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# DNA taxonomy reveals two new species records of *Hyalinobatrachium* (Anura: Centrolenidae) for Bolivia

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We collected two specimens of the genus *Hyalinobatrachium* during fieldwork expeditions to the Departamento Pando—the northernmost region of Bolivia situated in the south-western Amazonian basin, within the zone of tall evergreen lowland rainforest. The specimens are deposited in the Colección Boliviana de Fauna, La Paz (CBF 6453) and in the National Museum, Prague (NMP6V 74059). Because species identification within *Hyalinobatrachium* based only on morphological characters is in many cases problematic (Kok & Castroviejo-Fisher 2008; Castroviejo-Fisher *et al*. 2009), we took advantage of published sequences of *Hyalinobatrachium* to identify our samples. Our results show that each specimen belongs to a different species (*H. mondolfii* and *H. munozorum*), none of them previously known to occur in Bolivia. The taxonomic implications of our discovery are briefly discussed.

## Methods

For molecular phylogenetic analyses we sequenced the complete mitochondrial gene 12S and a fragment of approximately 875 bp from the mitochondrial gene 16S from the two specimens of *Hyalinobatrachium* collected in Pando. We follow Castroviejo-Fisher *et al*. (2009) for laboratory protocols. We then compared these sequences with those deposited in public databases for the genus *Hyalinobatrachium* (Appendix I). We performed alignments, model selection, maximum likelihood phylogenetic analysis and bootstrap (BSS) replicates as explained in Castroviejo-Fisher *et al*. (2009). We used a combined dataset including specimens for which both 12S and 16S sequences were ≥ 90 % of the length of the sequences from the Pando specimens. We implemented a GTR+I+G model. We used sequences of *Celsiella vozmedianoi* (Ayazagüena & Señaris, 1996), and *Celsiella revocata* (Rivero, 1985) as outgroups (Guayasamin *et al*. 2008, 2009). Intra- and interspecific genetic p-distances between 59 sequences of the 16S marker were calculated using the software package PAUP* 4.0b10. We focused on 16S sequences because (i) there are more sequences available in GenBank for this marker than for 12S (Appendix I) and (ii) this marker is widely used as a DNA barcode for amphibians (e.g. Vences *et al*. 2005a,b).

The advertisement call of *H. munozorum* was recorded using a Marantz PMD660 compact flash recorder and a Stage Line ECM-950 directional microphone and that depicted for *H. mondolfii* with a Sony WM D6C tape recorder and a Sennheiser Me 80 directional microphone (the sounds were digitized and edited at a sampling frequency of 44.1 KHz and 16 bit resolution with a Delta 66 digitalizing board and Peak 3.2 software in an Apple Macintosh computer). Air temperature of both records was 24 °C. The software Praat 4.5.02 for MacOS X (Boersma & Weenink 2006) was used to obtain numerical information and to generate audiospectrograms and oscillograms. Frequency information was obtained through Fast Fourier Transformations (FFT) (width, 1024 points).

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