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SHORT COMMUNICATION

New record of the rare Atlantic Forest rodent *Phyllomys lundi* (Mammalia: Rodentia)

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ABSTRACT. The arboreal echimyid rodent of the genus *Phyllomys* Lund, 1839 is found in the eastern Brazilian Atlantic forest, from the state of Ceará to the state of Rio Grande do Sul, reaching the São Francisco and Paraná river basins in the west. There are 13 species in the genus. *Phyllomys lundi* Leite, 2003, which until now was known from only two localities, is one of the four endemic Atlantic Forest species of *Phyllomys* with very restricted distribution. We provide additional data on the morphology, distribution and phylogeography (based on cytochrome b sequences) of the rare *P. lundi*. Our new record broadens the northern limit of the distribution *P. lundi* by approximately 250 km with respect to previous records.

KEY WORDS. Echimyidae, geographic distribution, Lund's Atlantic tree rat.

The Brazilian Atlantic rainforest is considered one of the most diverse and threatened biomes on the planet (Galindo-Leal & Camara 2005). Its level of endemism is up to 90% for some groups of organisms, and its overall 50% average endemism is surpassed only by the Amazon forest (Costa et al. 2003). Among the endemic Atlantic Forest mammals, there are several species of Echimyidae, the most diverse hystricognath rodent family in South America (Patton et al. 2015). The Atlantic Forest echimyids are grouped into five genera. Three are monoespecific, [*Kannabateomys amblyonyx* (Wagner, 1845), *Callistomys pictus* (Pictet, 1843) and *Euryzygomatomys spinosus* (G. Fisher, 1814)], while the other two are speciose (*Phyllomys* Lund, 1839 and *Trinomys* Thomas, 1921) (Loss & Leite 2011).

Phyllomys belongs to an arboreal radiation of echimyid rodents distributed in forested areas in eastern Brazil, from the states of Paraíba and Ceará (ca. 7°S) in the Northeast, to Rio Grande do Sul (ca. 30°S) in the South, reaching the São Francisco and Paraná river basins to the west at approximately 54°W (Leite 2003). Throughout this large distribution, *Phyllomys* dwells predominantly in the broadleaf evergreen forests of the coastal Atlantic Forest and associated habitats such as Araucaria forests and mangroves. Additionally, it inhabits the semi deciduous forests of the Caatinga and Cerrado (Leite 2003, Campos & Percequillo 2007). There are 13 described species of *Phyllomys*, four of which, based on records of very few specimens, seem to have restricted distribution: *Phyllomys kerri* (Moojen, 1950), with three collected specimens in 1941 at Ubatuba, state of São Paulo, *Phyllomys lundi* Leite, 2003 with two collected specimens, and *Phyllomys mantiqueirensis* Leite, 2003 and *Phyllomys unicolor* (Wagner, 1842), both known only from their respective holotypes (Loss & Leite 2011). The limited numbers of collected specimens result from the fact that collecting these arboreal and elusive species is difficult. Herein we report on the capture of the third known specimen of *P. lundi*, and present the results of a phylogenetic analysis of *Phyllomys*.

One adult female specimen (field number MBF 340) of *P. lundi* was captured in the municipality of Alto Jequitibá, at the protected area "Reserva Particular do Patrimônio Natural (RPPN) Refúgio dos Sauás", in eastern Minas Gerais, southeastern Brazil, near the border with Espírito Santo and Rio de Janeiro, latitude -20.4868 and longitude -42.0399, altitude around 400 m (Fig. 1). The animal was collected using a Sherman live trap placed 1.5 meters high in the forest understory. It was deposited in the Museu de Zoologia Newton Baião de Azevedo (MZNB), Universidade do Estado de Minas Gerais (EUMG) in Carangola, Minas Gerais, Brazil, catalog number 271. This specimen was

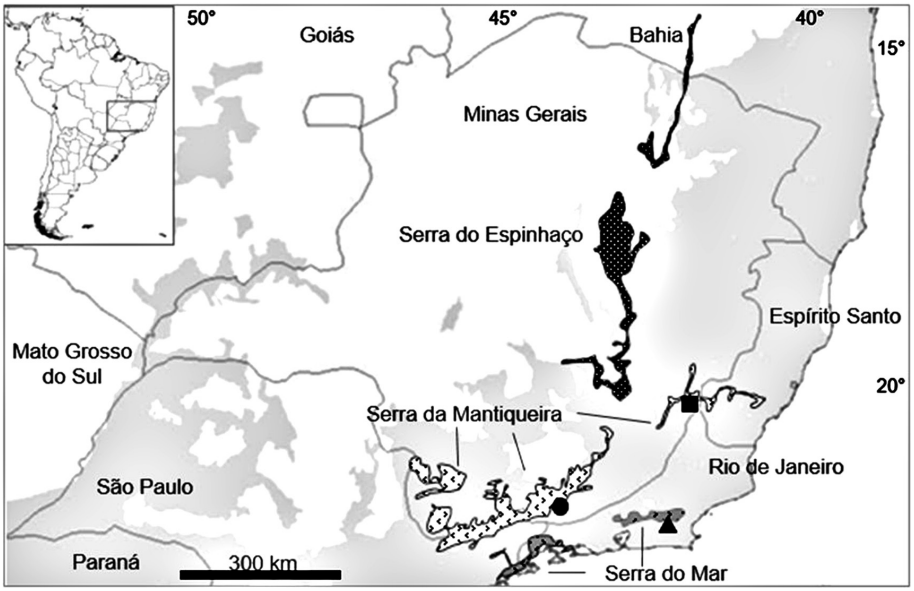

Figure 1. Collecting localities of *P. lundi* including the type locality (circle) and new locality of occurrence (square). Gray area corresponds to Atlantic Forest.

collected under permit number 41959-1, issued by Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio), and handled according to recommended safety procedures. A total of 23 measurements were taken with a digital caliper following previous work (Leite 2003, Table 1).

To confirm the morphological identification, a phylogenetic analysis was carried out using DNA cytochrome b sequences of the captured specimen and GenBank sequences (GenBank number KU756488) of *P. lundi* (EF608183), *Phyllomys nigrispinus* (Wagner, 1842) (EF608184), *Phyllomys dasythrix* Hensel, 1872 (EF608185), *Phyllomys blainvilii* (Jourdan, 1837) (JF297836), *Phyllomys brasiliensis* Lund, 1840 (EF608182), *Phyllomys lamarum* (Thomas, 1916) (EF608181), *Phyllomys pattoni* Emmons, Leite, Kock and Costa, 2002 (JF297839), *P. mantiqueirensis* (EF608179), *P. unicolor* (EF608188). Sequences of *Echimys chrysurus* (Zimmermann, 1780) (L23341) and *Makalata didelphoides* (Desmarest, 1817) (GI996078) were used as outgroups.

DNA was isolated from liver tissue preserved in 100% ethanol with the standard phenol-chloroform protocol (Sambrook & Russel 2001). The complete (1,143 bp) mitochondrial Cytochrome b gene (mt-Cytb) was PCR-amplified using the primers L14724 (Irwin et al. 1991) and mt-Cytb Rev (Casado et al. 2010), following a pre-denaturation step at 94°C for 2 min and 35 cycles 94°C for 1 min, annealing at 52°C for 1 min, 72°C for 1:30 min, and final extension of 72°C for 5 min. Amplicons were purified using the GFX PCR DNA and Gel Band Purification Kit (GE Healthcare) and labeled with the PCR primers and the internal primers SOT in1 and SOT in2 (Cassens et al. 2000) and mt-CytbAOT (Menezes et al. 2010). Electropherograms were manually checked in Chromas version 1.45 (Maccarthy 1998), Chromas PRO version 1.41 (Technelysium Pty Ltd.) and MEGA version 6.0 (Tamura et al. 2007). The sequences were manually aligned in MEGA version 6 (Tamura et al. 2007). Genetic distances were

Table 1. Cranial measurements of *Phyllomys lundi*. Measurements of holotype (MN62392) are from Leite (2003).

Measurements	MZNB271	Holotype
Greatest skull length	45.75	47.7
Rostral breadth	6.58	6.7
Rostral length	15.89	16.6
Nasal length	12.69	13.4
Orbital length	12.74	13.2
Interorbital constriction	10.69	11.1
Mastoid breadth	18.84	17.9
Zygomatic breadth	22.38	22.5
Condyloincisive length	41.31	41.9
Basilar length	36.51	36.0
Diastema length	11.46	10.1
Maxillary toothrow length	11.28	11.0
Incisive foramina length	4.80	4.7
Palatal length b	8.95	8.6
Palatal length a	19.85	17.8
Mesopterygoid fossa width	2.96	3.1
Max B	8.82	7.4
OccW	8.83	8.7
Bullar length	9.29	8.9
Postpalatal length	21.32	19.7
Rostral depth	9.03	10.4
Cranial depth at M1	14.12	13.6
Cranial depth	17.04	17.8

estimated with MEGA using the Kimura 2-parameter. The DNA substitution models were selected using MODELGENERATOR version 0.85 (Keane et al. 2006). The phylogenetic reconstructions by maximum likelihood (ML) and Bayesian inference (BI) based on the mt-Cytb were constructed using the HKY model (Hasegawa et al. 1985), with a proportion of invariable sites and gamma distributed rates. ML was carried out with PhyML version 3.0 (Guindon & Gascuel 2003), and branch support was calculated using the approximate likelihood ratio test (SH-aLRT) with Shimodaira-Hasegawa-like interpretation (Anisimova & Gascuel 2006). BI was carried out using MrBayes, version 3.1.2 (Ronquist & Huelsenbeck 2003). For BI, two chains were run for 1.000.000 generations, and one tree per 100 generations was sampled. Convergence and mixing were evaluated using TRACER version 1.5 (Rambaut & Drummond 2007), with initial 10% runs discarded (burn-in). A majority rule consensus phylogram was subsequently constructed.

The specimen collected by us presented the general morphological characters, as described for the species (Fig. 2), and the following external measurements in mm: head-body 398, tail 208, feet with 38 and without claws 35, ear 12; and weight 174,6 g. Dorsal pelage coloration is predominantly orange intermixed with black and the neck, thighs markedly orange, and spines conspicuous from neck to tail. Ventral hairs cream with white base, providing an overall washed aspect. Tail brown and hairy from base to tip; forefeet covered with brown yellow

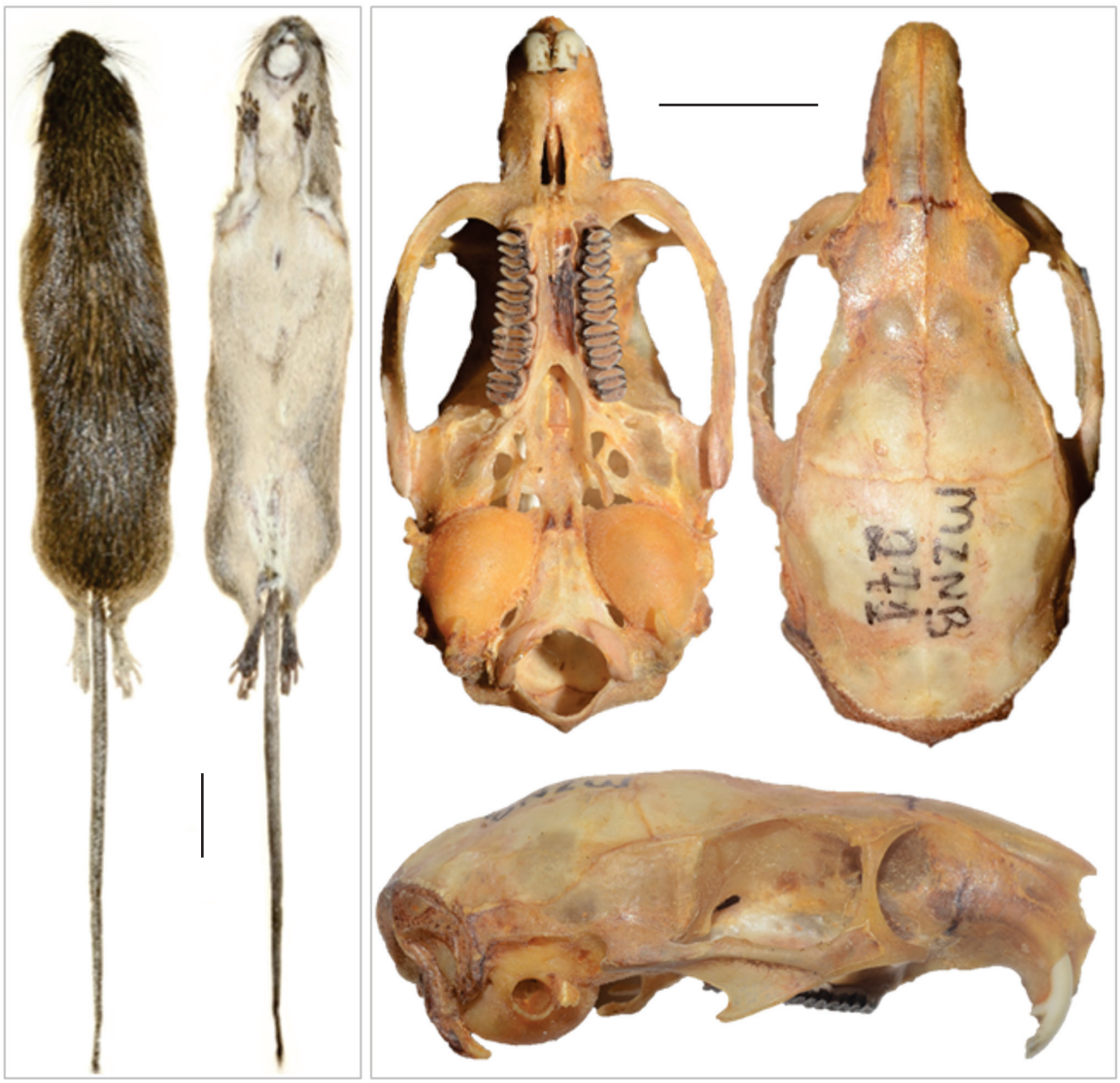

Figure 2. Dorsal, ventral and lateral views of skull and skin of *P. lundi* female MZNB 271. Scale bars: 1 cm.

hairs, except for gray-white fingers. Dorsal surfaces of hind feet covered with golden-creamy hairs and toes with silver hair. Skull delicate, with relatively narrow and long rostrum, and wide and convex interorbital region. Mandible with short coronoid process and a shallow sigmoid notch. There was some variation in characters of the skull with respect to previous description of the species (Leite 2003), such as rostrum narrow and elongated, with distal and proximal regions of similar width, and anterior part of the zygomatic plate surpassing the suture between nasal and frontal (Fig. 2). The skull measurements of the collected specimen are in Table 1.

The topologies obtained with maximum likelihood (ML) and Bayesian inference (BI) were similar (Fig. 3) and confirmed the morphological identification of the specimen collected by us, clustering it together with the other of *P. lundi*, with 0.4% of genetic distance between them (Table 2). The position of the *P. lundi* clade as sister to the clade [*P. blainvilli* (*P. brasiliensis*, *P. lamarum)*] is moderately supported (76% Bootstrap and 0.7 posterior probably), corroborating previous work (Loss & Leite 2011), and contrasting with the parsimony analysis that grouped *P. lundi* with *P. nigrispinus* (Leite 2003), but in agreement with the ML analysis of this same paper.

The Atlantic Forest is an area with complex topography over short geographical distances, and is generally characterized by strong seasonality, sharp environmental gradients, and the presence of the high mountain ranges of Serra do Mar and Serra da Mantiqueira (IBGE 1993). Serra da Mantiqueira and the complex topography of its Atlantic Forest apparently limit the distribution of *P. lundi* to the north and west, and the Atlantic Ocean limits it, to the south and east. This species occurs from the lowlands at Poço das Antas, state of Rio de Janeiro, to 680 m at the type locality (Fazenda do Boné, Passa Vinte, state of Minas Gerais, Brazil, Table 3). This is in accordance with the Montane Isolate Hypothesis, which postulates that the isolation

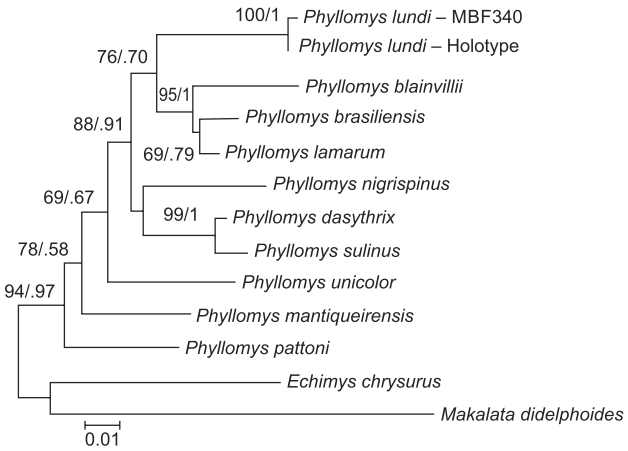

Figure 3. Maximum likelihood topology based on cytochrome b sequences. Numbers near branches are bootstrap values ≥ 70 for ML (above) and posterior probabilities for BI (below).

of montane rainforest remnants during climatic dry periods led to divergence and speciation, with new species subsequently expanding their range to the lowlands (Moreau 1966). The role of Serra da Mantiqueira mountain range in determining population structure, and even speciation in vertebrates is well documented for reptiles (Grazziotin et al. 2006), birds (Cabanne et al. 2007, 2008, d'Horta et al. 2011), amphibians (Fitzpatrick et al. 2009), and invertebrates (Batalha-Filho et al. 2010).

Phyllomys lundi was listed as endangered because it occurs at an area that is less than 5,000 km², all known individuals were collected in fewer than five locations, and there has been a continuing decline in the extent and quality of its habitat (Leite & Patterson 2008). It is now known from three localities (Table 3) 250 to 340 km far from one another in fragmented

Table 2. Genetic distance estimates (K2p), in percentage, between *Phyllomys* species with mt-Cytb data.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1 *P. lundi* MBF340																	
2 *P. lundi* EF608183	0.4																
3 *P. pattoni* JF297839	12.7	12.9															
4 *P. pattoni* JF297831	13.1	13.3	0.2														
5 *P. nigrispinus* EF608184	10.1	10.2	12.9	12.9													
6 *P. blainvillii* JF297836	12.5	13.0	11.5	11.9	12.3												
7 *P. blainvillii* EF608180	12.5	13.0	11.5	11.9	12.3	0.0											
8 *P. dasythrix* EF608185	11.5	12.0	11.4	11.4	10.0	10.3	10.3										
9 *P. dasythrix* JF297832	11.5	12.0	11.4	11.4	10.0	10.3	10.3	0.0									
10 *P.mantiqueirensis* EF608179	10.6	11.1	8.9	9.2	11.9	8.4	8.4	10.9	10.9								
11 *P.mantiqueirensis* EU313246	10.6	11.1	8.9	9.2	11.9	8.4	8.4	10.9	10.9	0.0							
12 *P. lamarum* EF608181	9.6	10.1	11.9	12.2	9.4	6.4	6.4	9.3	9.3	9.6	9.6						
13 *P. lamarum* JF297816	10.4	10.9	12.1	12.4	10.1	7.2	7.2	9.6	9.6	10.1	10.1	0.9					
14 *P. brasiliensis* EF608182	9.4	9.9	10.9	10.9	9.8	7.8	7.8	9.5	9.5	9.6	9.6	2.6	3.4				
15 *P. brasiliensis* EU313241	9.4	9.9	10.9	10.9	9.8	7.8	7.8	9.5	9.5	9.6	9.6	2.6	3.4	0.0			
16 *P. sulinus* EF608184	12.5	13.0	12.6	12.6	10.5	11.1	11.1	2.4	2.4	11.3	11.3	9.9	9.9	10.5	10.5		
17 *P. sulinus* JF297834	12.6	13.2	12.7	12.7	10.6	11.3	11.3	2.5	2.5	11.4	11.4	10.1	10.1	10.6	10.6	0.1	
18 *P. unicolor* EF608188	12.6	13.1	12.1	12.1	11.2	11.4	11.4	9.7	9.7	11.8	11.8	9.4	9.2	9.9	9.9	10.1	10.2

forests. This finding increases the northern limit of *P. lundi* by approximately 250 km (Fig. 1), and also expands its occurrence to an area of 23,757 km², which is more than the threshold used to categorize threatened species (20,000 km²) using IUCN's B criterion (geographic range). It does not, however, change the conservation status of the species, which is still known from less than five localities, all of which continue to shrink and decline in habitat quality.

Table 3. *Phyllomys lundi* specimens in mammal collections, with catalog number, and collecting localities. MN = Museu Nacional, Universidade Federal do Rio de Janeiro, UFMG = Departamento de Zoologia, Universidade Federal de Minas Gerais, MZNB = Museu de Zoologia Newton Baião de Azevedo. The Brazilian states are Minas Gerais (MG) and Rio de Janeiro (RJ).

Number	Locality
MNRJ62392	MG, Passa Vinte, Fazenda do Bené (-22.2333, -44.2000)
RBPDA2228	RJ, Reserva Biológica de Poço das Antas (-22.5175, -42.2835)
MZNB271	MG, Alto Jequitibá (-20.4868, -42.0399)

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