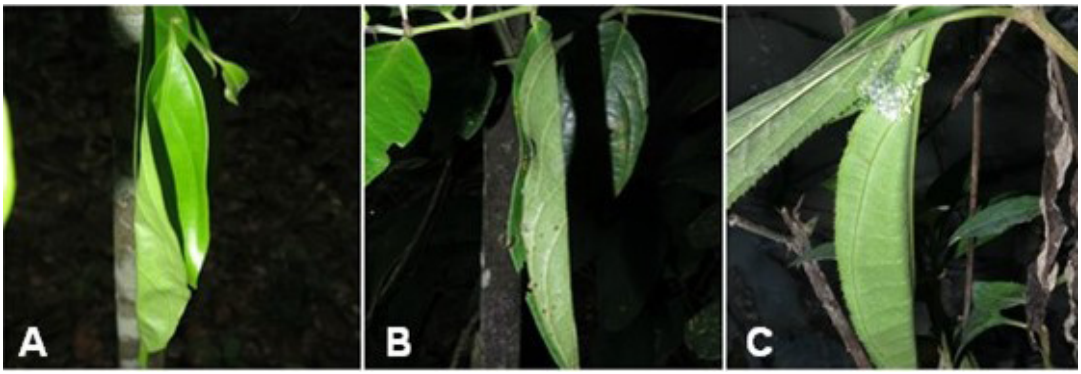

Figure 6. Variations in closure nests of *Callimedusa tomopterna*: **A** and **B:** Partially closed nests; **C:** Totally enclosed nest. (Photos: Lucas Ferrante).

to the structure of the leaves inspected by the females and external interferences as intruder males. In addition, the first amplexus had greater displacement intervals in relation to the other 3 amplexus in which the presence of the observers was less regular. The first amplexus also sought a higher altitude for the construction of the nest. We attribute the lowest displacement rate and highest nest building height also to our regular presence in the area, which can be considered from the animals' point of view as a potential predator.

The nest, spawning and embryology of the larval phase.—In all the nests (N = 4), a gelatinous mass was observed it unites the edges of the leaf and covering the eggs to avoid desiccation. Eggs that were not covered by this mass suffered desiccation at the pre-hatching stage, becoming yellowish and dying, as cited by Agar (1910) for *Phyllomedusa sauvagii*. Similarly, Pyburn (1980) and Wogel (2004) mention this gelatinous mass as eggless capsules for other species of the family Phyllomedusidae. We observed that this mass forms a buffer at the top and bottom of the leaf (jelly plugs) as observed by Downie et al. (2013) for *Phyllomedusa trinitatis*. According to Downie et al. (2013), the jelly plugs help protects the nest against predators and water entry. The arrangement of jelly plugs varied in length, closing partially (Fig.6a, b) or totally the leaf (Fig.6c).

To the first amplexus (amplexus 1), the leaf was 17.3 cm in length and was glued up until 9.1 cm. A total of 64 eggs were deposited inside the leaf, and all were fertilized showing signs of embryonic development. After 30 hours (second day), the eggs presented opaque colouration and well defined polar and apolar poles, the eggs had an average of 4 mm (Fig. 7a). After 60 hours of spawning end (third day), the embryos had well defined bilateral symmetry and neural tube formation (Fig. 7b). On the fourth day, after 94 hours, the embryos presented definite formation, appearance of the pharyngeal arches, the vitelline sac was perfectly visible, apparent eyes and the embryos showed movement (Fig. 7c). After 132 hours it was possible to observe the formation of the vascular system, and pigmentation of the blood vessels, in this period the embryos showed little or no activity (Fig. 7d). With one week, the embryos already had a clear cornea, visible organs, a perfectly visible lateral line and although it was still visible with a yolk sac already broke the egg membrane to free itself (Fig. 7e). The gills were not visible at hatching time compared to other species of the same family, as *Phitecopus azureus* and *Phyllomedusa sauvagii* reported by Salica et al. (2011), although the hatching time is in agreement with other species of the same family.

In a population of *C. tomopterna* for French Guiana, rain has influenced the premature hatching of tadpoles at different stages of development (Lescure, 1975). For *Phyllomedusa trinitatis* it was reported by able to hatch prematurely with the absence of external gills and embryos develop normally (Kenny, 1968). Also for *P. trinitatis*, Downie et al. (2013) verified that the humidity influences the pre-hatching stage of the eggs. According to Lescure (1995), the natural hatching of the eggs for *C. tomopterna* occurred at the end of a week (stage 23 according to Gosner, 1960) when the tadpoles had fully developed external branchia. Further studies on the development of tadpoles after hatching and metamorphosis, may help to understand

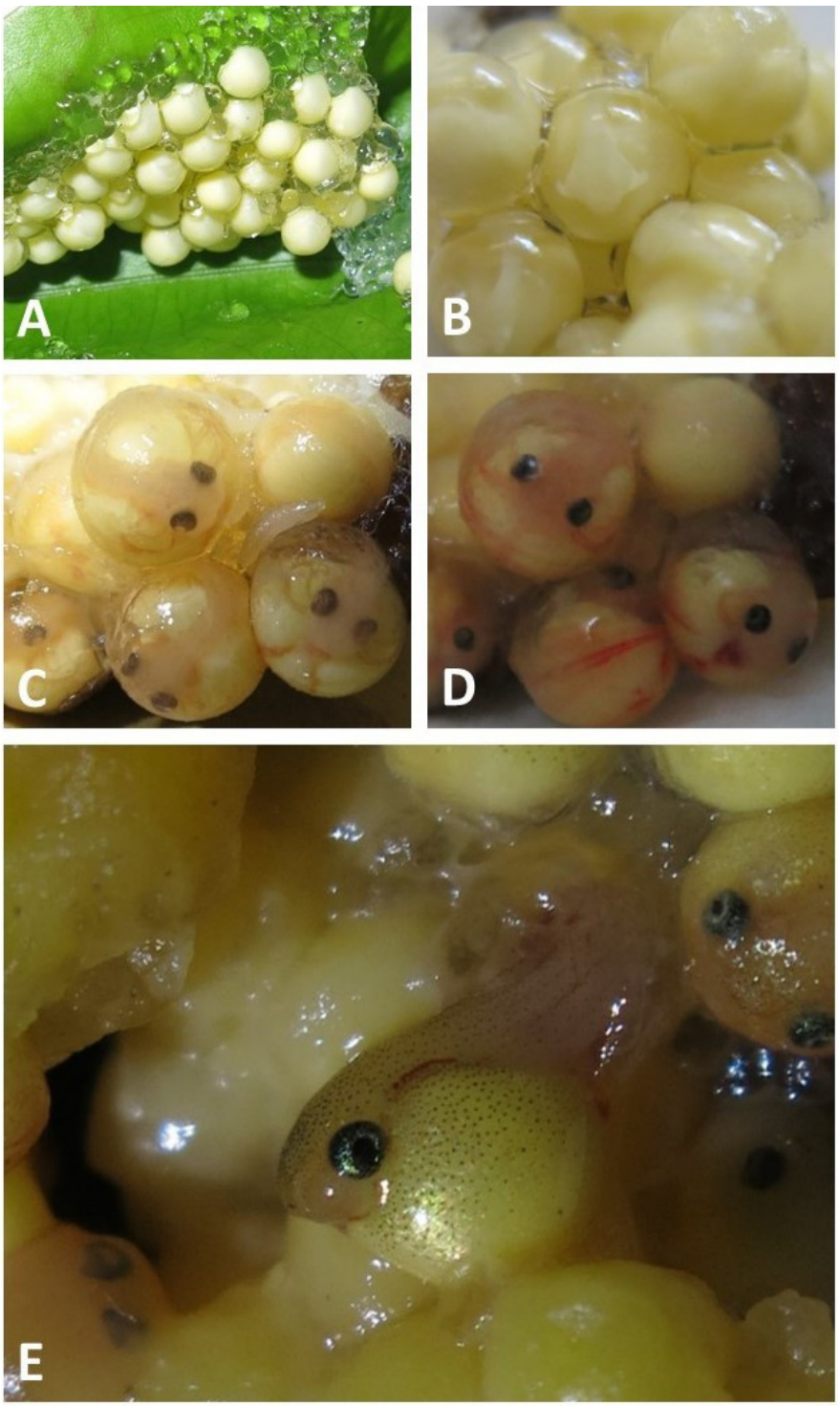

Figure 7. A: Eggs of *Callimedusa tomopterna* with opaque coloration and well defined polar and apolar poles; **B:** Embryos had well defined bilateral symmetry and neural tube formation; **C:** Embryos 94 hours after the fecundation; **D:** Embryos 156 hours after the fecundation showed vascular systems and pigmentation of the blood vessels; **E**: Tadpoles hatching of the eggs**.** (Photos: Lucas Ferrante).

the development of *C. tomopterna* and complete the information on the natural history for this species. The distribution of *C. tomopterna* and other species of the genus *Callimedusa*, includes regions from Amazonian slopes of the Andes from Ecuador to central Peru, upper Amazon Basin from Brazil, Colombia, Venezuela, Bolivia and Guianan Region (Duellman, 1974; Barrio-Amorós, 1998; Barrio-Amorós, 2009; Frost, 2017). This clade was pointed by Ron et al. 2013 for having originated in the Amazon Basin. Thus, the information reported here sheds some light on the reproduction of *Callimedusa* genus in the region of origin and group radiation.

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