

NOTA

## First record on an amphibian in the canopy of temperate rainforests of southern South America: *Eupsophus calcaratus* (Cycloramphidae)

Primer registro de un anfibio en el dosel del bosque templado del sur de Sudamérica: *Eupsophus calcaratus* (Cycloramphidae)

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### SUMMARY

We record for the first time arboreal habits in amphibians from the temperate rainforests of southern South America. In April 2006 we collected an individual of *Eupsophus calcaratus* at 16 m height, in a large *Eucryphia cordifolia* tree of old-growth forest of Chiloé Island, southern Chile. This species was considered until now as a terrestrial species, and the similarities between forest ground and epiphytic layer may allow this species inhabit the canopy. However, the level of association among these frogs and the forest canopy is unknown. The canopy of southern South America forests remains barely known, and as other forest canopies, may support high species diversity.

*Key words:* arboreal frogs, forest canopy, Chiloé temperate rainforest.

### RESUMEN

Este es el primer registro de hábitos arbóreos en una especie de anfibio de los bosques templados del sur de Sudamérica. En abril de 2006 fue colectado un individuo de *Eupsophus calcaratus* a 16 m de altura, en un gran árbol de la especie *Eucryphia cordifolia* en bosques antiguos de la Isla de Chiloé. Hasta ahora, esta especie era considerada como un animal terrestre. Similitudes entre el material epifito y el piso del bosque podrían permitir que esta especie habite en el dosel del bosque, aunque el nivel de asociación entre esta especie y el dosel aún no se conoce. El dosel de los bosques de Sudamérica es escasamente explorado y, tal como ocurre en otros ecosistemas forestales, podría albergar una gran riqueza de especies.

*Palabras clave:* sapos arbóreos, dosel del bosque, bosques templados de Chiloé.

### INTRODUCTION

The temperate rainforests of South America (STF) are mainly distributed in Chile and westernmost Argentina, and represent a biogeographical island separated from other forest ecosystems by over 1500 km of geographical barriers (the Andes and the Atacama and Patagonia deserts) during more than 1.5 million years (Armesto *et al.* 1998). This long term isolation has caused a high percentage of endemism in many taxa (Villagrán and Hinojosa 1997, Armesto *et al.* 1998), including the 80 % of the amphibian species (Formas 1979, Armesto *et al.* 1996). The amphibians of STF belong to the families Bufonidae, Calyptocephalellidae, Ceratophryidae, Cycloramphidae and Leiuperidae, with most species considered as terrestrial, associated to a variety

of habitats including swamps, ravines, shrubs and forest ground in old-growth and secondary forests (Ceí 1962, Méndez *et al.* 2005, Rabanal and Núñez 2008).

Arboreal frogs are frequent in northern temperate rainforests and reach a high diversity in tropical forests (Duellman and Trueb 1994). However, frogs with arboreal habits have not been recorded in STF (Formas 1979). Ceí (1962) suggested arboreal habits based on anatomical criteria for the endemic STF genera *Batrachyla* and *Hylorina*, but these species have been observed on the ground and only up to four meters above, in trees, bushes or stumps (Busse 1971, Formas 1979). In this note, we document the first record of arboreal habits in the terrestrial *Eupsophus calcaratus* (Günther 1881) frog, from old-growth forest of southern temperate rainforests of Chiloé Island, southern Chile.

## METHODS

The study area was located in the north west of Chiloé Island, at Punta Huechucuicui (41° 47' S, 74° 00' W). This area is characterized by a hilly landscape, with a large fragment of 300 ha of undisturbed and unpolluted old-growth temperate rainforest, surrounded by extensive forests with different degrees of human disturbance, completing a total area of around one thousand ha. Forests are dominated by broad-leaved evergreen trees, with scattered emergent trees of *Eucryphia cordifolia* Cav. (Monimiaceae) and a canopy dominated by *Laureliopsis philippiana* Mol. (Monimiaceae), *Aextoxicon punctatum* R. et P. (Aextoxicaceae), *Amomyrtus luma* Mol. (Myrtaceae), *A. meli* (Phil.) Legr. et Kaus. (Myrtaceae) and *Luma apiculata* (DC.) Burret (Myrtaceae). Climate is temperate with oceanic influence (DiCatri and Hajek 1976). The nearest meteorological station in Punta Corona (41° 47' S, 73° 52' W) has an annual average of 2,444 mm of rainfall and a mean annual temperature of 10.7° C.

In this area, we accessed to the canopy of a large *E. cordifolia* tree 30 m tall and 1.3 m diameter at breast height (Díaz *et al.* 2010). Large *E. cordifolia* trees are usually densely covered by epiphytes, including Gesneriaceae such as *Sarmienta scandens* (Brandis) Seeman, Bromeliaceae such as *Fascicularia bicolor* (Ruiz *et Pav.*) Mez, many species of ferns and mosses and the hemi-epiphytic tree *Raukua laetevirens* (Gay) Franche (Gutiérrez *et al.* 2008, Díaz *et al.* 2010). This tree was climbed using ropes and arborist techniques following the protocols described by the Tree Climbing USA (2006). Traps for invertebrates were located on the tree crown, as a part of a larger study of canopy biodiversity in this forest (Díaz 2009). Two eclector traps designed for the capture of walking invertebrates (Basset *et al.* 1997) were located at 16 m above the ground. Invertebrate surveys were monthly conducted during three to four days per month, extending the survey for over a year from March 2006 to May 2007. During March and April 2006 the traps were filled with alcohol 70 %, but since May 2006, alcohol was not used anymore and a capsule of Diazinon was located instead, a dry poison which unlike alcohol, does not damage flying insects such as moths.

The identity of the amphibian specimen found was defined comparing a fragment of the mitochondrial gen 16S with available sequences at GenBank. Total DNA was extracted using a salt method, from a finger fragment from the posterior right leg. Primers used, PCR protocol and sequence editing, were described in Correa *et al.* (2006). Comparison was conducted using BLAST (Basic Local Alignment Search Tool) from the National Center for Biotechnology Information (2009).

## RESULTS

In April 2006, during the austral fall an individual of *Eupsophus calcaratus* was captured in an Eclector trap filled with alcohol. The individual was probably an adult (snout-vent length = 31 mm), and was deposited in the Herpetological Collection of the Departamento de Biología Celular y Genética of Universidad de Chile (DBGUCH), with collection number 0604056 (figure 1). The genetic sequence showed the highest level of identity (98.3 %, 535 nucleotides compared) with the gene 16S of *Eupsophus calcaratus* (access number DQ864552).

## DISCUSSION

Arboreal frogs are very common in tropical forests from the Neotropical Region (e.g. families Centrolenidae and Hylidae) and in the North American temperate forests, where the family Hylidae is also present (Duellman and Trueb 1994). However, arboreal habits for frogs in STF



**Figure 1.** Individual of *Eupsophus calcaratus* collected at 16 m height in the canopy of a large *Eucryphia cordifolia* tree in Chiloé Island, southern Chile. (A) Dorsal view, (B) ventral view, (C) lateral view.

Individuo de *Eupsophus calcaratus* colectado a 16 m en el dosel de un gran árbol de la especie *Eucryphia cordifolia* en la Isla de Chiloé, Chile. (A) Vista dorsal, (B) vista ventral, (C) vista lateral.

have not been recorded before. Several authors describe climbing habits in *Batrachyla leptopus* (Bell) and *Hylorina sylvatica* Bell (Ceí 1962, Busse 1971, Formas 1979), but most observations were recorded less than three or four meters from ground level. Concerning the particular case of *Eupsophus*, this genus is endemic from STF (Formas 1995) and presents a large geographical distribution from 36° S to 50° S (Méndez *et al.* 2005); all known species are strongly associated with the forest ground, living below logs and under rocks in small ravines (Ceí 1962, Ortiz and Ibarra-Vidal 2005). For Chiloé Island only two *Eupsophus* species have been reported: *E. calcaratus* and *E. emiliopugini* (Rabanal and Núñez 2008, IUCN 2009). The species *Eupsophus calcaratus* is considered a common species, that reproduce on the ground, where the female deposits the eggs covered by a protective mucosity, and the development of the embryos is triggered by pond inundation (Formas and Vera 1980). The male presents parental care behavior, and after the laying, the male remains by the clutch, and later on also by the tadpoles, in small ponds in the ground (Úbeda and Nuñez 2006). Tadpoles develop quickly, with structures that keep their larval condition beyond the end of larval life (Vera Candioti *et al.* 2005).

In the canopy of old-growth forest of Chiloé Island, trees are evergreen and densely covered by epiphytes, resembling tropical forests. Large trees present a big and straight trunk and a crown that can overtake the forest canopy. Trunks and large branches present many small cavities, sometimes filled with water, and are densely covered by a large number of epiphytes reaching over 500 kg fresh weigh, mostly organic detritus (Díaz *et al.* 2010). The organic detritus of the epiphytic layer presented similarities with the organic horizon of the forest soil (Pérez *et al.* 2005), and may permit the survival of frogs in the canopy. There, holes filled with water are frequent in branches, and can represent similar habitats for egg laying than do inundated ponds at the ground level. Also, vegetation composed by vines and bromeliads create an heterogeneous habitat where frogs can hide and live, and conduct part of their life cycle.

Canopy environment has been little explored in STF, and arboreal habits of forest frogs have not been studied. During our investigations, big clumps of epiphytes from two *E. cordifolia* trees were removed and let fall onto the ground (Díaz *et al.* 2010). Less than an hour after epiphyte removal, we observed several individuals of *E. calcaratus* walking on the forest floor, near removed epiphyte clumps. This observation, along with the captured individual at 16 m reported here, makes us suspect the presence of *E. calcaratus* in the canopy could be frequent. In support to our findings, one recent discovery of eggs of *Batrachyla leptopus* in a tree at 13 m height provides strong evidence

of the arboreal habits in this frog species<sup>1</sup>. All these observations suggest that frogs are present in forest canopies of southern temperate rainforest; however, the diversity, abundance, reproductive habits, and the strength of the association between frogs and tree crowns remain unknown. The canopy of southern South America forests remains barely known and, as well as other forest canopies, may support high species diversity.

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