



## A new species of *Osteocephalus* (Anura: Hylidae) from Amazonian Bolivia: first evidence of tree frog breeding in fruit capsules of the Brazil nut tree

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### Abstract

A new species of *Osteocephalus* is described from lowland Amazonia of the Departamento Pando, northern Bolivia. The new species is most similar to *Osteocephalus planiceps* but differs by its smaller size (SVL 47.8–51.3 mm in males, 47.7–63.3 mm in females), absence of vocal slits, lack of sexual dimorphism in dorsal tubercles, single distal subarticular tubercle on the fourth finger, absence of dark spots on flanks, and by bicoloured iris with fine dark reticulate to radiate lines. The new species inhabits terra firme rainforest, breeds in water-filled fruit capsules of the Brazil nut tree and has oophagous tadpoles. Estimations of phylogenetic relationships within *Osteocephalus* based on mitochondrial DNA sequences show that the new species is closely related to *O. planiceps* and *O. deridens*.

**Key words:** Amphibia, Anura, Bolivia, Hylidae, Molecular Phylogeny, New Species, Oophagy, *Osteocephalus castaneicola*

### Introduction

Hylid frogs of the genus *Osteocephalus* represent typical anuran forms adapted to arboreal mode of life in rainforests of South America. They are excellent climbers and many of them evolved different reproductive adaptations to decrease competition and predator pressure. In this respect, the most specialized species call from or breed in bromeliads or other phytotelmata and provide biparental care to oophagous tadpoles (Jungfer & Schiesari 1995, Jungfer & Weygoldt 1999, Jungfer *et al.* 2000, Jungfer & Lehr 2001, Jungfer & Hödl 2002). Currently, the genus *Osteocephalus* comprises 20 recognized species distributed in the Amazon basin, Guianas and upper drainages of Río Magdalena and Río Orinoco in Colombia and Venezuela (Frost 2009). Nevertheless, *Osteocephalus* alpha taxonomy is far from stable. Existence of several unnamed species is mentioned by Jungfer & Hödl (2002).

Currently, four species of *Osteocephalus* are known to be present in Bolivia: *O. buckleyi* Goin, *O. pearsoni* Gaige, *O. taurinus* Steindachner and an undescribed *Osteocephalus* sp. (A) (sensu Jungfer & Lehr 2001). The latter one was originally associated with the name *O. lepieurii* (Duméril & Bibron) and its formal description remains under process of publication since long ago (see De la Riva *et al.* 2000, Jungfer & Lehr 2001, Jungfer & Hödl 2002). Apart from this, recent field research in the Departamento Pando (the northernmost region of Bolivia, situated in the south-western Amazonian basin within the zone of tall

evergreen lowland rainforest) revealed that at least two other taxa of *Osteocephalus* occur in Bolivian Amazonia. One, *Osteocephalus* sp. (B), is morphologically similar to *O. lepreurii* (see Moravec & Aparicio 2004). The second taxon, *Osteocephalus* sp. (C) represents a morphologically well differentiated, still unnamed species, which reproduces in abandoned water-filled fruit capsules of the Brazil nut tree. This contribution is aimed at the description of the latter.

## Materials and methods

Collected specimens (for exact localities see type specimens, Appendix, and Fig. 4) were fixed and stored in 70 % ethanol. Measurements are given in millimetres (mm) and were taken to the nearest 0.1 mm using a dissecting microscope and electronic digital calipers. Notes on colour in life were taken from field notes and colour photographs. Webbing formulae follow the standards of Myers & Duellman (1982), whereas all other terminology is that of Duellman (1970). Measurement abbreviations used throughout the text are: EN, eye to nostril distance; ED, horizontal eye diameter; ELW, upper eyelid width; FL, foot length as the distance from the heel to the tip of the fourth toe; HL, head length as the straight line distance from the posterior edge of the jaw articulation to the tip of the snout; HW, greatest head width; IOD, interorbital distance; SVL, snout-vent length; TD, horizontal tympanum diameter; and TL, tibia length. Specimens morphologically examined are listed in the Appendix. Institutional acronyms used are those listed in Leviton *et al.* (1985) with the following additions and corrections: CBF, Colección Boliviana de Fauna, La Paz; NMP6V and NMP6d, National Museum Prague.

For purpose of genetic analyses, tissue samples from 13 specimens of five *Osteocephalus* species, including *O.* sp. (B) and *O.* sp. (C), were taken from preserved voucher specimens (Table 1). We targeted a 1943 bp fragment of mitochondrial DNA (mtDNA) comprising partial 12S rRNA (12S), complete transfer RNA-Valin (tRNA-Val) and partial 16S rRNA (16S) genes. Our own DNA sequences were compared to and evaluated together with sequences of comparable mtDNA fragments of nine *Osteocephalus* species (14 individuals) obtained from GenBank. Additional sequences from five individuals of four species of genera *Tepuihyla*, *Itapotihyla*, *Osteopilus* and *Acris* were also taken from GenBank and used as outgroups (for their outgroup position see Faivovich *et al.* 2005, Wiens *et al.* 2006, Moen & Wiens 2009). For overview of all samples, their coverage, and GenBank accession numbers see Table 1.

Total genomic DNA was extracted from tissue samples using a commercial kit following the manufacturer's protocol. The whole portion of the targeted mtDNA was amplified using primers 12Sa [5'-CTGGGATTAGATACCCCACTA-3'; adapted from Kocher *et al.* (1989)] and 16SH1 [5'-CCGGTCTGAACTCAGATCACGT-3'; Palumbi *et al.* (1991)]. However, we were able to obtain only shorter separate fragments of the 12S (352 bp) and 16S (549 bp) genes in five samples due to low quality of their DNA using two pairs of primers: 12Sa / 12Sbs [12Sbs: 5'-TGAGGAGGGTGACGGGCGGT-3', adapted from Kocher *et al.* (1989)] and 16SL1 / 16SH1 [16SL1: 5'-CGCCTGTTTAACAAAACAT-3', adapted from Palumbi *et al.* (1991)]. Amplification of all fragments involved an initial cycle of denaturation at 94 °C for 15 min, and 35 subsequent cycles of 94 °C for 30 s, 55 °C for 30 s and 72 °C for 1 min, followed by a final extension step of 72 °C for 10 min. Sequencing was carried out using the 12Sa and 16SH1 primers and newly designed internal primers 16SLin (5'-AGTACCGYAAGGGAAAG-3') and 16SinH (5'-TCTTCTTGTTACTAGTT-3') by Macrogen Inc. (Seoul, Korea, <http://www.macrogen.com>). The sequences obtained have been deposited in GenBank (FJ965291–FJ965308).

Alignment was made by ClustalW (Thompson *et al.* 1994) as implemented in BioEdit 7.0 (Hall 1999) and checked by eye. The best-fit model of sequence evolution was selected using jModelTest 0.1.1 (Posada 2008) using maximum likelihood optimized trees calculated by the implemented PhyML algorithm (Guindon & Gascuel 2003). Both, the Akaike information criterion (AIC; Akaike, 1974) and the Bayesian information criterion (BIC; Schwarz 1978) selected the same best-fit model: transitional model 2 with gamma rate variation among sites (TIM2+G; Posada 2003). Phylogenetic trees were built using maximum likelihood

**TABLE 1.** *Osteocephalus* and outgroup species included in the molecular phylogenetic analyses.

Species	Code	Country	Locality	Museum Number	GenBank Accession Number			Note/Reference
					12S rRNA	tRNA-Val	16S rRNA	
<i>O. alboguttatus</i>		Ecuador	Sucumbios	KU 143119	DQ380347	-	-	Wiens <i>et al.</i> 2006
<i>O. buckleyi</i>		Ecuador	Napo: Jatun Sacha, 420 m	LAC 2216	DQ380378	-	EU034082	Wiens <i>et al.</i> 2006; Moen & Wiens 2009
<i>O. cabrerai</i>		Brazil	Acre, 5 km N Porto Walter, inland from Rio Juma	JPC 13178; LSUMZ H-13720	AY843705	AY843705	AY843705	Faivovich <i>et al.</i> 2005
<i>O. deridens</i>	der1	Peru	Loreto: 40 km SW of Iquitos	NMP6V 71262/2	FJ965304	-	FJ965291	this study
<i>O. lepricourii</i>		French Guiana	Creek of Margot		-	-	EF376066	Salducci <i>et al.</i> 2005
<i>O. "lepricourii"</i>		Venezuela	Amazonas: Neblina Base Camp on Río Mawarinuma (= Río Baria)	AMNH-A 1312546	AY549361	AY549361	AY549361	Faivovich <i>et al.</i> 2004
<i>O. mutabor</i>		Peru	Loreto: 1.5 km N Teniente López, elev. range 310–340 m	KU 221930	DQ380379	-	-	Wiens <i>et al.</i> 2006
<i>O. "oophagus"</i>		French Guiana	Kaw Road, 04°42' N / 52°18' W	MNHN 2001.0828	AY843708	AY843708	AY843708	Faivovich <i>et al.</i> 2005
<i>O. "oophagus"</i>		French Guiana	Mountain of Kaw		-	-	AF467267	Salducci <i>et al.</i> 2002
<i>O. planiceps</i>	pla1	Peru	Loreto: Puerto Almendras	NMP6V 71174/1	FJ965305	-	FJ965292	this study
<i>O. planiceps</i>	pla2	Peru	Loreto: Anguilla	NMP6V 71264/1	FJ965306	-	FJ965293	this study
<i>O. planiceps</i>	pla3	Peru	Loreto: Anguilla	NMP6V 71264/2	FJ965307	-	FJ965294	this study
<i>O. planiceps</i>		Peru	Loreto: San Jacinto: 175 m	KU 221933	DQ380380	-	-	Wiens <i>et al.</i> 2006
<i>O. taurinus</i>	tau1	Bolivia	Pando: Santa Crucito	CBF collections	FJ965296	FJ965296	FJ965296	this study
<i>O. taurinus</i>		French Guiana	Saül		-	-	EF376067	Salducci <i>et al.</i> 2005
<i>O. taurinus</i>		Peru	Loreto: Teniente López, 310 m	KU 221941	AY819380	-	AY819512	Wiens <i>et al.</i> 2005
<i>O. taurinus</i>		Peru	Madre de Dios: Cusco Amazónico	KU 205406; WED 55452	AY326041	AY326041	AY326041	Darst & Cannatella 2004

continued next page.

TABLE 1. (continued)

Species	Code	Country	Locality	Museum Number	GenBank Accession Number	Note/Reference
<i>O. taurinus</i>		Venezuela	Amazonas, Neblina Base Camp on Río Mawarinuma (= Río Baria), 140 m	AMNH-A 131245	-	'AY843709' ** Faivovich <i>et al.</i> 2005
<i>O. verruciger</i>		Ecuador	Napo: Río Azuela, 9.5 km W Reventador, 1630 m	KU 217751	DQ380381	Wiens <i>et al.</i> 2006
<i>O. sp. (B)</i>	spB1	Bolivia	Pando: Palmira	NMP6d 41/2009	FJ965297	FJ965297 this study
<i>O. sp. (B)</i>	spB2	Bolivia	Pando: Canadá	NMP6V 73105	FJ965298	FJ965298 this study
<i>O. sp. (B)</i>	spB3	Bolivia	Pando: Nacebe	NMP6V 72173/1	FJ965299	FJ965299 this study
<i>O. sp. (B)</i>	spB4	Bolivia	Pando: Nacebe	NMP6V 72173/3	FJ965308	FJ965295 this study
<i>O. sp. (C) = castaneicola</i>	spC1	Bolivia	Pando: San Antonio de Filadelfia	CBF 6051	FJ965300	FJ965300 <b>holotype</b> ; this study
<i>O. sp. (C) = castaneicola</i>	spC2	Bolivia	Pando: San Antonio de Filadelfia	NMP6V 73810/3	FJ965301	FJ965301 this study
<i>O. sp. (C) = castaneicola</i>	spC3*	Bolivia	Pando: San Antonio de Filadelfia	NMP6d 28/2009	FJ965302	FJ965302 this study
<i>O. sp. (C) = castaneicola</i>	spC4	Bolivia	Pando: San Antonio del Matti	NMP6V 73820	FJ965303	FJ965303 this study
<i>Tepuithyla edelcae</i>		Venezuela	Estado Bolívar, Auyantepui (2015 m)	MNHNP 1998-311	AY843770	AY843770 Faivovich <i>et al.</i> 2005
<i>Osteopilus septentrionalis</i>		Cuba	Guantánamo, Guantánamo bay	USNM 317830	AY843712	AY843712 Faivovich <i>et al.</i> 2005
<i>Itapotihyla langsdorffii</i>		Argentina	Misiones, General Belgrano, 10 Km N Bernardo de Irigoyen, Salto Andresito	MACN 38643	AY843706	AY843706 Faivovich <i>et al.</i> 2005
<i>Itapotihyla langsdorffii</i>		Brazil	São Paulo: Estação Ecológica de Jureia, N of Jureia	USNM 303287	AY819379	AY819379 Wiens <i>et al.</i> 2005
<i>Acris crepitans</i>		USA	Alabama, De Kalb Co., Powerline access Rd., 1/10 mi W of Lookout Mt. Boys Camp Rd.	LSUMZ H-2164	AY843559	AY843559 Faivovich <i>et al.</i> 2005

\* tadpole from a water-filled fruit capsule of the Brazil nut tree

\*\* AY843709 sequence is a chimera; the 12S rRNA part corresponds fully to AY843707 (*O. "lepteurii"*; Faivovich *et al.* 2005), which is, moreover, the same sequence of the same individual as AY549361 (Faivovich *et al.* 2004); we used only the 16S rRNA part

method (ML) by PhyML 3.0 (Guindon & Gascuel 2003), and for comparison by RAxML 7.0 (Stamatakis 2006), and using Bayesian analysis (BA) by MrBayes 3.2 (Huelsenbeck & Ronquist 2001, Ronquist & Huelsenbeck 2003). Parameters were set in PhyML as follows: base frequencies A = 0.35, C = 0.21, G = 0.18, T = 0.26, substitution rate matrix AC = AT = 5.31, AG = 10.77, CG = GT = 1.00, CT = 37.85 and gamma shape rate variation among sites  $\alpha = 0.151$ . BioNJ tree was used as an initial tree, the best of the nearest neighbour interchange (NNI), and the 'new' subtree pruning and regrafting algorithm (SPR; Hordijk & Gascuel 2005) of branch swapping was used as a tree topology search, with options to optimize the topology and branch lengths. Bootstrap values were computed based on 1000 resampled data sets (Felsenstein 1985). ML using RAxML was computed with the general time-reversible model with rate heterogeneity (GTR+G; Tavaré 1986), with parameters estimated during the run. The same was done in the case of BA. The BA was performed with two runs and four chains for each run for  $6 \times 10^6$  generations, and sampling every  $100_{th}$  tree. The first 300 trees (burn-in value) were discarded, as log-likelihood scores of sampled trees plotted against the generation time showed that stationarity was fully achieved after the first 20,000 generations. A majority rule consensus tree was then produced from the remaining trees after discarding the burn-in trees, and the posterior probabilities (BPP) calculated as the frequency of samples recovering any particular clade (Huelsenbeck & Ronquist 2001). The BA was run four more times with random starting trees and the results were compared to check for local optima. Genetic uncorrected  $p$ -distances were calculated in PAUP\* (Swofford 2003).

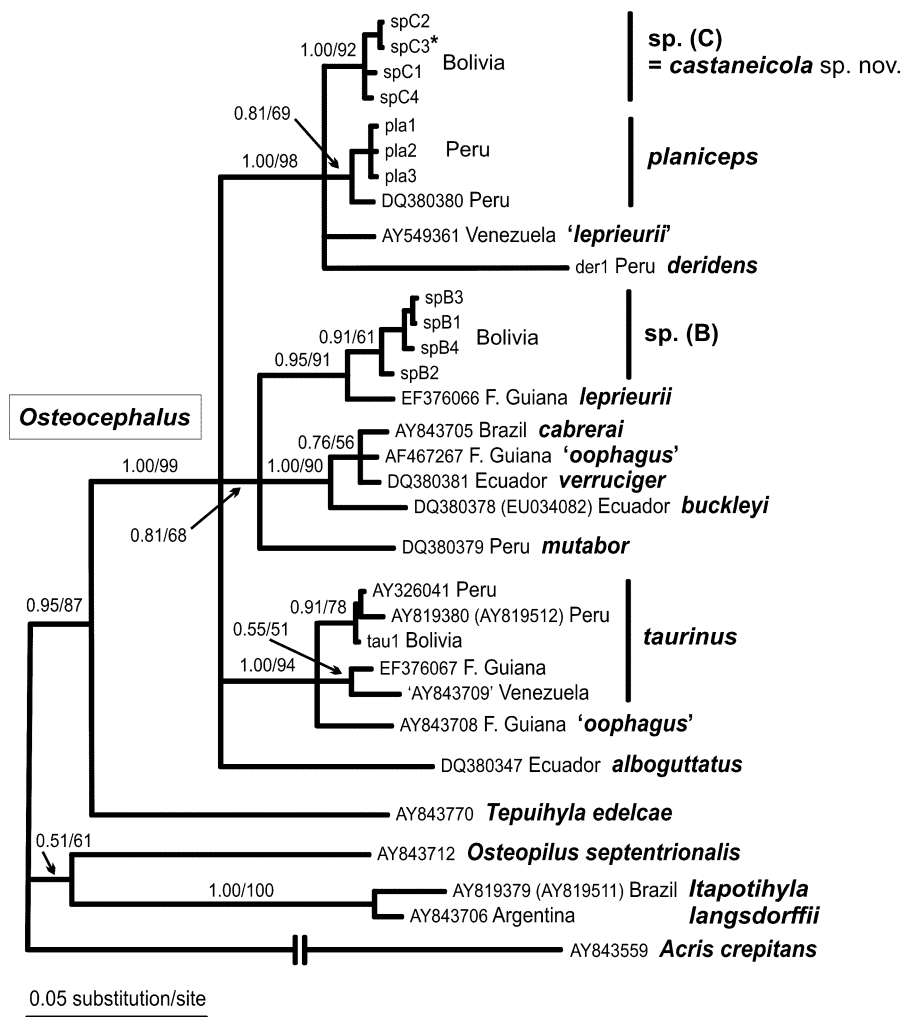
## Results

Both ML analyses, PhyML (Guindon & Gascuel 2003) and RAxML (Stamatakis 2006), resulted in most likely trees with the same topology with log likelihoods ( $\ln L$ ) = -7486.82 and -7489.98, respectively. Although, the bootstrap support of the relationships among main clades and most of species was very low. All independent BA runs resulted in essentially identical topologies and likelihood estimates. The majority rule consensus Bayesian tree (mean  $\ln L$  = -7534.54; Fig. 1) had the same topology as both ML trees, when branches of the ML trees with the bootstrap support below 50% were collapsed. The estimation of phylogenetic relationships within the genus *Osteocephalus* shows that four main phylogenetic lineages can be distinguished within the studied species: (1) individual lineage formed by *O. alboguttatus* (Boulenger); (2) lineage comprising *O. "oophagus"* Jungfer & Schiesari from French Guiana and *O. taurinus* (support 1.00/94 = BPP/ML bootstrap), however, the latter is further structured forming a sublineage from Peru and Bolivia, while next samples from French Guiana and Venezuela form a polytomy within the whole clade (low support of their relationships); (3) clade of not very high statistical support (0.81/68) comprising three well supported sublineages: (i) *O. mutabor* Jungfer & Hödl, (ii) *O. buckleyi*, *O. cabrerai* (Cochran & Goin), *O. verruciger* Werner and another sample of *O. "oophagus"* from French Guiana showing remarkably low mutual genetic differentiation (support 1.00/90), and (iii) Bolivian population of *Osteocephalus* sp. (B) clustering close to *O. leprieurii* from French Guiana (support 0.95/91); (4) clade (support 1.00/98) consisting of four sublineages represented by (i) *O. deridens* Jungfer, Ron, Seipp & Almendáriz, (ii) *O. "leprieurii"* from Venezuela, (iii) *O. planiceps* Cope, and (iv) the unnamed Bolivian *Osteocephalus* sp. (C).

The representatives of the two known populations of *Osteocephalus* sp. (C) form a well supported (1.00/92) separate lineage within the fourth clade. *O. planiceps* seems to be the closest relative of *Osteocephalus* sp. (C), with 3.0 % of mean uncorrected  $p$ -distances in 16S rRNA (Table 2), which is concordant with suggested interspecific level in this molecular marker in frogs (Fouquet *et al.* 2007, Vieites *et al.* 2009). This fact also corresponds to the unique morphology and life history of this species and justifies us to describe it as a new species herein.

**TABLE 2.** Uncorrected *p*-distances in percentage among *Osteocephalus* species and outgroup genera included in the phylogenetic analysis. Below diagonal are genetic distances based on the 352bp 12S rRNA fragment, above diagonal are distances based on the 380 bp 16S rRNA fragment, and on diagonal within species mean uncorrected *p*-distances, if applicable.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	
1 <i>O. alboguttatus</i>	-																								
2 <i>O. buckleyi</i>	5.1	-																							
3 <i>O. cabrerai</i>	4.8	1.4	-	8.2	5.6	6.8	-	5.5	0.8	6.1	-	5.5	5.3	-	5.5	5.8	-	5.7	6.9	11.6	10.0	11.6	-	13.4	
4 <i>O. deridens</i>	6.8	8.0	8.0	-	8.2	4.5	-	8.4	8.5	3.4	-	8.4	8.7	-	8.4	9.2	-	8.6	5.3	12.4	9.7	13.4	-	15.0	
5 <i>O. leprieurii</i> F. Guiana	-	-	-	-	-	7.7	-	6.1	5.3	5.8	-	5.3	5.3	-	5.3	5.8	-	1.2	6.8	11.6	10.8	11.6	-	14.5	
6 <i>O. "leprieurii"</i> Venezuela	5.4	3.7	4.5	5.7	-	-	-	7.9	6.6	2.4	-	7.9	7.6	-	7.9	8.7	-	7.8	4.4	10.5	9.5	12.1	-	14.7	
7 <i>O. mutabor</i>	4.3	2.8	2.6	8.0	-	4.8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
8 <i>O. "oophagus"</i> AY843708	5.1	4.5	4.8	8.2	-	4.8	4.0	-	5.3	7.1	-	2.1	1.6	-	2.1	2.6	-	6.7	7.6	10.8	10.0	11.8	-	14.7	
9 <i>O. "oophagus"</i> AF467267	-	-	-	-	-	-	-	-	-	5.8	-	5.3	4.7	-	5.3	5.6	-	5.1	6.6	10.8	9.5	10.6	-	12.9	
10 <i>O. planiceps</i> plal-pla3	4.8	3.7	4.0	5.4	-	1.7	4.3	4.3	-	<b>0.00,0</b>	-	6.8	6.9	-	6.8	7.6	-	6.4	3.0	10.3	8.7	11.6	-	14.5	
11 <i>O. planiceps</i> DQ380380	4.5	3.4	3.7	5.4	-	2.0	4.0	4.0	-	0.3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
12 <i>O. taurinus</i> tau1	4.5	4.0	4.3	7.4	-	4.3	3.4	0.9	-	3.7	3.4	-	1.6	-	0.0	2.1	-	5.9	7.4	11.1	9.5	10.0	-	14.5	
13 <i>O. taurinus</i> F. Guiana	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.6	1.1	-	5.7	7.4	10.8	9.2	10.8	-	14.2	
14 <i>O. taurinus</i> Peru AY819380	4.5	4.5	4.8	7.4	-	4.8	4.0	1.4	-	4.3	4.0	0.6	-	-	-	-	-	-	-	-	-	-	-	-	
15 <i>O. taurinus</i> Peru AY326041	4.3	4.3	4.5	7.1	-	4.5	3.7	1.1	-	4.0	3.7	0.3	-	0.3	-	2.1	-	5.9	7.4	11.1	9.5	10.0	-	14.5	
16 <i>O. taurinus</i> Venezuela	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6.4	8.2	11.8	9.7	11.3	-	14.7	
17 <i>O. verruciger</i>	5.4	0.9	0.6	8.2	-	4.0	3.1	4.8	-	4.0	3.7	4.3	-	4.8	4.5	-	-	-	-	-	-	-	-	-	
18 <i>O. sp. (B)</i> spB4	5.5	3.1	3.9	8.6	-	4.9	3.1	4.6	-	4.3	4.0	4.0	-	4.6	4.3	-	3.9	<b>0.40,5</b>	7.4	12.0	10.7	11.7	-	14.6	
19 <i>O. sp. (C)</i> = <i>castaneicola</i> spC1-spC4	4.5	3.3	4.2	5.3	-	1.9	3.9	3.9	-	1.4	1.1	3.3	-	3.9	3.6	-	3.6	4.0	<b>0.20,6</b>	10.4	8.6	11.5	-	14.6	
20 <i>Tepithyla edelcaae</i>	6.3	5.1	5.4	8.5	-	5.7	5.4	5.1	-	5.4	5.1	4.3	-	4.8	4.5	-	5.4	6.0	4.8	-	9.2	11.6	-	13.2	
21 <i>Osteopilus septentrionalis</i>	4.8	4.8	4.5	8.0	-	5.7	4.8	4.3	-	5.1	4.8	3.7	-	4.3	4.0	-	5.1	5.5	4.8	5.1	-	11.1	-	12.4	
22 <i>Itapothyla langsdorffii</i> Argentina	8.0	6.0	6.0	10.2	-	8.0	6.8	6.8	-	7.4	7.1	6.5	-	7.1	6.8	-	6.3	6.6	7.0	5.7	6.0	-	-	12.6	
23 <i>Itapothyla langsdorffii</i> Brazil	8.0	6.0	6.0	10.0	-	7.7	6.8	6.8	-	7.1	6.8	6.5	-	7.1	6.8	-	6.3	6.6	6.8	5.7	6.0	-	-	-	
24 <i>Acris crepitans</i>	9.1	8.8	8.8	11.6	-	9.1	9.7	9.9	-	8.8	8.8	9.4	-	9.9	9.7	-	9.1	9.7	9.0	9.1	8.2	10.2	9.9	-	



**FIGURE 1.** The majority rule consensus tree of the Bayesian phylogeny of the frog genus *Osteocephalus*, using mitochondrial 12S rRNA – tRNA-Val – 16S rRNA data. Nodal support, Bayesian posterior probabilities (BPP) and maximum likelihood (PhyML) bootstrap (1000 pseudoreplicates), are indicated. Collapsed branches were supported < 50 % bootstrap and < 0.50 BPP. The asterisk denotes the tadpole sample from a water-filled fruit capsule of the Brazil nut tree. *Osteocephalus* “*oophagus*” no. AF467267 may represent *O. cabrerai* according to Fouquet *et al.* (2007, Supporting Information).

***Osteocephalus castaneicola* sp. n.**

Figs. 2(A–E), 3(A–B)

**Holotype.** CBF 6051, adult male from the vicinity of the settlement of San Antonio de Filadelfia, 11°18' S, 67°23' W, ca. 200 m a.s.l., Provincia Manuripi, Departamento Pando, Bolivia, collected on 22 November 2007 by J. Moravec, M. Guerrero-Reinhard and G. Calderón.

**Paratopotypes.** NMP6V 73810/1–3, two adult males and an adult female, same locality and collecting data as holotype; CBF 6052, adult female, same locality and collecting data as holotype;

**Paratypes.** CBF 6053–6054, adult male and adult female from San Antonio del Matti, 11°30'S, 68°53'W, ca. 270 m a.s.l., Provincia Manuripi, Departamento Pando, Bolivia, collected on 27 November 2007 by J. Moravec, M. Guerrero-Reinhard and G. Calderón; NMP6V 73820, adult female, same locality and collecting data as CBF 6053–6054.

**Diagnosis.** A medium-sized species of *Osteocephalus* as revealed from mtDNA analyses, which can be distinguished by the following combination of characters: (1) medium size, SVL 47.8–51.3 mm in males,

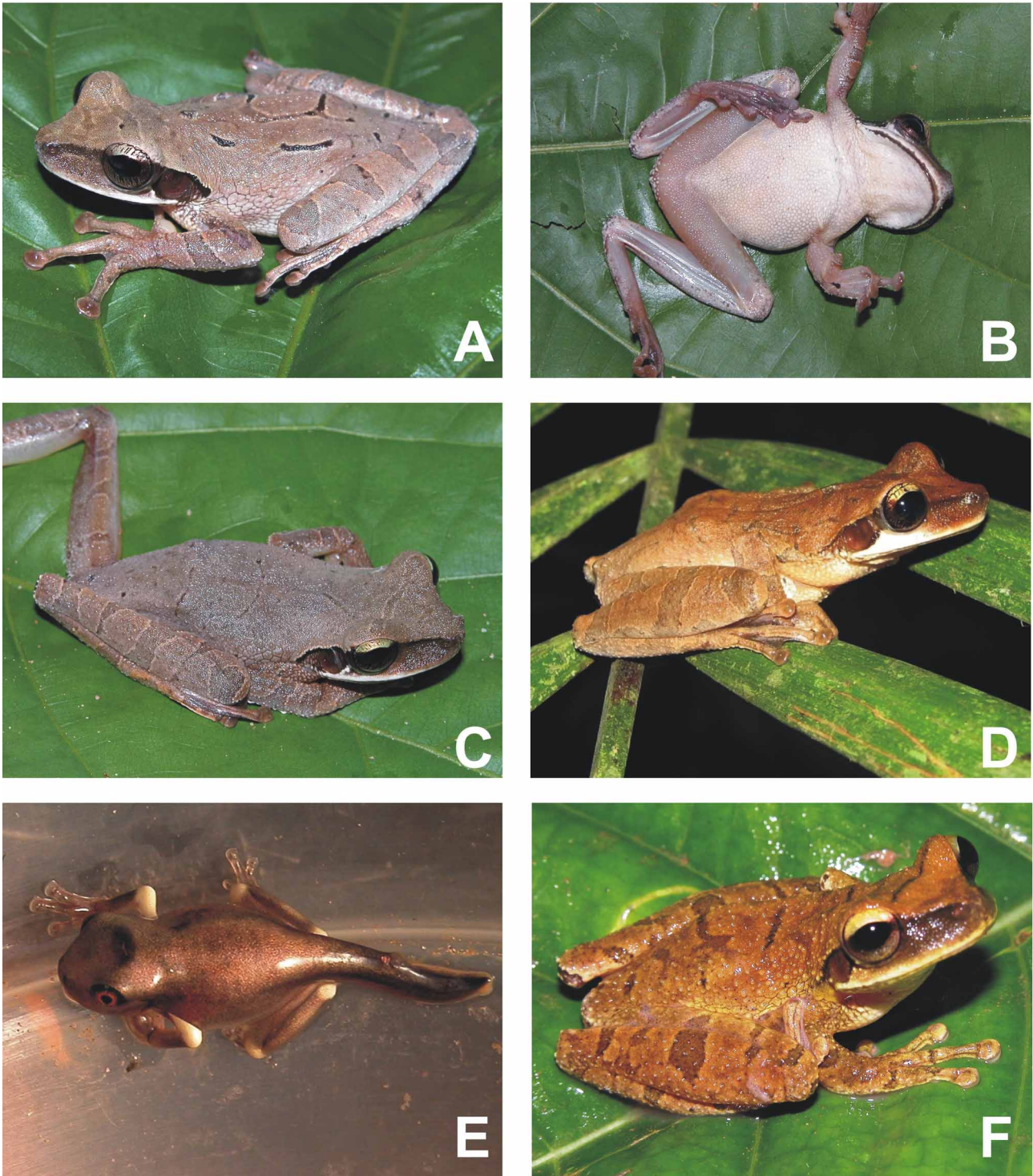
47.7–63.3 mm in females; (2) snout rounded in dorsal view, rounded and slightly inclined posteroventrally in lateral view; (3) canthus rostralis distinct, angular, distinctly curved medially; loreal region concave; (4) low frontoparietal ridges well-marked in large individuals; (5) tympanum large, round to oval, about 62.5–76.5% of eye diameter, tympanic annulus distinct; supratympanic fold markedly developed; (6) vocal slits absent, vocal sac indistinct; (7) vomerine odontophores large, prominent, angular, narrowly separated or in contact medially, between oblique choanae, bearing 6–14 vomerine teeth each; (8) skin on dorsal surfaces with numerous minute tubercles; (9) low tarsal and ulnar tubercles present, slightly larger than dorsal tubercles; (10) axillary membrane absent; (11) basal webbing on hand [webbing formula II (2<sup>-</sup>–2<sup>+</sup>)—(3<sup>-</sup>–3<sup>+</sup>) III (3<sup>-</sup>–3)—(2<sup>2/3</sup>–3<sup>-</sup>) IV]; toes about three fourths webbed [webbing formula I (1–1<sup>1/4</sup>)—(1<sup>2/3</sup>–2<sup>-</sup>) II (1–1<sup>+</sup>)—(2<sup>-</sup>–2) III (1–1<sup>+</sup>)—(1<sup>2/3</sup>–2) IV (1<sup>2/3</sup>–2<sup>-</sup>)—(1<sup>-</sup>–1) V]; (12) single round distal subarticular tubercle under the fourth finger; (13) dark keratinous excrescences restricted to prepollex; (14) in life, dorsum tan, pale brown to purple brown, with scarce narrow irregular dark brown markings; a narrow pale supralabial line expanding in a subocular spot; flanks pale, without markings; hidden surfaces of thighs light brown; throat and belly creamy white; a narrow dark line along the mandible; ventral surfaces of thighs fleshy pink; iris bicoloured with a dark horizontal stripe, golden above, bronze below, both parts with fine dark reticulate to radiate lines; tibiae green or white; (15) in life, newly metamorphosed juveniles light brown dorsally, with a dark interorbital spot, bright orange iris, and creamy white upper arms, knees and heels.

**Comparisons.** Morphologically, *O. castaneicola* can be distinguished from all other Amazonian species of *Osteocephalus* by absence of vocal slits and by the following combinations of characters: from *O. alboguttatus* by more extensive webbing and by colouration (*O. alboguttatus*: toes two thirds webbed, light brown dorsum with small blackish dots, flanks and upper surface of thighs with small round white spots, beneath whitish with dark reticulation) (Boulenger 1882, Duellman 1978); from *O. buckleyi* by absence of large tarsal tubercles, absence of patagium and by eye colouration (*O. buckleyi*: large tubercles along the tarsus, well developed patagium, light iris without conspicuous dark pattern) (Boulenger 1882, Cochran & Goin 1970; examined specimens listed in the Appendix); from *O. cabrerai* by absence of large dorsal, ulnar and tarsal tubercles, absence of patagium and by colouration (*O. cabrerai*: large wart-like tubercles on head and dorsum, large tubercles along the ulna and tarsus, small patagium, irregularly mottled dorsal pattern, light iris with very fine vermiculation) (Cochran & Goin 1970; examined specimens listed in the Appendix); from *O. carri* (Cochran & Goin) by colouration (*O. carri*: dense large irregular dark spots on the dorsum, black spots on flanks, fuscous throat and chest) (Cochran & Goin 1970); from *O. deridens* by larger size and by colouration (*O. deridens*: SVL up to 34.9 mm in males and 50.6 mm in females, dorsum light or dark tan with or without irregular darker or lighter markings, golden yellow iris with a dark horizontal stripe and regular dark radiation (Jungfer *et al.* 2000; examined specimens listed in the Appendix); from *O. elkejungingerae* (Henle) by skin texture and by colouration (*O. elkejungingerae*: conspicuous tubercles with keratinized tips in breeding males, dorsum with broad light dorsolateral stripes in juvenile and subadult specimens (Henle *et al.* 1983; Jungfer *et al.* 2000; examined specimens listed in the Appendix); from *O. fuscifacies* by larger size and by colouration (*O. fuscifacies*: SVL up to 45.6 mm in males and 53.2 in females, dorsum light or dark tan with or without irregular darker or lighter markings, light subocular spot absent, venter dark with creamy white granules or creamy white, golden iris with a dark horizontal stripe and regular dark radiation (Jungfer *et al.* 2000; examined specimens listed in the Appendix); from *O. heyeri* Lynch by larger size and by colouration (*O. heyeri*: SVL up to 36.1 mm in males and 47.7 mm in females, dorsum brown with darker markings and pale spots, flanks with pale spots, hidden surfaces of limbs dark brown with pale spots, iris dark) (Lynch 2002); from *O. leoninae* Jungfer & Lehr by larger size and by colouration (*O. leoninae*: SVL up to 42.0 mm in males and 53.2 mm in females, upper part of iris yellow without dark markings, unpigmented nuptial pads, bold dorsal pattern) (Jungfer & Lehr 2001, Chávez *et al.* 2008); from *O. leprieurii* by nuptial excrescences restricted to prepollex, skin texture and by colouration (*O. leprieurii*: prepollical and subdigital nuptial excrescences, numerous conspicuous tubercles with keratinized tips in breeding males, golden iris with dark vermiculation, white supralabial stripe in juveniles) (Jungfer & Hödl 2002); from *O. mutabor* by skin texture



and by colouration (*O. mutabor*: numerous conspicuous tubercles with keratinized tips in breeding males, bold dark transverse markings, golden yellow iris with dark vermiculation, white dorsolateral stripes in juveniles) (Jungfer & Hödl 2002; examined specimens listed in the Appendix); from *O. oophagus* by head shape and by colouration (*O. oophagus*: truncate snout in dorsal view, white mottling or reticulation on posterior half of the flanks, golden iris with regular black radiation, orange spots on elbow, knee and heel in juveniles) (Jungfer & Schiesari 1995; examined specimens listed in the Appendix); from *O. pearsoni* by skin texture and by colouration (*O. pearsoni*: small nonspinous tubercles in males, black reticulation on the venter, dark iris) (Trueb & Duellman 1971, Jungfer & Schiesari 1995, Jungfer & Lehr 2001); from *O. planiceps* by smaller size, skin texture, keratinous excrescences restricted on prepollex and by colouration (*O. planiceps*: SVL up to 65.9 mm in males and 88.2 mm in females, numerous conspicuous tubercles with keratinized tips in breeding males, keratinous excrescences extending laterally to disc of thumb, dark spots on flanks, iris with regular black radiation) (Cope 1874, Duellman & Mendelson 1995, Jungfer & Lehr 2001, examined specimens listed in the Appendix); from *O. subtilis* Martins & Cardoso by larger size and by colouration (*O. subtilis*: SVL up to 38.8 mm in males, dark iris) (Martins & Cardoso 1987); from *O. taurinus* by smaller size, less webbing on the hands and by colouration (*O. taurinus*: SVL up to 81.0 mm in males and 94.1 in females, fingers one-half webbed, dark spots on flanks, small brown flecks on the throat, chest and sides of the belly, greenish gold iris with regular black radiation) (Duellman 2005; examined specimens listed in the Appendix); from *O. verruciger* by skin texture and by colouration (*O. verruciger*: numerous conspicuous tubercles with keratinized tips in breeding males, uniform reddish brown iris) (Trueb & Duellman 1971, Jungfer *et al.* 2000, Jungfer & Hödl 2002); from *O. yasuni* by skin texture and by colouration (*O. yasuni*: numerous conspicuous tubercles with keratinized tips in breeding males, yellow venter in adults, iris with irregular dark reticulation, intense yellow-orange venter and webbing in juveniles) (Ron & Pramuk 1999, Jungfer *et al.* 2000, Jungfer & Hödl 2002, Cisneros-Heredia 2007).

There are seven available names in the synonymy of four *Osteocephalus* species: *Hyla festae* Perraca, 1904 (type locality: Ecuador: “Valle de Santiago” (= lower Río Zamora) Province of Morona-Santiago) in the synonymy of *O. buckleyi*; *Hyla leprieurii britti* Melin, 1941 (type locality: Brazil: “Río Uaupés (north of the Río Japú”, Amazonas) and *Osteocephalus ayarzaguenai* Gorzula & Señaris, 1997 (type locality: Venezuela: “Campamento Airo, Valle del Río Karuay”, Estado Bolívar) in the synonymy of *O. leprieurii*; *Osteocephalus flavolineatus* Steindachner, 1862 (type locality: Brazil: “Cocuy” (= Cucuí), Amazonas) and *Hyla depressa* Andersson, 1945 (type locality: Ecuador: “Río Pastaza, Watershed”) in the synonymy of *O. taurinus*; and *Hyla riopastazae* Andersson, 1945 (type locality: Ecuador: “Baños, Río Pastaza, Provincia Tungurahua”) and *Hyla orcesi* Funkhouser, 1956 (type locality: Ecuador: “[Río] Pacayacu, a stream that flows into the Cotapino, drainage of the Suno, Río Napo region”) in the synonymy of *O. verruciger*. The new species differs from all of them by the following combination of characters: from *Hyla festae* by smaller size and by colouration (female holotype of *H. festae*: SVL 75.0 mm, large median longitudinal dark brown blotch on the dorsum, dark brown spots on flanks, throat and belly) (Trueb & Duellman 1971); from *Hyla leprieurii britti* by nuptial excrescences restricted to prepollex and by skin texture (male holotype of *H. l. britti*: prepollical and subdigital nuptial excrescences and tuberculate dorsum) (Trueb & Duellman 1971, Jungfer & Hödl 2002), from *Osteocephalus ayarzaguenai* by colouration (*O. ayarzaguenai*: golden iris with dark vermiculation) (Jungfer & Hödl 2002; examined specimen listed in the Appendix); from *Osteocephalus flavolineatus* by smaller size and colouration (female holotype of *O. flavolineatus*: SVL 81.8 mm, light middorsal stripe, spots on the flanks) (Cochran & Goin 1970, Trueb & Duellman 1971); from *Hyla depressa* by smaller size, skin texture, and by colouration (male holotype of *H. depressa*: SVL 68.9 mm, tuberculate dorsum, light middorsal stripe) (Cochran & Goin 1970, Trueb & Duellman 1971); from *Hyla riopastazae* by colouration (*H. riopastazae*: brown spots and mottling on throat, chest and belly) (Trueb & Duellman 1971); and from *Hyla orcesi* by skin texture and by colouration (*H. orcesi*: tuberculate dorsum, ventral surfaces dirty brown) (Cochran & Goin 1970, Trueb & Duellman 1971).



**FIGURE 2.** Holotype of *Osteocephalus castaneicola* **sp. n.** (CBF 6051) in life, (A) dorsal, and (B) ventral views. (C) Adult female paratype of *Osteocephalus castaneicola* **sp. n.** (CBF 6052) in life. (D) Night colouration of adult male paratype of *Osteocephalus castaneicola* **sp. n.** (NMP6V 73810/2) under natural conditions. (E) Newly metamorphosed juvenile of *Osteocephalus castaneicola* **sp. n.** (F) Adult male of *Osteocephalus* sp. (B) (NMP6V 73105) from Canadá (Bolivia, Pando) in life.

**Description of the holotype.** Adult male 51.3 mm SVL. Head narrower than body, slightly longer than wide; snout rounded in dorsal view, moderately protruding in lateral view; distance from nostril to eye shorter than diameter of eye; canthus rostralis distinct, angular, curved medially; loreal region concave; internarial

area slightly depressed; nostrils moderately protuberant, directed laterally; interorbital area flat, IOD 112.2% of ELW; lateral margins of the frontoparietals barely visible through skin; eye large, strongly protuberant, its diameter about five times depth of lip below eye; tympanic membrane clearly evident, large, slightly wider than high, about two third of eye length, separated from eye by ca. 50% of its diameter; tympanic annulus distinct; supratympanic fold conspicuous, covering upper edge of tympanum, continuing above insertion of arm. Arm slender, axillary membrane absent; small low tubercles scattered along ventrolateral edge of forearm; relative length of fingers I<II<IV<III; fingers bearing large, oval discs, that of third finger about half of tympanum diameter; subarticular tubercles prominent, round, single; supernumerary tubercles present; palmar tubercle large, flat, disunited distally; prepollical tubercle large, flat, elliptical; prepollex enlarged; large dark keratinous nuptial excrescences covering inner surface of prepollex up to subarticular tubercle of thumb (Fig. 3); webbing rudimentary between fingers I and II; webbing formula of fingers II<sup>2</sup>—3 III<sup>3</sup>—3<sup>+</sup> IV. Legs moderately long, slender; heels overlapping when limbs flexed perpendicular to the axis of body; small raised tubercles on the outer edge of tibiotarsal articulation; small low tubercles scattered along the ventrolateral edge of foot; toes moderately long, bearing oval discs slightly smaller than those of fingers; relative length of toes I<II<V<III<IV; outer metatarsal tubercle distinct, small, round; inner metatarsal tubercle large, ovoid; subarticular tubercles single, round, protuberant; supernumerary tubercles present; toes three fourths webbed; webbing formula of toes II<sup>+</sup>—2<sup>+</sup> III<sup>1</sup>—2<sup>+</sup> IV<sup>2</sup>—1<sup>+</sup> V. Skin on dorsum, head, and dorsal surfaces of limbs smooth, with numerous minute tubercles; skin on flanks shagreen; skin on venter coarsely granular; skin on throat slightly granular; proximal two thirds of lower surfaces of thighs slightly granular. Cloacal opening directed posteriorly at upper level of thighs; short simple cloacal sheath covering cloacal opening; rounded tubercles around vent and on posterior surface of proximal third of thigh. Tongue ovoid, widely attached to floor of mouth; vomerine odontophores angular, separated medially, between choanae, bearing 8 and 9 (left/right) vomerine teeth; choanae rhomboidal, oblique; vocal slits absent; vocal sac indistinct.

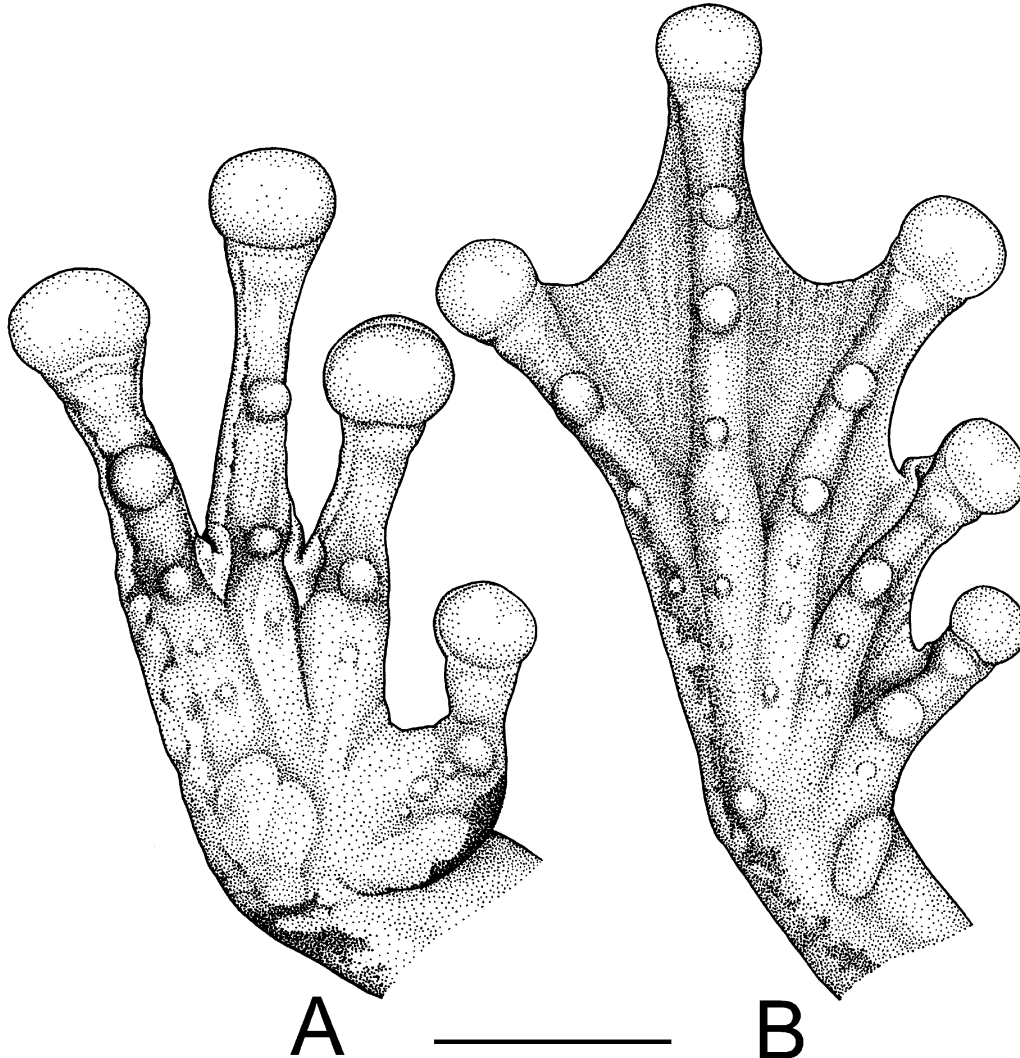
Measurements of the holotype: SVL 51.3; HL 17.7; HW 16.6; EN 5.3; ED 6.1; TD 4.0; ELW 4.9; IOD 5.4; TL 27.3; FL 33.4.

In alcohol, head and dorsum tan with several narrow irregular darker tan to dark brown markings (including an indistinct interorbital stripe) narrowly outlined by pale brown line; dorsal surfaces of limbs tan with darker tan crossbars outlined by a pale brown line. A narrow pale supralabial line expanding in a subocular spot; a dark canthal stripe extending from nostril to the anterior margin of eye; a broad dark brown postocular stripe extending from posterior margin of eye across the tympanum to insertion of arm. Flanks pale with several inconspicuous small darker markings; a dark supraclacal spot; hidden surfaces of thighs tan. Throat and belly creamy white; a narrow dark line along the lower jaw; ventral surfaces of thighs yellowish white; plantar surfaces pale brown. Tibiae green.

In life, dorsal and lateral colouration differed only slightly from the preserved specimen in having a slight purple-red tint by day. Ventral surfaces of forearms and thighs fleshy pink; tibiae green. Iris bicoloured with dark brown horizontal stripe, golden above, bronze below, both parts with fine dark reticulate to radiate lines (Fig. 2A).

**Variation.** Variation of measurements of the type series is given in Table 3. *Osteocephalus castaneicola* exhibits sexual dimorphism in body size, but sexual dimorphism of dorsal skin texture is absent. Both breeding males and females bear similar minute flat to round tubercles on dorsal surfaces of head, body and limbs. The most conspicuous dorsal tubercles are present in female paratopotype CBF 6052 (Fig. 2C), having SVL 47.7 mm and containing numerous small immature eggs. The new species shows considerable variation in number of vomerine teeth (6–14 on each odontophore). Vomerine odontophores are separated in holotype, paratopotype NMP6V 72810/1 and paratypes CBF 6054 and NMP6V 73820, but in contact in the remaining types. Some variation seems to be evident in distinctiveness of lateral margins of the frontoparietals. They are not visible through skin in smaller individuals (SVL up to 47 mm; paratopotype CBF 6052 and paratype CBF 6053) and best pronounced in largest individuals (SVL above 59 mm; female paratopotype NMP6V 73810/3

and female paratypes CBF 6054 and NMP6V 73820). Some differences can be found in shape of distal subarticular tubercle of the fourth finger. It is single in holotype and four other type specimens, but it shows a slight tendency to bifidity in the paratopotype NMP6V 73810/3 and paratypes CBF 6053 and NMP6V 73820. The finger and toe webbing formulae vary as follows: II (2<sup>-</sup>–2<sup>+</sup>)—(3<sup>-</sup>–3<sup>+</sup>) III (3<sup>-</sup>–3) —(2<sup>2/3</sup>–3<sup>-</sup>) IV and I (1–1<sup>1/4</sup>) —(1<sup>2/3</sup>–2<sup>-</sup>) II (1–1<sup>+</sup>)—(2<sup>-</sup>–2) III (1–1<sup>+</sup>)—(1<sup>2/3</sup>–2) IV (1<sup>2/3</sup>–2<sup>-</sup>)—(1<sup>-</sup>–1) V.



**FIGURE 3.** (A) Palmar, and (B) plantar views of right hand and foot of the holotype of *Osteocephalus castaneicola* **sp. n.** (CBF 6051). Scale bar equals 5 mm.

General dorsal colouration in alcohol varies from light tan to dark tan with purple-red tint or to reddish-brown. Dorsal pattern varies mostly regarding distinctness and shape of the irregular darker markings. A more or less distinct interorbital streak narrower than the diameter of the eye is present in all individuals. Dorsal markings are fused in a large, irregular, indistinct dorsal spot in the male paratype CBF 6053, whereas dorsal pattern of paratopotypes CBF 6052, NMP6V 73810/1, 73810/3 and paratype 73820 is almost missing. Ventral colouration in alcohol varies from cream white to yellowish-white. A fine dark brown mottling is present on the throat and pectoral area of the female paratype NMP6V 73820. Colour of tibiae seems to vary independently of age or size of individual specimens. The bones are green in the holotype and paratopotypes NMP6V 73810/1–3 (SVL 48.4–59.1 mm) and white in paratopotype CBF 6052 and paratypes CBF 6053, 6054 and NMP6V 73820 (SVL 47.7–63.3 mm).

In life, dorsal colouration varies from tan to brown. A slight purple-red tint observed in most specimens by day turns into ochre by night (Fig. 2D). Newly metamorphosed juveniles are light brown dorsally with a dark interorbital spot, bright orange iris, and creamy white upper arms, knees and heels (Fig. 2E).

**TABLE 3.** Variation of measurements (in mm) of the type series of *Osteocephalus castaneicola* sp. n.). See text for abbreviation.

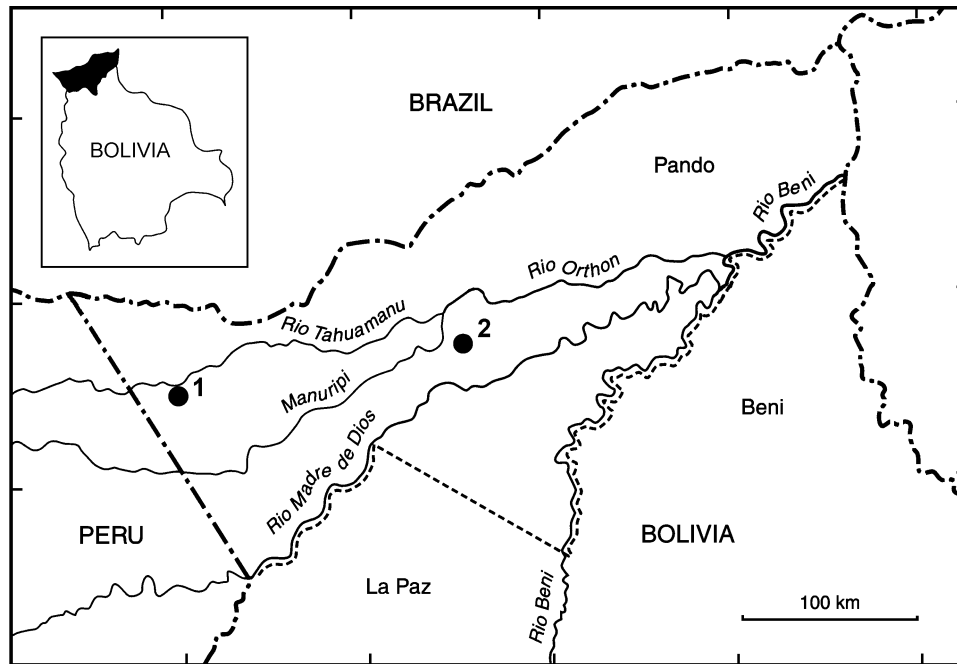
Measurement	Males (N=4) Mean $\pm$ SD; Range	Females (N=4) Mean $\pm$ SD; Range
SVL	49.3 $\pm$ 1.56; 47.8–51.3	57.6 $\pm$ 6.83; 47.7–63.3
HL	16.9 $\pm$ 0.87; 15.7–17.7	19.4 $\pm$ 2.18; 16.6–21.9
HW	16.2 $\pm$ 0.67; 15.4–16.9	18.4 $\pm$ 1.92; 15.6–20.0
EN	5.0 $\pm$ 0.36; 4.5–5.3	6.1 $\pm$ 0.87; 4.9–7.0
ED	5.9 $\pm$ 0.29; 5.1–6.1	6.1 $\pm$ 0.71; 5.1–6.8
TD	3.7 $\pm$ 0.22; 3.5–4.0	4.3 $\pm$ 0.70; 3.5–5.2
ELW	4.8 $\pm$ 0.08; 4.7–4.9	5.5 $\pm$ 0.79; 4.6–6.4
IOD	5.0 $\pm$ 0.30; 4.7–5.4	5.8 $\pm$ 0.83; 4.8–6.6
TL	26.5 $\pm$ 0.70; 25.7–27.3	31.9 $\pm$ 3.28; 27.2–32.9
FL	32.5 $\pm$ 1.35; 30.5–33.4	38.5 $\pm$ 4.46; 31.9–41.3

**Distribution, ecology and threat status.** The known localities of *Osteocephalus castaneicola* lie in western and central part of the Departamento Pando, northern Bolivia (Fig. 4). This area is located in the south-western Amazon basin within the zone of tall evergreen lowland rainforest. *O. castaneicola* was encountered in more or less undisturbed terra firme forest with frequent occurrence of large climax forest trees [e.g. *Bertholletia excelsa* Humb. & Bonpl., *Ceiba pentandra* (L.) Gaertn., *Cedrela odorata* L., *Ficus* sp.]. The forest was characterised by relatively well defined tree strata and a dense canopy at ca. 25–35 m above the ground. The understory was dominated by various tree seedlings, young trees, herbaceous lianas, palms and ferns. The forest floor was covered by leaf litter with scattered large fruit capsules of the Brazil nut tree (*Bertholletia excelsa*) and other species of Lecythiadaeae. All observed individuals of *O. castaneicola* were sitting on vegetation in ca. 0.5–2 m height. No calling males were located. Other hylid species found in sympatry with *O. castaneicola* included *Hypsiboas lanciformis* Cope, *H. punctatus* (Schneider), *Phyllomedusa camba* De la Riva, *P. tomopterna* (Cope), *P. vaillantii* Boulenger, *Trachycephalus coriaceus* (Peters), and *T. resinificatrix* (Goeldi). *O. castaneicola* is apparently known (as *O. sp.*) to occur also in the Region Madre de Dios (exact localities not provided) in adjacent southern Peru (von May *et al.* 2007).

Life history of *O. castaneicola* is closely associated with fruit capsules of the Brazil nut tree, which are opened by agoutis (*Dasyprocta* sp.) or indigenous Brazil nut collectors and abandoned on the forest floor. At both known localities of *O. castaneicola* some of water-filled capsules contained tadpole assemblages numbering up to tens of individuals. Rarely the same tadpoles were found also in water-filled palm bracts lying on the ground. In some cases the assemblages consisted of larvae of markedly different sizes and different stage of development. The largest tadpoles reached a total length of 33–35 mm. Occasionally, white ingested eggs were visible through the transparent venter of the larger larvae. The tadpoles were raised until metamorphosis (Fig. 2E) and their determination was verified by genetic comparison with the adult specimens (Fig. 1).

According to the sparse data available we here classify *O. castaneicola* as “Data Deficient” according to the IUCN red list criteria. In Peru, the species occurs within protected areas (von May *et al.* 2007).

**Etymology.** The specific name is a compound from the Latin *castanea* (Horse Chestnut, *Aesculus*) from which the Spanish *castaña* (vernacular name of the Brazil nut tree) was derived and the Latin *colō* (to inhabit). The name is used as a noun in apposition and refers to the life history of the new species.



**FIGURE 4.** Schematic map of northern Bolivia showing the known distribution of *Osteocephalus castaneicola* sp. n. in Bolivia: (1) San Antonio del Matti, (2) San Antonio de Filadelfia (type locality).

## Discussion

The obtained phylogeny demonstrates existence of several distinct lineages within the genus *Osteocephalus*. Nevertheless, the fact that representatives of two nominal species (*O. leprieurii* and *O. oophagus*) cluster independently within two different clades indicates that more complete sampling and accurate determination of analysed taxa are necessary to obtain a more exact picture of phylogenetic relationships within the given genus. Some of the GenBank sequences represent obviously misidentified species or even a chimerical sequence (see Table 1). Also the high genetic similarity of the GenBank sequences of *O. cabrerai*, *O. verruciger* and *O. "oophagus"* argue more likely for erroneous original determination of the sequenced animals than for close or even nearly identical phylogenetic positions of these morphologically well differentiated species. However, despite of the rather preliminary character of the obtained phylogeny of the genus, it is evident that *O. castaneicola* forms a separate monophylum with *O. deridens*, *O. planiceps* and the GenBank sample from Venezuela determined as *O. leprieurii* (sensu Faivovich *et al.* 2004, 2005) (Fig. 1). Both *O. castaneicola* and *O. deridens* lay eggs in phytotelmata and take care of their oophagous tadpoles. Similarly, *O. planiceps* breeds in various phytotelmata (pers. obs. KHJ). In contrary, *O. leprieurii* forms explosive breeding congregations around temporal free waters (Jungfer & Hödl 2002). Considering these huge differences in reproductive mode, the position of Venezuelan "*leprieurii*" in this clade seems to be questionable. In the past, the name *O. leprieurii* has been widely used for many forms of *Osteocephalus* and it is likely that the Venezuelan sample represents a misidentified taxon (this sample was used later also by Wiens *et al.* 2005, 2006, Fouquet *et al.* 2007 and Moen & Wiens 2009). This possibility is supported by the fact that the GenBank sample of *O. leprieurii* from French Guiana (EF376066; Salducci *et al.* 2005), where the type locality of this species (Cayenne) is located, is embedded in a different clade together with morphologically similar *O. mutabor*, *Osteocephalus* sp. (B) and other species. A similarly doubtful situation can be found in the case of two GenBank samples of *O. "oophagus"* from French Guiana (Salducci *et al.* 2002, Faivovich *et al.* 2005; the latter sample was used also by Wiens *et al.* 2006, and Moen & Wiens 2009), which cluster separately in two different clades. However, the GenBank sample AF467267 was labelled without further

explanation as *O. cabrerai* in Fouquet *et al.* (2007), contrary to the original (Salducci *et al.* 2002, 2005) and GenBank data. The sample really clusters with *O. cabrerai* (AY843705; Faivovich *et al.* 2005), and thus, may represent rather *O. cabrerai* than *O. oophagus*. Anyway, both clades containing *O. "oophagus"* comprise species, which breed in free water bodies and do not take care of their tadpoles, although *O. oophagus* is known by reproducing in phytotelmata and feeding its tadpoles with fertilized eggs (Jungfer & Weygoldt 1999). Considering egg deposition in free water plesiomorphic (Duellman & Trueb 1986), we can suppose the tendency to utilize phytotelmata as breeding place to be a derived state. Therefore, one may expect that *Osteocephalus* species with this derived reproductive mode could cluster together. However, this pattern is not supported by current data considering any of the ambiguous positions of *O. "oophagus"* in our tree, suggesting that this reproductive strategy may have evolved more than once within *Osteocephalus*. Nevertheless, specific determination of the "*oophagus*" samples should be verified to have a clearer picture on the life history evolutionary scenario in this genus.

Breeding in phytotelmata is one of many possible ways to avoid competition and predator pressure in the tropics (e.g. Duellman 1978, Krügel & Richter 1995). In this respect, fruit capsules of the Brazil nut tree may offer an excellent shelter. In addition, water trapped in the capsules dries up much more slowly than free water in small puddles appearing on the forest floor after heavy rains. Therefore, frogs specialized to breeding in abandoned Brazil nut tree capsules may profit both from the protection and from relatively stable water conditions provided by these unusual phytotelmata. Although *O. castaneicola* represents the first evidence of a hyloid frog breeding in fruit capsules of the Brazil nut tree, at least three other frog species are known to use the same breeding place: two dendrobatids, *Adelphobates castaneoticus* (Caldwell & Myers) and *A. quinquevittatus* (Steindachner), and one bufonid, *Rhinella castaneotica* (Caldwell) (Caldwell 1993, Lötters *et al.* 2007). Complex life history responses to predation are described in two of these species by Caldwell (1993). There are no similar available data in the case of *Osteocephalus castaneicola*.

Absence of vocal slits and absence of an obvious vocal sac is the most characteristic morphological feature of the males of *O. castaneicola*. Jungfer & Hödl (2002) suppose that the relatively small subgular vocal sac of bromeliad-breeding species of *Osteocephalus* might have evolved from ancestral (for the genus) lateral or both lateral and subgular vocal sac as an adaptation towards limited space in a narrow leaf axil. Therefore, an interesting question is, if disappearance of vocal slits as well as a distinct vocal sac can be seen as an advanced adaptation for breeding in limited space inside the Brazil nut tree fruit capsule. Observation of sporadic breeding also in fallen water-filled palm bracts indicates some degree of plasticity in the breeding strategy of the new species. It appears that at least in the case of *Rhinella castaneotica* the fruit capsules are not obligatory for the breeding. This species has subgular vocal sac and can reproduce also in small water-filled holes in the soil (see Köhler & Lötters 1999). Therefore, more detailed research should be further done to understand the unusual life history of *O. castaneicola*.

As mentioned in the introduction, the taxonomic status of the Bolivian populations referred to as *Osteocephalus* sp. (A) (Jungfer & Lehr 2001) remains to be solved. It was reported as *O. leprieurii* or *O. cf. leprieurii* from the Departamento Santa Cruz by De la Riva *et al.* (2000). According to the scarce information available, this taxon resembles *O. leprieurii*, but differs from it by its bicoloured iris with dark reticulation, yellow venter and absence of sexual size dimorphism (Jungfer & Hödl 2002). On the other hand, Jungfer & Lehr (2001) mentioned that the bicoloured iris of this species lacks reticulation and also the specimen from Los Fierros figured by De la Riva *et al.* (2000, p. 107) lacks an obvious reticulation in the iris. A formal description of this taxon should be in press since 2000 (see De la Riva *et al.* 2000, Jungfer & Lehr 2001, Jungfer & Hödl 2002). Unfortunately, no tissue samples of *Osteocephalus* sp. (A) were at disposal for our molecular comparison with populations of similar *Osteocephalus* sp. (B) from Pando (Fig. 2F). The latter form shows relatively high variation in iris colouration (bicoloured to uniform with dark horizontal stripe and dark vermiculation). It has creamy to yellowish white venter and it is slightly dimorphic in sexual size (Moravec & Aparicio 2004). Morphologically, *Osteocephalus* sp. (B) cannot be differentiated clearly from *O. leprieurii*, redescribed by Jungfer & Hödl (2002). It appears to be conspecific with the Brazilian population discovered recently ca. 600 km east of the Bolivian border in municipality of Aripuanã (10°09' S, 59°28' W;

state of Mato Grosso) and determined as *O. lepreurii* (Santana *et al.* 2008). According to our molecular results, *Osteocephalus* sp. (B) forms a discrete unit with the sample of *O. lepreurii* from French Guiana. Nevertheless, it is difficult to judge if it is really conspecific with *lepreurii* or not (obtained divergence 1.3–1.8 % uncorrected *p*-distances in 16S rRNA; 1.2 % in the among-all species comparable fragment, Table 2). Therefore, a more thorough study focused on phylogeography and bioacoustics of *O. lepreurii* including populations of *Osteocephalus* sp. (A) and *Osteocephalus* sp. (B) is necessary to solve the systematic status and mutual position of these forms.

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## Appendix. Additional specimens examined

- Osteocephalus* “*ayarzaguenai*”: VENEZUELA: Amazonas: Marakapiwei, MBUCV 6632.
- Osteocephalus buckleyi*: BOLIVIA: Pando: Santa Crucito, NMP6V 73945; Pando: CBF 1262, 2150–51.
- Osteocephalus cabrerai*: PERU: Loreto: 21 km W of Iquitos, NMP6V 71144/1–2.
- Osteocephalus castaneicola* sp. n.: BOLIVIA: Pando: San Antonio de Filadelfia, NMP6d 28/2009.
- Osteocephalus deridens*: PERU: Loreto: Anguilla, NMP6V 71263; 35 km SW of Iquitos, NMP6V 71262/1–5.
- Osteocephalus elkejungingerae*: PERU: Huánuco/Ucayali: El Boquerón del Padre Abad, ZFMK 33352 (holotype), 36319 (paratype).
- Osteocephalus fuscifacies*: ECUADOR: Napo: Aliñahuí (5 km W of Ahuano), ZFMK 68660 (paratype).
- Osteocephalus mutabor*: ECUADOR: Napo: Río Chaloyacu on Carretera Narupa – Coca, ZFMK 66237 (paratype).
- Osteocephalus oophagus*: BRAZIL: Amazonas: Reserva Forestal Adolfo Ducke, ZFMK 57137–38 (paratypes).
- Osteocephalus planiceps*: PERU: Loreto: Anguilla, NMP6V, 71264/1–2; 21 km W of Iquitos, NMP6V 71204/1–2; Puerto Almendras, NMP6V 71174/1–5.
- Osteocephalus taurinus*: BOLIVIA: Pando, Nacebe, NMP6V 72172/1–2; Pando: CBF 1281, 1300–02, 2147–48, 43333; PERU: Loreto: Puerto Almendras, NMP6V 71184.
- Osteocephalus* sp. (B): BOLIVIA: Pando: Canadá, NMP6V 73105; Nacebe, CBF 5589–93, NMP6V 72173/1–4; Palmira, NMP6d 41/2009.