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How integrative taxonomy can save a species from extinction: The supposedly extinct mouse opossum *Cryptonanus ignitus* (Diaz, Flores and Barquez, 2000) is a synonym of the living *C. chacoensis* (Tate, 1931)

Pablo Teta[a,*], Juan F. Díaz-Nieto[b]

[a] *División Mastozoología, Museo Argentino de Ciencias Naturales "Bernardino Rivadavia", Avenida Ángel Gallardo 470, C1405DJR, Buenos Aires, Argentina*
[b] *Departamento de Ciencias Biológicas and Grupo de Investigación BEC, Universidad EAFIT, Cr 49 No.7sur-50, Bloque 38, 050022, Medellín, Colombia*

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ABSTRACT

We reviewed the taxonomic status of the "extinct" mouse opossum *Cryptonanus ignitus* (Diaz, Flores and Barquez, 2000). This species is only known from the type, an adult male collected in the province of Jujuy, northwestern Argentina, in 1962. Based on both qualitative and quantitative external and cranial traits and the analysis of DNA sequences extracted from the 57-year-old type skin, we confirm the suggestion of previous researchers that *C. ignitus* is an elderly example of the living species *C. chacoensis* (Tate, 1931). Most of the supposedly diagnostic morphological traits of *C. ignitus* (e.g., zygomatic arches unusually wide and robust, postorbital process of the jugal massively developed, prominent temporal scars) are age-correlated in didelphids and were observed in other adult specimens of *C. chacoensis*. The clay coloration of the ventral pelage of *C. ignitus*, which allegedly sets apart this species from other congeners, is here reported for new examples of *C. chacoensis* from other localities. Our results suggest the need to carefully evaluate the taxonomic status of other species supposed to have become extinct in recent times in mainland South America.

Introduction

The genus *Cryptonanus* Voss, Lunde, and Jansa, 2005 includes five species of small mouse opossums (15–40 g) with dark facial masks and long prehensile tails (Voss et al., 2005). This genus is widely distributed across a variety of tropical, subtropical, and temperate ecoregions between 7° and 35 °S latitude, including open and forested regions (Voss et al., 2005). Most species of *Cryptonanus* are poorly known, lacking basic information about their taxonomy, ecogeographic distribution, and natural history (Voss et al., 2005). The case of the red-bellied mouse opossum *C. ignitus* (Diaz, Flores and Barquez, 2000) is a good example, because this species is only known from the type specimen, an adult male collected in the province of Jujuy, northwestern Argentina, more than fifty years ago.

The red-bellied mouse opossum was listed as Extinct by the International Union for the Conservation of the Nature (Flores, 2016), because it has not been recorded since 1962, and because the entire area where the type specimen was collected has been converted to agriculture and industry; extensive searches over the last 44 years have failed to find this species again. After a detailed inspection of the type specimen, Voss et al. (2005) suggested that it might be just an elderly example of *C. chacoensis* (Tate, 1931), indicating that most of the qualitative and quantitative external and cranial traits supposedly diagnostic of *C. ignitus* are age-related. However, these authors preferred to maintain *C. ignitus* as a valid taxon on the basis of some unusual features of the holotype, such as its clay-colored ventral pelage, which was quite unlike the ventral coloration of other specimens of *C. chacoensis* they examined (Voss et al., 2005).

The taxonomic status of *Cryptonanus ignitus* has several implications for mammalian conservation biology. In fact, if it were a valid species, it would be one of the nine mammal species to have become extinct over the past 500 years in mainland South America (cf. Teta et al., 2014; Prevosti et al., 2015). In this contribution we analyze molecular and morphological evidence bearing on the taxonomic situation of *C. ignitus* and confirm its status as a junior synonym of *C. chacoensis*.

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[*] Corresponding author.
E-mail address: pablot@macn.gov.ar (P. Teta).

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Table 1
List of CYTB sequences of *Cryptonanus* included in this report.

Species	Genbank #	Locality	Voucher	Tissue	bp	Source
agricolai	HQ622148	Brazil, Piaui (8)	UUPI 167	SSL-2010	784	Loss et al. (2011)
agricolai	KT334295	Brazil, Rio de Janeiro (9)	MZUSP 35409		1149	Delciellos et al. (2016)
chacoensis	MK604524	Argentina, Entre Ríos (1)	CNP 1898	LTU533	1149	This study
chacoensis	MK604525	Argentina, Formosa (2)	CNP 1899	LTU566	1149	This study
chacoensis	KF313984	Brazil, Goias (7)	LBCE 7486		1149	Faria et al. (2013)
chacoensis	MK604526	Paraguay, Alto Paraguay (13)	TTU 118130	TK61072	1149	This study
chacoensis	MK604527	Paraguay, Alto Paraguay (13)	TTU 118132	TK61103	1149	This study
chacoensis	KM188474	Paraguay, Alto Paraná (14)	FMNH	TK129269	801	de la Sancha and D'Elía (2015)
chacoensis	KM188473	Paraguay, Alto Paraná (14)	FMNH	TK129281	801	de la Sancha and D'Elía (2015)
chacoensis	KM188476	Paraguay, Alto Paraná (14)	FMNH	TK129546	801	de la Sancha and D'Elía (2015)
chacoensis	KM188472	Paraguay, Alto Paraná (14)	TTU 116569	TK145238	801	de la Sancha and D'Elía (2015)
chacoensis	KM188484	Paraguay, Boquerón (15)	TK	TK130933	534	de la Sancha and D'Elía (2015)
chacoensis	KM188482	Paraguay, Boquerón (15)	TK	TK30969	767	de la Sancha and D'Elía (2015)
chacoensis	MK604528	Paraguay, Canindeyú (17)	TTU 104512	TK67402	1149	This study
chacoensis	KM188480	Paraguay, Canindeyú (17)	FMNH	TK129488	801	de la Sancha and D'Elía (2015)
chacoensis	KM188479	Paraguay, Canindeyú (18)	FMNH	TK129422	801	de la Sancha and D'Elía (2015)
chacoensis	KM188475	Paraguay, Itapúa (19)	FMNH	TK129395	801	de la Sancha and D'Elía (2015)
chacoensis	KM188470	Paraguay, Itapúa (19)	TK	TK129396	801	de la Sancha and D'Elía (2015)
chacoensis	KM188471	Paraguay, Itapúa (19)	FMNH	TK129415	801	de la Sancha and D'Elía (2015)
chacoensis	MK604529	Paraguay, Presidente Hayes (20)	FMNH 164089	CJY1211	1149	This study
chacoensis	KM188481	Paraguay, Presidente Hayes (21)	TK	TK122110	767	de la Sancha and D'Elía (2015)
chacoensis	KU171187	Paraguay, Caazapá (16)	MNHNP	GD521	1149	Díaz-Nieto et al. (2016)
guahybae	KM403640	Brazil, Rio Grande do Sul (10)	MCNU 1725		803	Dias et al. (2015)
guahybae	KM403639	Brazil, Rio Grande do Sul (11)	MCNU 2808		803	Dias et al. (2015)
guahybae	KM403638	Brazil, Santa Catarina (12)	UFSC 4854		803	Dias et al. (2015)
ignitus	MK604530	Argentina, Jujuy (3)	AMNH 167852		603	This study
unduaviensis	KT437822	Bolivia, Pando (4)	AMNH 262401	NK14234	1149	Díaz-Nieto et al. (2016)
unduaviensis	KM188469	Paraguay, Alto Paraguay (13)	TTU 118131	TK61074	791	de la Sancha and D'Elía (2015)
unduaviensis	KU171189	Bolivia, Santa Cruz (5)	AMNH 260032	NK12313	724	Díaz-Nieto et al. (2016)
unduaviensis	MK604531	Bolivia, Santa Cruz (6)	AMNH 260031	NK11825	708	This study

Material and methods

Taxon and gene sampling for molecular analyses

Because our main objective was to assess the taxonomic status of *Cryptonanus ignitus*, our designated ingroup included this taxon and all of the other currently recognized species of the genus: *C. agricolai, C. chacoensis, C. guahybae,* and *C. unduaviensis* (following Voss and Jansa, 2009). For such purposes, we obtained a tissue sample from the holotype of *Cryptonanus ignitus* (AMNH 167852), which was kindly harvested by Robert S. Voss at the AMNH following the methods described in Díaz-Nieto et al. (2016). We also obtained preserved tissue, kindly made available to us by Sharon A. Jansa at the University of Minnesota, for 6 different specimens from Argentinian and Paraguayan localities of *C. chacoensis* and 1 sample of *C. unduaviensis* from Bolivia. We completed our taxon sampling by downloading 22 sequences of the mitochondrial gene Cytochrome *b* (CYTB) from Genbank corresponding to four different ingroup species. However, Genbank sequence KR190438, obtained from fragmentary osteological material and which was not identified beyond the genus level (Rocha et al., 2015), was not included in our data matrix. We obtained full-length CYTB sequences for three samples (CJY1211, TK61072, TK61073) with shorter sequences uploaded to Genbank (corresponding to accession numbers KM188483, KM188477, KM188478). After inspecting the Genbank data, we also recognized two pairs of sequences corresponding to duplicates, of which we discarded the shorter and kept the longer sequence from each (denoted with bolded letters): GQ911596 and KU171187 (both obtained from sample GD521), and HM583366 and KT437822 (sequenced from sample NK14234). Finally, we used three species of the thylamyine genera *Gracilinanus, Lestodelphys, and Thylamy* (represented by Genbank accessions KU171191, HM583364, HM583378) as outgroups. All ingroup sequences included in our data matrix are found in Table 1 and the localities from which the corresponding specimens were collected are mapped in Fig. 1.

Laboratory methods

Ancient DNA extraction followed the methods described in Giarla et al. (2010), and procedures for DNA extraction from fresh tissue can be found in Voss and Jansa (2009). All the new sequences were obtained based on PCR amplifications. Although we did our best to obtain the complete CYTB sequence from all of our samples, it was not always possible to do so with the low molecular weight "ancient" DNA obtained from the holotype of *Cryptonanus ignitus*. Therefore, we designed a new reverse primer (Cytb-F609-Cryptonanus: 5′-GTTGGGTTGCTGGAGCCT-3′) that, paired with primer CYTB-F1-Didelphidae (from Giarla et al., 2010), was useful for obtaining approximately the first half of the CYTB gene. PCR amplification using ancient DNA as a template (from *C. ignitus* holotype) was performed in a 25 µl reaction using Platinum Taq DNA polymerase (Invitrogen) to enhance specificity and recommended concentrations of primers, dNTPs, buffer, and $MgCl_2$. Thermal cycling conditions involved an initial denaturation at 94 °C for 2 min followed by a single stage of 35 cycles of denaturation at 94 °C for 30 s, annealing at 59 °C for 30 s, extension at 72 °C for 1 min, and a final 7-min extension at 72 °C. For PCR amplification of CYTB from fresh tissue samples, we used the primers CYTB-F1-Didelphidae and CYTB-R1-Didelphidae (from Voss et al., 2013) and thermal cycling conditions described in Giarla et al. (2010). All the obtained amplicons were sequenced using amplification primers and dye-terminator chemistry on an ABI-3730xl automated sequencer. Sequences were edited, assembled, and examined with reference to translated amino-acid sequences using GENEIOUS PRO 7.0 (http://www.geneious.com, Kearse et al., 2012).

Phylogenetic analyses

A total of 30 ingroup sequences and 3 outgroup sequences were aligned with MUSCLE (Edgar, 2004) to produce a CYTB matrix that was partitioned by codon position. We used the Bayesian Informa-

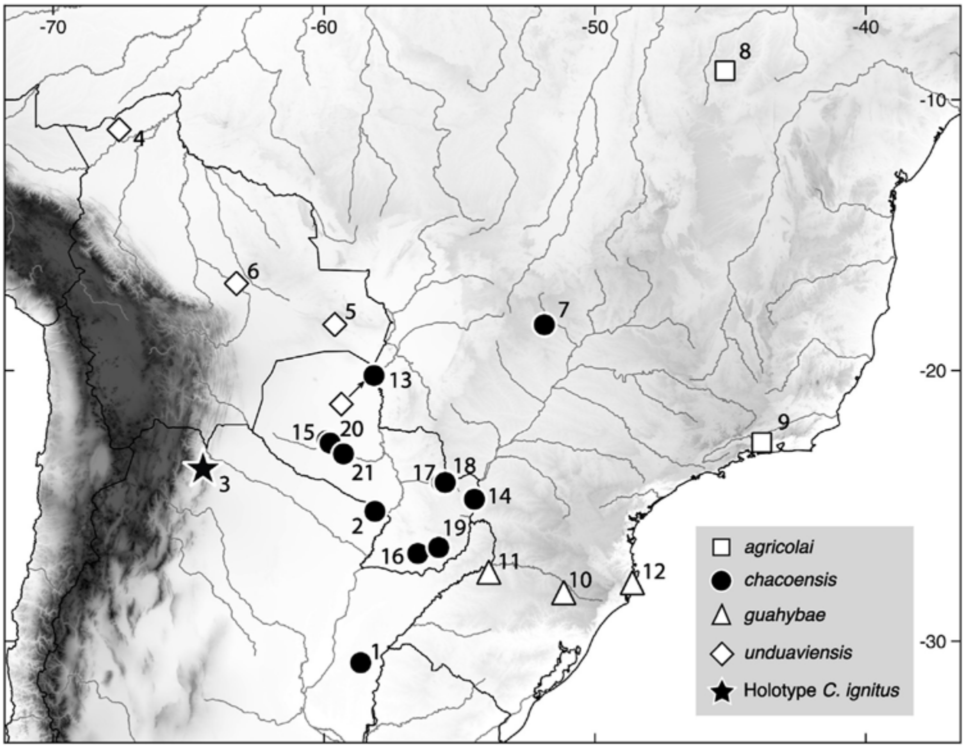

Fig. 1. Collection localities for sequenced specimens of *Cryptonanus*.

tion Criterion in PartitionFinder2 (Lanfear et al., 2016) to find the partition scheme and substitution model that best fitted our data implementing the greedy search algorithm (Lanfear et al., 2012) and PhyML as phylogenetic reconstruction method (Guindon et al., 2010). Subsequently, we performed maximum-likelihood analyses in GARLI 2.0 (Zwickl, 2006) using five independent searches and including the best partition scheme and substitution models obtained from PartitionFinder2. Lastly, we evaluated nodal support based on a bootstrap analysis of 1000 pseudoreplicated datasets. Bootstrap Support (BS) values were summarized on the best ML tree using Sumtrees version 3.3.1 (Sukumaran and Holder, 2010). All the previously described phylogenetic analyses were implemented in the CIPRES Science Gateway (Miller et al., 2010). Uncorrected intra- and inter-specific distances (*p*-distances) were estimated with MEGA7 (Kumar et al., 2016).

Specimens examined for morphological comparisons

The specimens we examined for morphological analysis are preserved in the following collections (listed in order of their standard institutional abbreviations): AMNH, American Museum of Natural History (New York, United States); FMNH, Field Museum of Natural History (Chicago, USA); MACN-Ma, Museo Argentino de Ciencias Naturales "Bernardino Rivadavia" (Buenos Aires, Argentina).

Morphological analyses

Anatomical terminology follows Voss et al. (2005) and Voss and Jansa (2009). Craniodental measurements were taken with digital calipers and recorded to the nearest 0.01 mm. The following dimensions were measured according to the definitions provided by Díaz et al. (2002) and Voss et al. (2005): condylo-incisive length (CIL), measured from the occipital condyles to the anterior surface of the first upper incisors; occipito-nasal length (ONL), measured from the posteriormost projection of occipital condyles to the anteriormost point of nasals; breadth of braincase (BB), measured at the widest point of the cranium just posterior to the zygomatic arches; least interorbital breadth (LIB), measured at the narrowest point across the frontals between the orbits; zygomatic breadth (ZB), measured at the widest point across both zygomatic arches; mastoidal breadth (MB), measured as width across the mastoids; length of rostrum (LR), measured from the anterior border of the orbit to the anterior border of the ipsolateral nasal; length of the nasal (LN), measured from the posteriormost point to the anteriormost point of nasals; length of maxillary toothrow (LMxT), measured from the anterior margin of the alveolus of the upper canine to posterior margin of alveolus of the ipsilateral fourth molar (M4); palatal length (PL), measured from the anteriormost point of the premaxillae to the postpalatine torus, including the postpalatine spine (if present); width across canines (CC), measured across the outer alveolar margins of the upper canines; width across molars; (MM), measured across the labial margins of the right and left M4 crowns, at or near the stylar A position.

Principal component analyses (PCA) were used to identify the main axes of cranial morphometric variation among specimens referable to *Cryptonanus chacoensis* (including the holotype of *C. ignitus*, as recovered in our molecular results). Principal components (PCs) were extracted from the variance-covariance matrix, after the $\log_{10}$-transformation of the original data (Strauss, 2010). Statistical analyses were conducted using the software Infostat (Di Rienzo et al., 2008).

Results

Cytochrome-b sequence data

Our final CYTB dataset includes 30 ingroup sequences and 3 outgroup sequences ranging in length from 534 to 1149 bp, resulting in 89.11% nucleotide coverage overall (Table 1). The best-fitting partition scheme recovered by PartitionFinder2 corresponded to 3 partitions, with the nucleotide substitution models for each par-

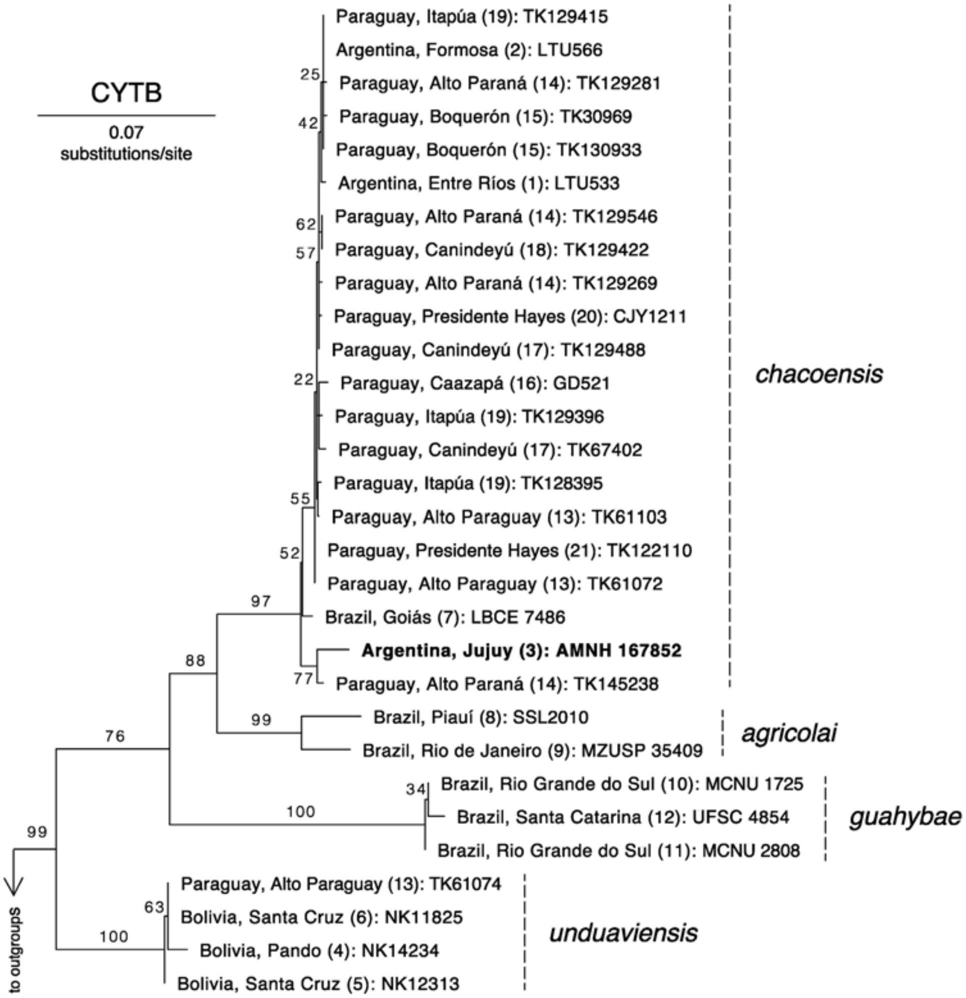

Fig. 2. Maximum-likelihood topology of CYTB sequences of *Cryptonanus* species. Numbers on branches correspond to bootstrap support values. Each terminal is identified by country of origin and an alphanumeric specimen identifier (see Table 1). Bolded terminal corresponds to the holotype of *C. ignitus*.

tition: first position, SYM+Γ; second position, HKY; third position, HKY+ Γ.

This is the first phylogenetic reconstruction that includes all the currently recognized species of *Cryptonanus,* and our analysis resulted in a topology that provides strong support for the monophyly of this genus and for most of the relationships among its species (Fig. 2). More importantly, our results show that the haplotype we obtained from the holotype of *C. ignitus* is part of a strongly supported clade of sequences corresponding to the species *C. chacoensis*. There is no clear phylogeographic structure within the species *C. chacoensis*, with most of the haplotype relationships only weakly supported and very low intraspecific divergence observed (Fig. 3). Nonetheless, our sequence of *C. ignitus* is sister to a haplotype from eastern Paraguay, although such relationship is only marginally supported. The remaining species (*C. agricolai, C. guahybae,* and *C. unduaviensis*) are each recovered as strongly supported haplogroups. Sequences of *C. unduaviensis* are sister to a marginally supported clade including *C. agricolai, C. chacoensis,* and *C. guahybae*. Within the latter clade, haplogroups corresponding to *C. agricolai* and *C. chacoensis* are reciprocally monophyletic with strong support.

Qualitative morphology

Ventral coloration in examples of *Cryptonanus chacoensis* varied between self-white or self-cream to clay colored (Fig. 4A–D). A small patch of pure white on an otherwise clay-colored venter was observed in one subadult female from Corrientes, northwestern Argentina (Fig. 4B). Skull traits supposedly diagnostic of *C. ignitus* (e.g., zygomatic arches unusually wide and robust, postorbital process of the jugal massively developed, prominent temporal scars; Fig. 5) were also observed in other old adult specimens of *C. chacoensis* (e.g., MACN-Ma 22445, MACN-Ma 22461, FMNH 226362).

Quantitative morphology

The first principal component extracted from the covariance matrix of $\log_{10}$-transformed measurements of specimens of *Cryptonanus chacoensis* (including *C. "ignitus"* as per our molecular results) explained almost all of the variance (83.0%) in these data, and uniformly positive coefficients for this vector suggests that this is a general size factor (Table 2). The hypothesis of multivariate isometry for these data (Jolicoeur, 1963) would correspond to a vector with coefficients all equal to 0.29, so coefficients much larger than this value (e.g., for CC and ZB) and much smaller than this value (e.g., for LIB and BB) indicate positive and negative allometry, respectively. Consistent with its advanced age, the holotype of *ignitus* scores high on PC1 (Fig. 6), and its relatively narrow braincase and wide zygomatic arches conform to the allometries described by this factor (Table 3).

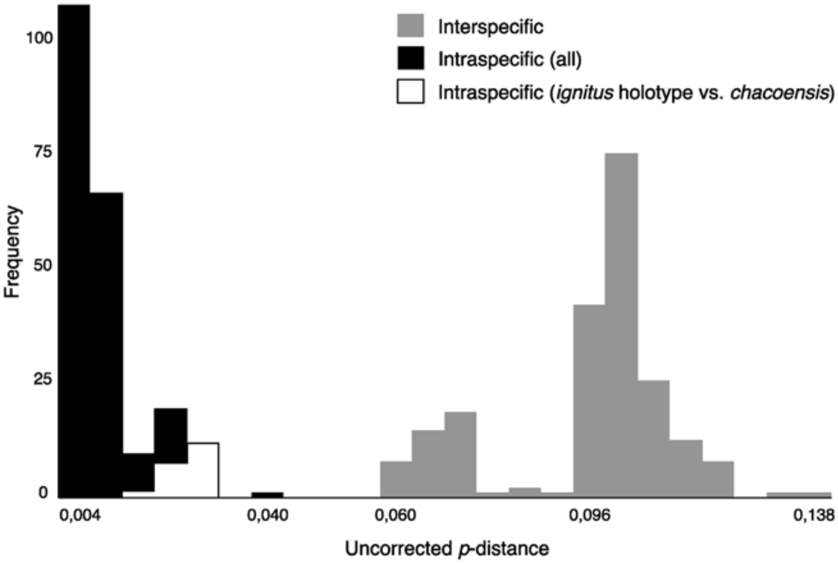

Fig. 3. Histogram of intra- and inter-specific CYTB *p*-distances for *Cryptonanus* specimens sequenced in this study.

Fig. 4. Ventral coloration of the holotype of *Cryptonanus ignitus* (A, AMNH 167852) and three specimens of *C. chacoensis* (B–D, from left to right: MACN-Ma 22463, MACN-Ma 22449, and MACN-Ma 22461). Specimens A, B and C have self-clay-colored ventral fur; specimen in D has the typical creamy-white ventral coloration of most individuals of *C. chacoensis*. Abbreviations: wp = white pectoral patch.

Discussion

All of our methodological approaches and their associated results—phylogenetic analyses of mitochondrial DNA, qualitative morphology, and quantitative morphological traits—confirm the suggestion of previous researchers (cf. Voss et al., 2005) that *C. ignitus* is just an elderly example of the living species *C. chacoensis*. Voss et al. (2005) were the first to discuss this hypothesis, indicating that "Large ontogenetic series of most opossums, for example, show a tendency for older animals to have better-developed temporal scars, lambdoidal crests, and more massive zygomatic arches, but relatively smaller braincases than younger conspecifics. In addition, the canine fangs of old adult male didelphids are often extruded from their alveoli to a much, greater extent than in conspecific females and younger males". These conditions are certainly true for the holotype of *C. ignitus*, a very old adult male with heavily worn molars and fused basioccipital/basisphenoid suture (cf. Voss et al., 2005). Despite these suggestions, Voss et al. (2005) maintained *C. ignitus* as a valid species mostly due to its self-orange ventral fur, not seen at this time in other examples of *Cryptonanus*. Our examination of large series of Argentinian individuals allows us to expand the diagnosis of *C. chacoensis* to include examples with whitish ventral

Fig. 5. Lateral (above), ventral (below, left) and dorsal (below, right) views of the skull and labial view of the mandible (rotated) of the holotype of *Cryptonanus ignitus* (AMNH 167852), including some of its supposedly diagnostic features, such as unusually wide and robust zygomatic arches (za), massively developed postorbital process of the jugal (pp), prominent temporal scars (ts), very long upper canines (uc) and palatine fenestrae (pf, consisting of two small holes on each side of the palatine bone). Other abbreviations: lc = lamboideal crest; mp = maxillopalatine fenestra; po = postorbital constriction.

parts to others clay-colored. Indeed, small to large patches of self-white hairs are present in some individuals (e.g., MACN-Ma 22463), apparently independent of age or sex. Both color morphs are apparently found at the type locality of *C. ignitus*, as we deduced by the mention of another specimen from Yuto, Jujuy, with self-whitish ventral fur (Voss et al., 2005). In this report, we document a second case of such color polymorphism in Corrientes, northeastern Argentina (Fig. 3).

Our mitochondrial DNA evidence—like our morphological and morphometric results—shows strong support in favor of recog-

Table 2
Results for principal components (PC) 1 and 2 based on 10 log-transformed measurements. See Materials and methods for explanation of the abbreviations.

Variables	PCA loadings	
	PC1	PC2
CIL	0.28	−0.22
ONL	0.31	−0.1
BB	0.11	−0.1
LIB	0.02	−0.34
ZB	0.38	−0.36
MB	0.26	−0.09
LR	0.29	−0.03
LN	0.42	0.14
LMxT	0.14	0.23
PL	0.24	−0.25
CC	0.46	0.22
MM	0.21	0.7
Eigenvalue	0.01	4.50E-04
% Variance	0.83	0.06

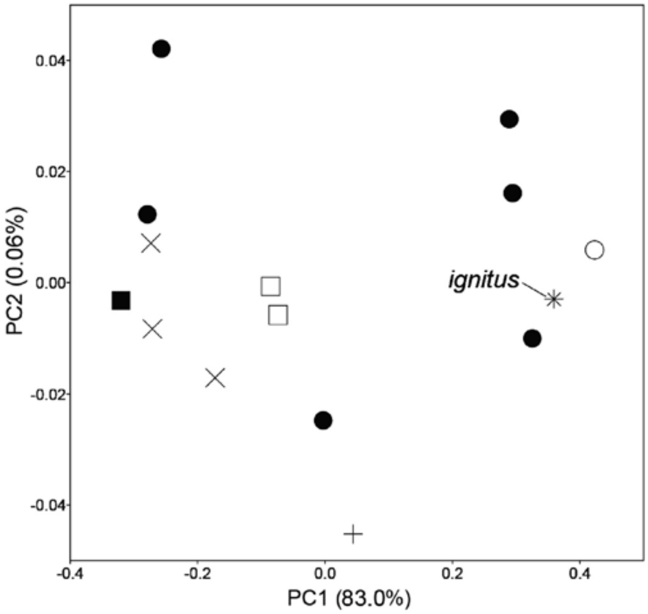

Fig. 6. Specimen scores for examples of *Cryptonanus* (N = 15) for principal components 1 and 2 based on log-transformed measurements. Symbols correspond to different geographic samples as follows: black square = Salta Province, Argentina; white square = Jujuy Province, Argentina; + = Misiones Province, Argentina; black circle = Corrientes Province, Argentina; x = Buenos Aires Province, Argentina; white circle = Alto Paraguay, Paraguay. The asterisk referred as *ignitus* corresponds to the holotype of this nominal form (AMNH 167852).

Table 3
Summary statistics [mean ± *SD* (range) n] of cranial measurements (in mm) of examples of *Cryptonanus chacoensis* and the holotype of *C. ignitus*. See Materials and Methods for explanation of abbreviations.

Variable	*C. chacoensis*	*C. ignitus*
CIL	25.2 ± 1.39 (23.31–27.42) 14	26.73
ONL	25.82 ± 1.53 (24.11–28.45) 14	27.8
BB	9.79 ± 0.26 (9.33–10.17 14	10.37
PC	5.17 ± 0.16 (4.86–5.46) 14	5.38
ZB	14.05 ± 1.02 (12.85–15.77) 14	15.67
MB	9.91 ± 0.49 (9.35–10.76) 14	10.6
LR	9.85 ± 0.62 (9.15–10.95) 14	10.3
LN	11.25 ± 0.93 (10.05–12.79) 14	12.26
LMxT	9.79 ± 0.35 9.25–10.31) 14	10.1
PL	13.21 ± 0.65 (11.98–14.25) 14	14.01
CC	3.95 ± 0.36 (3.50–4.75) 14	4.51
MM	7.94 ± 0.45 (7.36–8.95) 14	8.5

nizing *Cryptonanus ignitus* as a junior synonym of *C. chacoensis*. Although we lack dense sampling for most of the other species of *Cryptonanus*, the *p*-distance we computed between the haplotype of *ignitus* and other haplotypes of *chacoensis* falls within the intraspecific variation observed for all the species across the genus (Fig. 3). Moreover, such intraspecific variation does not overlap with the interspecific distances we obtained (i.e., those between specimens referable to different currently recognized species).

Following de Queiroz (1998, 2007), we recognize species as lineages, for which strongly supported mitochondrial-haplogroup membership and morphological diagnosability are widely accepted recognition criteria. By these criteria, *Cryptonanus chacoensis* (including *ignitus*) appears to be a valid species. For the practical purpose of identifying morphological material, it can be diagnosed from other congeneric lineages (species) by the following unique combination of traits (modified from Voss et al., 2005): self-whitish to self-clay-colored ventral fur (versus ventral fur gray-based in *C. guahybae*), 4-1-4 = 9 abdominal-inguinal mammae (versus 7-1-7 = 15 mammae, of which the anteriormost two or three pairs are pectoral in *C. guahybae*), small molars (LM = 4.9–5.4 mm, versus LM mostly >5.4 mm in *C. unduaviensis*), and an incomplete anterior cingulum on M3 (versus M3 anterior cingulum narrow but complete in other forms).

Based on our results and previous records in the literature (cf. Flores et al., 2000; Voss et al., 2005), only one species of *Cryptonanus*, *C. chacoensis*, is found in northwestern Argentina. This mouse opossum is considered as of Least Concern by the International Union for the Conservation of the Nature (Teta and Martin, 2016), a view that strongly contrasts with the supposedly extinct status of *C. ignitus* (cf. Flores, 2016). This is not a minor issue, since some authors have suggested that South America is in the forefront (only behind Australia) of mainland mammalian extinctions during the last 500 years. According to Teta et al. (2014), at least nine species of mammals have disappeared from South America during this period. However, this number could be an overestimate, because taxonomic results similar to those presented here have been reported by previous authors with other allegedly extinct taxa. For example, Teta and Campo (2017) concluded that the cavy *Galea tixiensis*, a fossil species from the Holocene of central-eastern Argentina, is a synonym of the living and widely distributed *G. leucoblephara*. The same could also be true for the vizcacha *Lagostomus crassus*, a species only known by a single skull collected in Peru, which is possibly a synonym of *L. maximus* (see Spotorno and Patton, 2015). As was expressed by previous authors (e.g., Garbino et al., 2018), rigorous approaches towards historical and recent records are much needed when producing lists of globally or regionally extinct fauna, given the crucial importance of such lists for conservation biology. Part of that rigor must include integrative taxonomic studies involving different lines of evidences. Taxonomy is not a trivial pursuit (Rylands, 2007), and hasty taxonomic practice can have negative consequences for species conservation.

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Appendix A.

List of studied specimens and their collecting localities. Specimens consist primarily in skins with their associate skulls and are housed in the following museums: AMNH, American Museum of Natural History (New York, USA); FMNH, Field Museum of Natural History (Chicago, USA); MACN, Museo Argentino de Ciencias Naturales "Bernardino Rivadavia" (Buenos Aires, Argentina).

Cryptonanus chacoensis (n = 28): **Argentina**: Buenos Aires, Campana, Canal 6 y Paraná de Las Palmas (MACN-Ma 24301, 24456, 24608, 24610, 24623, 24624), Arroyo Las Piedras (MACN-Ma 24625); Zárate, Arroyo Ñacurutú (MACN-Ma 49.35); Corrientes, Departamento Capital, Laguna Pampín, Campo Romero Feris (MACN-Ma 22460, 22468), Pirayuí (MACN-Ma 22445, 22447), San Cayetano (MACN-Ma 22449, 22461, 22463), San Cosme, Santa Ana, Laguna González (MACN-Ma 22446, 22450), Santo Tomé, Virasoro, Las Marías, Escuela Agrotécnica (MACN-Ma 22448); Formosa; Pirané, El Colorado (MACN-Ma 24559); Chaco; Capitán Solari, Parque Nacional Chaco (MACN-Ma 22453); Jujuy, Ledesma, Yuto (AMNH 167852; Holotype of *Cryptonanus ignitus*); San Pedro, Ingenio La Esperanza (MACN-Ma 24541); Misiones; Cainguas (MACN-Ma 24288); Guaraní, Cuartel Río Victoria (MACN-Ma 25991) Iguazú, curso medio del arroyo Urugua-í, E Puerto Libertad; (MACN-Ma 49.325); Salta, Orán, Establecimiento Ledesma (MACN-Ma 22457), Establecimiento El Tabacal, Lote Inés (MACN-Ma 24560, 24607). **Paraguay**: Alto Paraná, Reserva Limoy (FMNH 226362).

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