

## Breeding sites of the leaf frog *Phyllomedusa tetraploidea* (Hylidae, Phyllomedusinae) in a forest remnant in southern Brazil

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**Abstract.** *Phyllomedusa tetraploidea* is a Neotropical leaf frog from the Atlantic forest, but also occurs in altered areas. We evaluated the reproductive sites used by individuals of this species in a forest remnant in southern Brazil. The study was conducted at Turvo State Park for two months during the species's reproductive period. Calling and oviposition sites were characterized according to vegetation type, height, and distance from the margin. Clutches were placed mainly on liana leaves, near the margins of ponds, at an average height of 135 cm. The maximum number of eggs per clutch was 190. Males called on the vegetation from 1 to 1.5 m high, mainly on small trees, and close to the margins of ponds, with intermediary values of Simpson's Index ( $B = 3.51$ ,  $B = 2.80$ ,  $B = 3.39$  respectively). Male's body length showed no relationship with quantitative variables of the calling sites. Our results suggest that the plasticity regarding reproductive sites exhibited by the species may be related to its occurrence in water bodies in disturbed habitats.

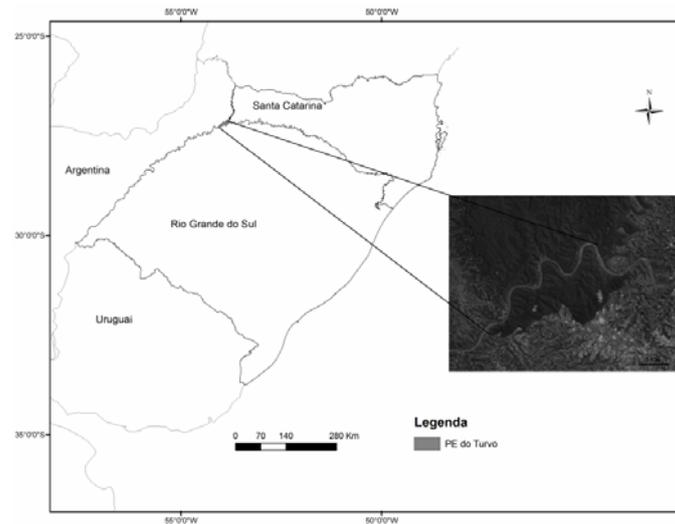
**Key words:** Anura, calling site, oviposition site, Atlantic forest.

Species of the genus *Phyllomedusa* utilize vegetation for vocalization and oviposition (Haddad & Prado 2005, Faivovich et al. 2010). Eggs are deposited on leaves hanging over the water and the female of the pair in amplexus wraps one or more leaves with posterior limbs for the nest formation. After hatching, tadpoles fall into the water to complete their development (Haddad & Prado 2005, Faivovich et al. 2010). *Phyllomedusa tetraploidea* Pombal & Haddad 1992 belongs to the *Phyllomedusa burmeisteri* group and is the only species in the group occurring in the interior of the Atlantic forest in Argentina, Paraguay and Brazil (Brunes et al. 2010, Iop et al. 2011, Frost 2011). It is typical from forested habitats, but can also occur in secondary forest and pastures (Machado et al. 1999, Kwet et al. 2004). Little is known about the natural history, ecology, and geographical distribution of this species. Herein, we characterize the calling and oviposition sites used by *P. tetraploidea* in a forest remnant.

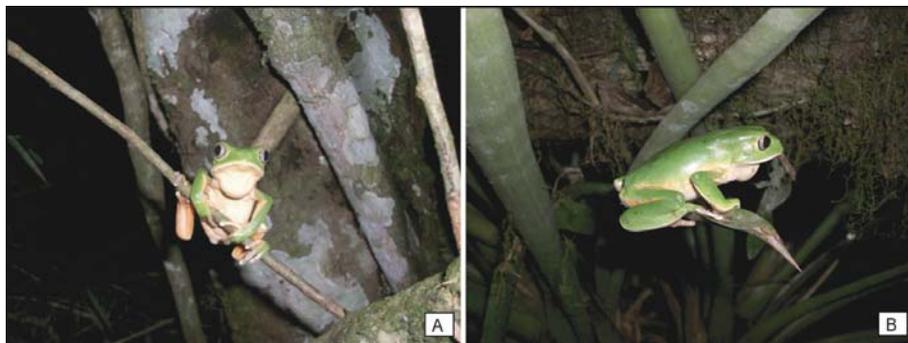
The study was conducted at Turvo State Park, a remnant of deciduous Atlantic forest in Rio Grande do Sul state (Ruschel et al. 2007), southern Brazil (27°07' - 27°16'S, 53°48' - 54°04'W) (Fig. 1). Climate is subtropical sub-humid, with rainfall well distributed throughout the year (Maluf 2000). We surveyed four water bodies during the day searching for clutches and at night searching for

males in calling activity (*sensu* Scott & Woodward, 1994), in October and November of 2011. To characterize the reproductive sites, we measured height and distance from the water of calling and oviposition sites. We only analyzed males and clutches found at the margin or inside the ponds because we considered inappropriate to group samples found at the same distance from water, but in opposite directions, especially regarding the reproductive mode of *Phyllomedusa* spp. We categorized the vegetation based on height and complexity according to Vidal & Vidal (2003): herbs (little or no lignifications), epiphytic orchids and epiphytic bromeliads, lianes (climbing vines), shrubs (branches from the base, less than 5 m), small trees (same tree architecture, with a maximum of 5 m height) and trees (clear trunk, more than 5 m). Additionally, we measured the snout-vent length (SVL) of the males and counted the number of eggs of four clutches. The diameter of ten eggs was measured, chosen randomly from each clutch. We used multiple regression to assess the relationship between SVL of males and the quantitative variables for site (height of the perch and distance from the water) (Zar, 1999). The Simpson's Index (Simpson, 1949) was used to identify the magnitude of variation in calling site use. This index was calculated separately for each site characteristic (vegetation type, perch height and distance from shore).

We were able to find a total of 36 calling sites and ten oviposition sites distributed in different types of vegetation considering all four water bodies. The height where males were found on the vegeta-



**Figure 1.** Study site, where the observations of breeding sites of *Phyllomedusa tetraploidea* were conducted, Turvo State Park, southern Brazil.



**Figure 2.** *Phyllomedusa tetraploidea* calling sites: A) male perched on a small tree and B) on epiphytic bromeliads, at Turvo State Park, southern Brazil.

**Table 1.** Number of males and clutches of *Phyllomedusa tetraploidea* observed in categories of values (cm) of height on the vegetation and distance from the margin of the pond (DM) at Turvo State Park, southern Brazil.

| cm      | Males  |    | Clutches |    |
|---------|--------|----|----------|----|
|         | Height | DM | Height   | DM |
| 0-50    | 0      | 14 | 0        | 5  |
| 51-100  | 8      | 3  | 1        | 0  |
| 101-150 | 17     | 5  | 7        | 0  |
| 151-200 | 2      | 1  | 2        | 1  |
| 201-250 | 2      | 0  | 0        | 1  |
| 251-300 | 5      | 1  | 0        | 0  |
| > 300   | 2      | 4  | 0        | 1  |
| Total   | 36     | 28 | 10       | 8  |

tion varied from 55 to 600 cm (mean = 165 cm  $\pm$  110, n = 36) and the distance from the water varied

from 0 to 800 cm (mean = 166 cm  $\pm$  231, n = 28 [we could not measure the distance from water for eight males] [Table 1]). The average SVL of males was 66 mm (range = 60 - 77 mm, n = 20) and there was no significant relationship between male SVL and quantitative variables of the calling sites ( $F = 1.72$ ,  $p = 0.2$ , n = 20). Clutches were found at 50 to 190 cm high on the vegetation (mean = 135 cm  $\pm$  42.5, n = 10) and the distance from the water ranged from 10 to 410 cm (mean = 113 cm  $\pm$  144, n = 8 [we could not measure the distance from water for two clutches] [Table 1]). Clutches were deposited almost exclusively on leaves of lianes (90%), but also on leaves of small trees (10%). The number of eggs per clutch and the average diameter (AD) of eggs were 137 (AD = 4.15 mm),

176 (AD = 3.17 mm), 177 (AD = 3.56 mm), and 190 (AD = 3.50 mm). Considering all 36 males found, the substrates most used as calling sites were small trees (55%), followed by trees (13%), epiphytic orchids and epiphytic bromeliads (11%), lianes (11%), herbaceous plants (5%), and shrubs (2,7%) (Fig. 2). Males varied in the use of vocalization sites; however, some heights on the vegetation, distance from the margin, and vegetation types were clearly more used than others, resulting in an intermediary Simpson's Index in the gradient specialist-generalist ( $B = 3.51$ ,  $B = 3.39$  and  $B = 2.80$ , respectively). The reproductive sites of *P. tetraploidea* were mainly placed close to the margins of ponds, on vegetation about 1 to 1.5 m height, where males called preferentially perched on small trees. These results differ from those reported in previous studies, where *P. tetraploidea* males were observed calling from grasses and other herbaceous plants (Pombal & Haddad 1992, Machado et al. 1999). Additionally, in this study, the clutches of *P. tetraploidea* were deposited on a single leaf, differing from previous observations, where clutches were deposited on more than one leaf and fixed together with eggless capsules (Pombal & Haddad 1992). These differences in calling site and leaf clutch nests may result from different vegetation type availability among sites and need further investigation. Our results suggest that the plasticity exhibited by *P. tetraploidea* regarding position in the vegetation and vegetation type used for reproduction may be related to its occurrence in water bodies in disturbed habitats (Machado et al. 1999, Kwet et al. 2004, Affonso & Delariva 2012).

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