Papéis Avulsos de Zoologia

Museu de Zoologia da Universidade de São Paulo

Volume 55(3):47-67, 2015 **www.mz.usp.br/publicacoes** **www.revistas.usp.br/paz** **www.scielo.br/paz** **ISSN impresso: 0031-1049** **ISSN *on-line:* 1807-0205**

On the occurrence of *Holochilus chacarius* (Cricetidae: Sigmodontinae) in Brazil, with taxonomic notes on *Holochilus* species

Marcus Vinícius Brandão[1]
Fabio Oliveira do Nascimento[2,3]

ABSTRACT

Currently four species of Holochilus *(Cricetidae: Sigmodontinae) are recognized. According to the literature, three species are recorded for Brazil:* H. sciureus, H. brasiliensis *and* H. chacarius. *Samples from western Brazil (Mato Grosso and Mato Grosso do Sul states) are usually reported as* H. sciureus *and, when referring to specimens from the Pantanal, as* H. chacarius. *However, the taxonomic status of specimens from this region has not been properly evaluated through detailed morphological analyses or voucher specimens. About 110 specimens of* Holochilus *deposited in Brazilian collections were qualitatively and quantitatively analyzed (univariate and multivariate tests). Our results suggested the existence of three species (*H. sciureus, H. brasiliensis *and* H. chacarius*) in Brazil, which present significant morphological and morphometric differences, thus confirming that the Mato Grosso and Mato Grosso do Sul samples, formerly misidentified as* H. sciureus, *belong to* H. chacarius. *This species differs from* H. sciureus *and* H. brasiliensis *by a series of pelage and skull characters, such as: the coloration of pelage, light orange-brown in the dorsum, light orangish in the flanks and white in the venter; ridges of the masseteric crest confluent at the level (or slightly above) of the mental foramen and at the anterior part of m1, alternated main molar cusps, lophids compressed and with acute outer margins (rarely prismatic), anteromedian fossetid labially displaced and subcircular (small to medium), metaflexid less developed (frequently not reaching the midline of the tooth), proto and hipoconid subrectangular in outline and transversely orientated, mesoflexid transversely orientated and mesoloph absent. The updated geographic distribution of* Holochilus *in Brazil shows that* H. sciureus *is present in the northern portion of the country, being the only species present at the Amazon and at the Caatinga and with limit in the Cerrado biome at the center of Goiás state.* Holochilus brasiliensis *is restricted to eastern Brazil, with its northern record at the Atlantic Forest of south Bahia.* Holochilus chacarius *occurs in the Pantanal areas of the Mato Grosso and Mato Grosso do Sul states. A new record extends the distribution of* H. chacarius *to the Cerrado biome at eastern Mato Grosso, confirming its presence in this biome.*

Key-Words: *Holochilus chacarius; Holochilus sciureus; Holochilus brasiliensis;* Mato Grosso; Brazil.

[1.] Universidade Federal de São Carlos, Campus Sorocaba, Departamento de Biologia, Laboratório de Diversidade Animal. Rodovia João Leme dos Santos, km 110 (SP-264), Itinga, CEP 18052-780, Sorocaba, SP, Brasil. E-mail to correspondence: puerabio@gmail.com
[2.] Museu de Zoologia, Universidade de São Paulo, Mastozoologia. Avenida Nazaré, 481, CEP 04263-000, São Paulo, SP, Brasil. E-mail: fabnasc@gmail.com
[3.] Museu Paraense Emílio Goeldi, Mastozoologia. Avenida Perimetral, 1.901, CEP 66017-970, Belém, PA, Brasil.
http://dx.doi.org/10.1590/0031-1049.2015.55.03

INTRODUCTION

Species of the genus *Holochilus* Brandt, 1835 are South American rodents of the Oryzomyini radiation easily distinguished from other genera by the presence of interdigital foot webs of the hind feet, large body and feet sizes, and orangish-brown dorsal pelage (Voss & Carleton, 1993; Patton *et al.,* 2000). The only revision of this genus was made by Hershkovitz (1955), who restricted all forms – except *H. magnus* Hershkovitz, 1955, now placed in *Lundomys molitor* (Winge, 1887) (see Voss & Carleton, 1993) – into a single species, *H. brasiliensis* (Desmarest, 1819), with 10 subspecies. Nowadays, *Holochilus* is considered to be composed by four species (Musser & Carleton, 2005; Pardiñas *et al.,* 2013): *H. brasiliensis* Wagner, 1842, *H. sciureus* Wagner, 1842, *H. chacarius* Thomas, 1906 and the recently described *H. lagigliai* Pardiñas, Teta, Voglino & Fernández, 2013. However, all species present an unclear taxonomy and geographic distribution (Voss & Carleton, 1993; Pardiñas & Teta, 2011; Pardiñas *et al.,* 2013) due to the lack of a recent and critical taxonomic revision.

Holochilus brasiliensis is the largest species of the genus and is found in southeastern Brazil, eastern Paraguay, Uruguay, and east/central Argentina (Musser & Carleton, 2005, Pardiñas *et al.,* 2013). In Brazil, this species is mostly associated to the Atlantic Forest, with poorly documented limits from the states of Rio Grande do Sul to Bahia (Massoia, 1981; Marques, 1988). Furthermore, there are scarce records of *H. brasiliensis* along riverine forests in the Cerrado biome (Carmignotto, 2005). Regarding the smallest species, *H. sciureus* is widespread in the northern limits of the genus, inhabiting remarkably different biomes that ranges from humid environments in Amazonia, through central Brazil and up to the Caatingavegetation on northeastern limits of Brazil (Moojen, 1952; Patton *et al.,* 2000; Carmignotto *et al.,* 2012). *Holochilus chacarius,* in turn, is widespread in the southwestern limits of the genus, where is commonly associated to the Chaco of Argentina, Paraguay and south Bolivia, whereas *H. lagigliai* is known only from the type locality at Mendoza Province, Argentina (Pardiñas *et al.,* 2013: fig. 2). As a result, field identification of *Holochilus* spp. is usually associated to their size and geographic distribution.

Except for *H. lagigliai,* all species of the genus were already mentioned to occur in Brazil (*e.g.,* Bonvicino *et al.,* 2008; Cáceres *et al.,* 2008, 2011; Oliveira & Bonvicino, 2006, 2011; Paglia *et al.,* 2012), stating that *H. chacarius* occurs in the Pantanal region of Mato Grosso do Sul state. However, none of these reports listed any voucher specimens or explained the decision of reporting the presence of this taxon in Brazil. Furthermore, there is no reference in literature that justifies with details the occurrence of *H. chacarius* in Brazil. Therefore, the northernmost vouchered record previously known for *H. chacarius* was made for Bolivia by Anderson (1997) – reported as *H. sciureus* but latter some specimens were reidentified by Pardiñas & Galliari (1998) – and almost all reports of a small-sized species of Brazilian *Holochilus* are restricted to *H. sciureus.*

We provide here a short review on morphological diagnostics characters in order to distinguish the species of *Holochilus* from Brazil currently recognized, and to discuss the presence of *H. chacarius* in this country, reporting a distribution extension for this taxon. Finally we provide updated records of *H. brasiliensis* and *H. sciureus,* reevaluating both distributional limits in Brazil.

MATERIAL AND METHODS

We have studied skins and skulls of *H. chacarius, H. sciureus* and *H. brasiliensis* deposited in the mammal collections of Museu de Zoologia da Universidade de São Paulo, São Paulo (MZUSP), and Museu Nacional, Universidade Federal do Rio de Janeiro, Rio de Janeiro (MN) (Appendix I).

The external qualitative characters refers to the pelage color pattern of the head, body, feet and tail. For the external quantitative characters, we used body measurements taken from the specimen's tags as follows: (1) head and body length (HB); (2) tail length (T); (3) hind foot length (HF); and (4) ear length (E). When only total length (TL) was provided, we subtracted the recorded tail length from total length to obtain the values of the head and body length.

The following craniodental measurements were taken: condyle-incisive length (CIL); length of the diastema (LD); length of the molar tooth row (without alveolus) (MTR); breadth of first upper molar (BM1); length of the incisive foramina (LIF); breadth of the incisive foramina (BIF); breadth of the palatal bridge (BPB); breadth of the zygomatic plate (BZP); length of the rostrum (LR); length of nasals (LN); interorbital breadth (LIB); breadth of the braincase (BB); breadth across the squamosal zygomatic processes (ZB); and zygomatic length (ZL). All craniodental measurements were based on Voss *et al.* (2001: fig. 33) and taken with digital calipers to the nearest 0.01 mm.

Three age classes were recognized based on maxillary tooth eruption and abrasion and fusion of the

skull sutures: juveniles, subadults and adults. Specimen is only classified as an adult if covered by full adult pelage – not the juvenile coat or the transitional molt from juvenile to adult fur – with permanent dentition (third molar fully erupted) and completely ossified spheno-occipital suture. In our analysis we used only adults that present all the mentioned characters.

Geographic data on *Holochilus* were obtained by direct examination of museum specimens. Locality data was obtained from published gazetteers (*e.g.,* Paynter Jr., 1994, 1995; Paynter Jr. & Trylor Jr., 1991) and online ones (*e.g.,* Global Gazetteer 2.2, www.fallingrain.com/world/index.html).

Statistical Analysis

We apply the Kolmogorov-Smirnov test for all variables of the studied species. When the variables were normally distributed, we performed Student's t-test to evaluate the existence of sexual dimorphism.

We performed one-way analysis of variance (ANOVA) and Tukey's *pos hoc* test to check whether are significant differences ($p < 0.05$) among the species on craniodental variables.

For the multivariate analysis, specimens with missing values were removed, reducing the sample from 65 to 48 specimens, and all craniodental variables were $\log_{10}$ transformed. Principal component analysis (PCA) was extracted from the covariance matrix and it applied as an exploratory tool for investigating the patterns of variation among the species of *Holochilus,* as well as to evaluate the degree of separation among them. Only principal components with eigenvalues greater than 1 were extracted and after they were rotated using the varimax criterion. Discriminant function analysis (DFA) was performed to investigate whether the three species of *Holochilus* studied could be distinguished based on skull morphology and also to construct a predictive pattern of different group memberships. All statistical analyses were performed with SPSS 17.0 software.

RESULTS AND DISCUSSION

The identity of the Mato Grosso specimens

In the course of a research on *Holochilus* specimens from Mato Grosso state, Brazil, which has a significant portion of Amazon rainforest in its north limit, we verified that many references found for small mammals assemblages from this biome and adjacent transitional areas (*e.g.,* Bezerra *et al.,* 2009; Patton *et al.,* 2000; Rocha *et al.,* 2011a, b) mentioned a small sized *Holochilus* species currently identified as *H. sciureus,* which Patton and collaborators (2000) distinctively described its ventral color as gray washed with orange or buff (Fig. 1B). Our hypothesis of the presence of a different species in Mato Grosso came from the fact that specimens of *Holochilus* from this state, although had a similar size, clearly present a self-white venter (Fig. 1A).

For that reason, we initially compared specimens from Amazonas, Rondônia and Pará state, both adjacent to Mato Grosso. All specimens from those areas present venter coloration as the one described by Patton and collaborators (2000), been remarkably different from the Mato Grosso specimens. Latter, we also find that all specimens from northeastern Brazil present a similar venter coloration to those from Brazilian Amazon (excluding Mato Grosso), just as the morphology described by Oliveira & Langguth (2004) for Paraíba e Pernambuco specimens. Specimens that present whitish venters that resembles those from Mato Grosso were from large-sized Atlantic Forest specimens, which clearly represent *H. brasiliensis* (Fig. 1C), the largest species. For this reason, this name could not be assigned to the Mato Grosso specimens. Additionally some of these specimens present self-white ventral fur only restricted to throat and inguinal areas (see details further in the text). The only specimens that matched both morphological and size features were from Mato Grosso do Sul, the Brazilian state adjacent to Mato Grosso at the south, and therefore also could not be assigned to *H. brasiliensis* and *H. sciureus.*

Studying records from species of *Holochilus* known from other countries, *H. lagigliai* and *H. chacarius,* we made progress on Mato Grosso specimens identification. The morphology of *H. lagigliai* is quite different from the Mato Grosso specimens (see details further in the text), refuting the hypothesis that they are different populations of the same species. However, *H. chacarius* has similar size and pelage characters similar to Mato Grosso specimens (Massoia, 1971, 1976, 1980). Furthermore, *H. lagigliai* is known only from the central western Argentina, while *H. chacarius* was already confirmed to inhabit Bolivia (Anderson, 1997; see Pardiñas & Galliari, 1998), Argentina and Paraguay (Massoia, 1971, 1976; Pardiñas & Teta, 2011; Pardiñas *et al.,* 2013: fig. 2), which are adjacent regions to Mato Grosso and Mato Grosso do Sul states.

In view of that, the evidences suggest that the specimens from Mato Grosso are in fact neither

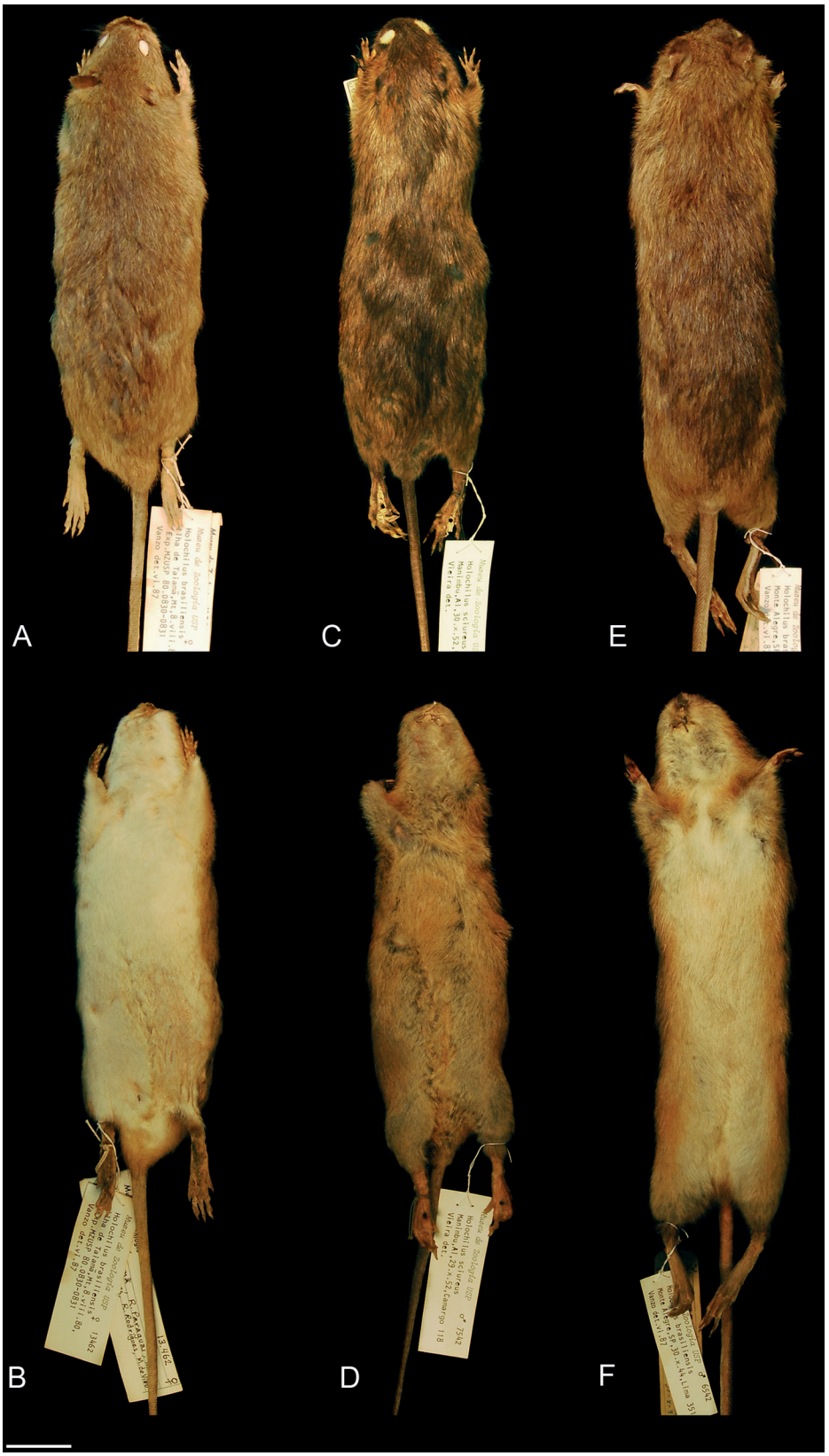

FIGURE 1: Dorsal and ventral views of skin of *Holochilus* spp. **A:** *H. chacarius* (MZUSP 13462). **B:** *H. sciureus* (MZUSP 7542). **C:** *H. brasiliensis* (MZUSP 6542). Scale bar: 20 mm.

H. sciureus nor *H. brasiliensis*. However, the Mato Grosso specimens present external similarity with both the other congeneric species in Brazil: while ventral pelage pattern resembles those of *H. brasiliensis,* the size is consistently different, whereas the opposite is observed when compared to *H. sciureus*. Therefore, in order to consistently distinguish Mato Grosso specimens from the mentioned Brazilian species, we investigate in details the craniodental and external characters.

Quantitative analysis

The Kolmogorov-Smirnov test (applied to test the normality of the sample) showed a normal distribution of all variables (results not included herein). According to Student's t-test, there are no significant differences between the sexes (results not included herein). Therefore, both males and females were included together in the descriptive statistics and the following tests.

The descriptive statistics (mean, standard deviation, minimum and maximum values, and number of specimens) are available in Table 1. The results of ANOVA revealed that 11 of 14 variables (CIL, LD, MTR, BM1, BIF, BZB, LR, LN, BB, ZB and ZL) (Table 2) exhibited differences among the species. The *pos hoc* Tukey test showed significant difference between *H. chacarius* and *H. brasiliensis* for 11 variables (CIL, LD, MTR, BM1, BIF, BPB, LR, LN, BB, ZB and ZL) (Table 2). *Holochilus chacarius* and *H. sciureus* were different for three variables (MTR, BIF, and ZB) (Table 2), whereas *H. brasiliensis* and *H. sciureus* were different for eight variables (CIL, BM1, BPB, BZP, LR, BB, ZB and ZL) (Table 2).

A total of 48 specimens (14 *H. chacarius,* 21 *H. brasiliensis* and 13 *H. sciureus*) with no missing data were included in the PCA. Table 3 shows the three first components resulting from the analysis, as well as their respective coefficients, eingevalues and variances. The first principal component was responsible for 60.59% of variance, and in combination with the second component, both were responsible for 69.90% of variance. All coefficients of the first component showed positive signal, indicating a positive correlation with each other, and it can be interpreted as a component usually associated to the overall size of the skull (Jolicoeur & Mosimann, 1960). The greatest coefficient is associated to the breadth of the palatal bridge (BPB), indicating it as the dominant craniodental measurement in the first component. Other variables, breadth across the squamosal zygo-

TABLE 1: Descriptive statistics for craniodental and external variables of adult specimens (males and females grouped) of *H. chacarius, H. brasiliensis* and *H. sciureus.* Legends: M = mean; SD = standard deviation; MIN = minimum; MAX = maximum; and N = number of specimens.

Variables		*Holochilus chacarius*			*Holochilus brasiliensis*			*Holochilus sciureus*		
		M ± SD	Min. – Max.	N	M ± SD	Min. – Max.	N	M ± SD	Min. – Max.	N
Skull	**CIL**	34.78 ± 1.98	32.37 – 38.68	17	38.19 ± 1.55	34.96 – 40.56	26	35.42 ± 1.47	32.95 – 38.10	17
	LD	10.78 ± 0.98	9.35 – 12.60	17	11.43 ± 0.71	9.96 – 12.66	26	10.95 ± 0.76	9.50 – 12.24	24
	MTR	6.89 ± 0.27	6.25 – 7.22	17	7.40 ± 0.21	7.10 – 7.80	26	7.23 ± 0.26	6.62 – 7.67	23
	BM1	2.18 ± 0.09	2.00 – 2.35	17	2.43 ± 0.11	2.23 – 2.61	23	2.17 ± 0.10	2.05 – 2.36	23
	LIF	7.18 ± 0.58	6.47 – 8.50	17	7.49 ± 0.36	6.78 – 8.22	26	7.51 ± 0.59	6.40 – 8.34	24
	BIF	2.32 ± 0.25	1.93 – 2.95	17	2.60 ± 0.20	2.30 – 3.08	26	2.58 ± 0.22	2.18 – 3.00	24
	BPB	2.71 ± 0.30	2.17 – 3.33	17	3.39 ± 0.33	2.65 – 3.95	26	2.85 ± 0.20	2.43 – 3.31	23
	BZP	4.95 ± 0.40	4.28 – 5.70	16	5.14 ± 0.34	4.38 – 5.90	26	4.82 ± 0.43	4.10 – 5.83	24
	LR	11.44 ± 0.73	10.00 – 12.72	16	12.69 ± 0.65	11.54 – 14.00	26	11.83 ± 0.65	10.50 – 13.18	22
	LN	13.73 ± 1.30	11.83 – 16.58	16	14.49 ± 0.85	12.87 – 16.40	26	14.34 ± 0.81	12.67 – 15.66	22
	LIB	4.79 ± 0.22	4.46 – 5.20	17	4.79 ± 0.37	4.14 – 5.60	26	4.65 ± 0.27	4.01 – 5.00	21
	BB	15.03 ± 0.45	14.27 – 15.64	17	15.91 ± 0.59	14.47 – 17.11	26	15.03 ± 0.44	14.19 – 15.66	17
	ZB	19.93 ± 1.12	18.40 – 22.50	14	22.85 ± 1.00	20.55 – 25.37	24	20.83 ± 1.03	18.36 – 22.40	22
	ZL	16.96 ± 0.99	15.55 – 19.08	17	18.62 ± 0.82	16.80 – 20.26	26	17.48 ± 0.79	15.83 – 19.16	22
External	**HB**	150.11 ± 11.81	130.37 – 166.00	9	186.11 ± 17.77	170.00 – 220.00	9	171.71 ± 14.53	135.00 – 200.00	34
	T	158.00 ± 17.32	130.00 – 187.00	8	178.83 ± 15.35	155.00 – 210.00	12	154.26 ± 16.40	120.00 – 175.00	34
	HF	37.94 ± 3.35	33.00 – 42.00	8	45.36 ± 3.27	35.00 – 50.00	14	39.18 ± 3.86	32.00 – 53.00	34
	E	17.11 ± 1.83	15.00 – 20.00	9	20.55 ± 1.13	19.00 – 22.00	11	16.80 ± 2.05	11.00 – 20.00	25

TABLE 2: ANOVA and Tukey test to evaluate the existence of differences in the craniodental variables in the three Brazilian species of *Holochilus.*

Variables	ANOVA	Tukey		
	F	*H. chacarius* / *H. brasiliensis*	*H. chacarius* / *H. sciureus*	*H. brasiliensis* / *H. sciureus*
CIL	26.158*	**0.000**	0.509	**0.000**
LD	3.977*	**0.030**	0.776	0.096
MTR	22.031*	**0.000**	**0.000**	0.052
BM1	48.087*	**0.000**	0.944	**0.000**
LIF	2.471	0.137	0.113	0.989
BIF	9.2223*	**0.000**	**0.002**	0.927
BPB	35.823	**0.000**	0.271	**0.000**
BZP	4.219*	0.299	0.542	**0.014**
LR	19.731*	**0.000**	0.178	**0.000**
LN	3.242*	**0.042**	0.133	0.869
LIB	1.670	1.000	0.301	0.230
BB	21.895*	**0.000**	1.000	**0.000**
ZB	40.675*	**0.000**	**0.037**	**0.000**
ZL	21.416*	**0.000**	0.157	**0.000**

Values in bold represent statistical difference at 5% in Tukey test and the * represent statistical differences at 5% in ANOVA.

matic processes (ZB), breadth of the zygomatic plate (BZP), length of the rostrum (LR), zygomatic length (ZL) and condyle-incisive length (CIL), in decreasing order, also contributed in a similar way to each other for the first component.

The variables that contributed to the second component were, in decreasing order, breadth of the incisive foramina (BIF), length of the incisive foramina (LIF), length of nasals (LN), breadth of the palatal bridge (BPB), length of the diastema (LD) and breadth across the squamosal zygomatic processes (ZB). The two first variables are related to size of incisive foramina, and the remaining variables (except ZB) are related to the anterior region of the skull. Moreover, regarding to the second component, there is a connection between form and size.

In the two first principal components plot (Fig. 2) is possible to observe that *H. sciureus* specimens are widely mixed to *H. chacarius,* mainly, and to *H. brasiliensis,* but these latter two species, in turn, show a certain overlapping between their respective specimens. Concerning to the first component, and

TABLE 3: Factor loadings, eingevalues and percentage of variance of Principal Component Analysis (PCA) for *Holochilus* spp. using 14 craniodental variables. Variables with high loading are marked with boldface.

Variables	Component	
	1	2
CIL	**0.020**	0.013
LD	0.019	**0.018**
MTR	0.006	0.007
BM1	0.019	0.003
LIF	0.008	**0.023**
BIF	0.010	**0.039**
BPB	**0.056**	**0.019**
BZP	**0.024**	0.007
LR	**0.024**	0.012
LN	0.015	**0.022**
LIB	0.000	-0.002
BB	0.011	0.004
ZB	**0.026**	**0.015**
ZL	**0.021**	0.011
Eingevalue	0.010	0.001
Variance (%)	60.59	9.31

TABLE 4: Discriminant function analysis (DFA) for *Holochilus* spp. using two first component scores. Variables with high loading are marked with boldface.

Variables	Function	
	1	2
CIL	0.461	**-0.723**
LD	**-0.776**	-0.022
MTR	0.419	**0.656**
BM1	0.474	-0.328
LIF	**-0.957**	**0.503**
BIF	**0.497**	0.001
BPB	**0.500**	0.220
BZP	0.245	-0.223
LR	0.248	-0.009
LN	**-0.514**	**0.409**
LIB	-0.089	-0.286
BB	0.372	**-0.571**
ZB	**0.618**	**0.392**
ZL	-0.288	0.097
Eingevalue	6.732	0.961
Variance (%)	87.51	12.49
Canonical Correlation	0.933	0.700

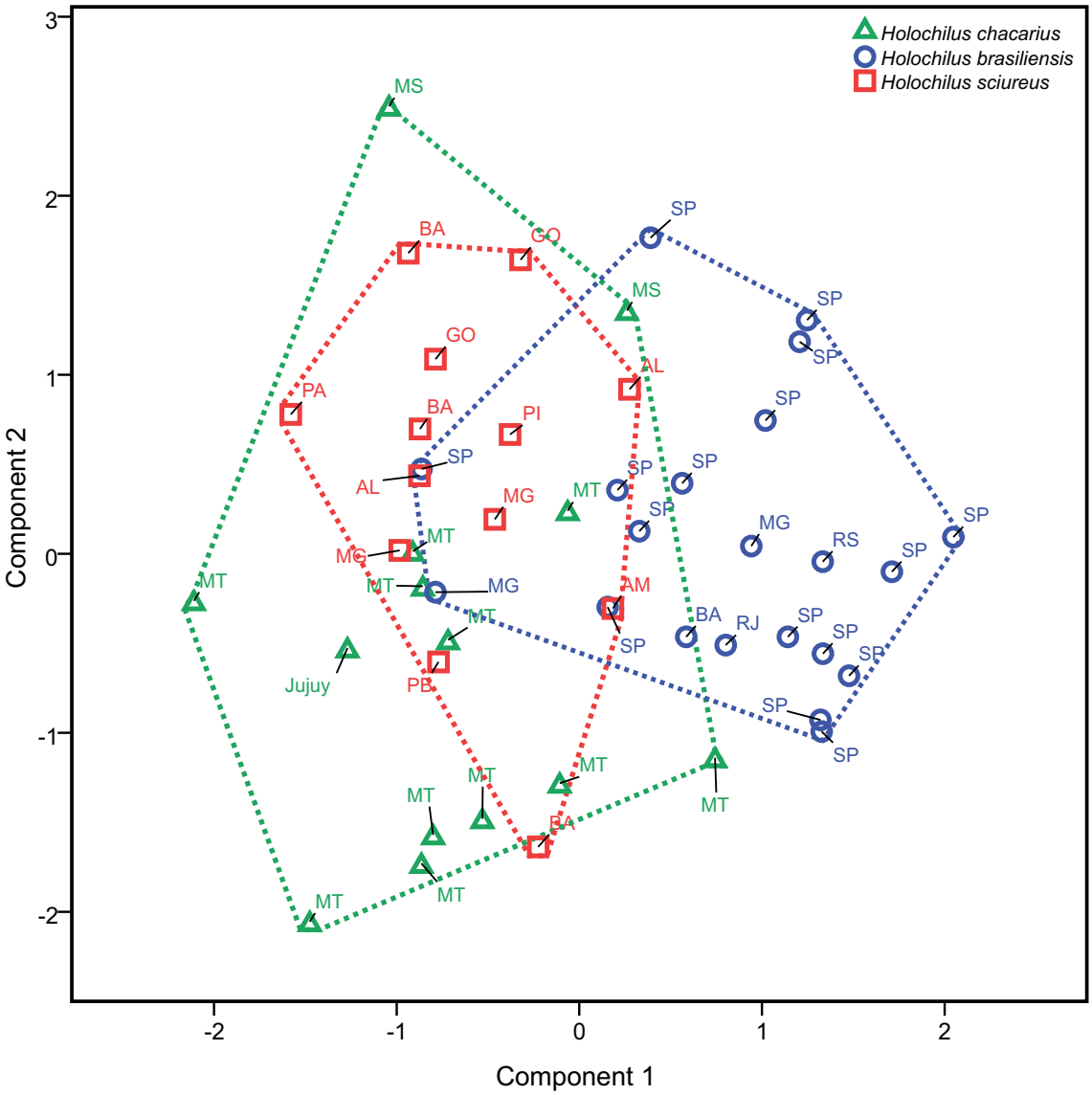

FIGURE 2: Distribution of the factorial scores in the first and second principal components of the craniometrical variables (in decimal logarithm) of *Holochilus* specimens.

also considering it related to the overall size of the skull, it is possible to affirm that there is a trend in *H. brasiliensis* specimens to have bigger skull dimensions, whereas the *H. chacarius* and *H. sciureus* specimens trend to show smaller ones, and furthermore, there are overlaps among the smallest *H. brasiliensis* specimens and the biggest *H. chacarius* and *H. sciureus* ones. With regard to the second component, the specimens of the three species are widely mixed.

The discriminant function analysis created two canonical functions from the original craniodental variables used in the analysis. The first canonical variable is responsible by 87.51% of total variance and the second by 12.49% (Table 4 and Fig. 3). The canonical correlation also shows a high relation between each discriminant function and the pre-defined groups of the analysis, indicating that both functions are useful to discriminate the groups (Table 4 and Fig. 3). Discriminant analysis shows a clear separation of each *Holochilus* species (Fig. 3), and *H. brasiliensis* is well separated from the two other species based on canonical coefficients of the first function, which are mainly related to the following variables, in decreasing order: length of the incisive foramina (LIF), length of the diastema (LD), breadth across the squamosal zygomatic processes (ZB), length of nasals (LN), breadth of the palatal bridge (BPB) and breadth of the incisive foramina (BIF) (Table 4; Fig. 3). Based on the canonical coefficients of the second function (Table 4), there is an overlapping among the individuals of the three

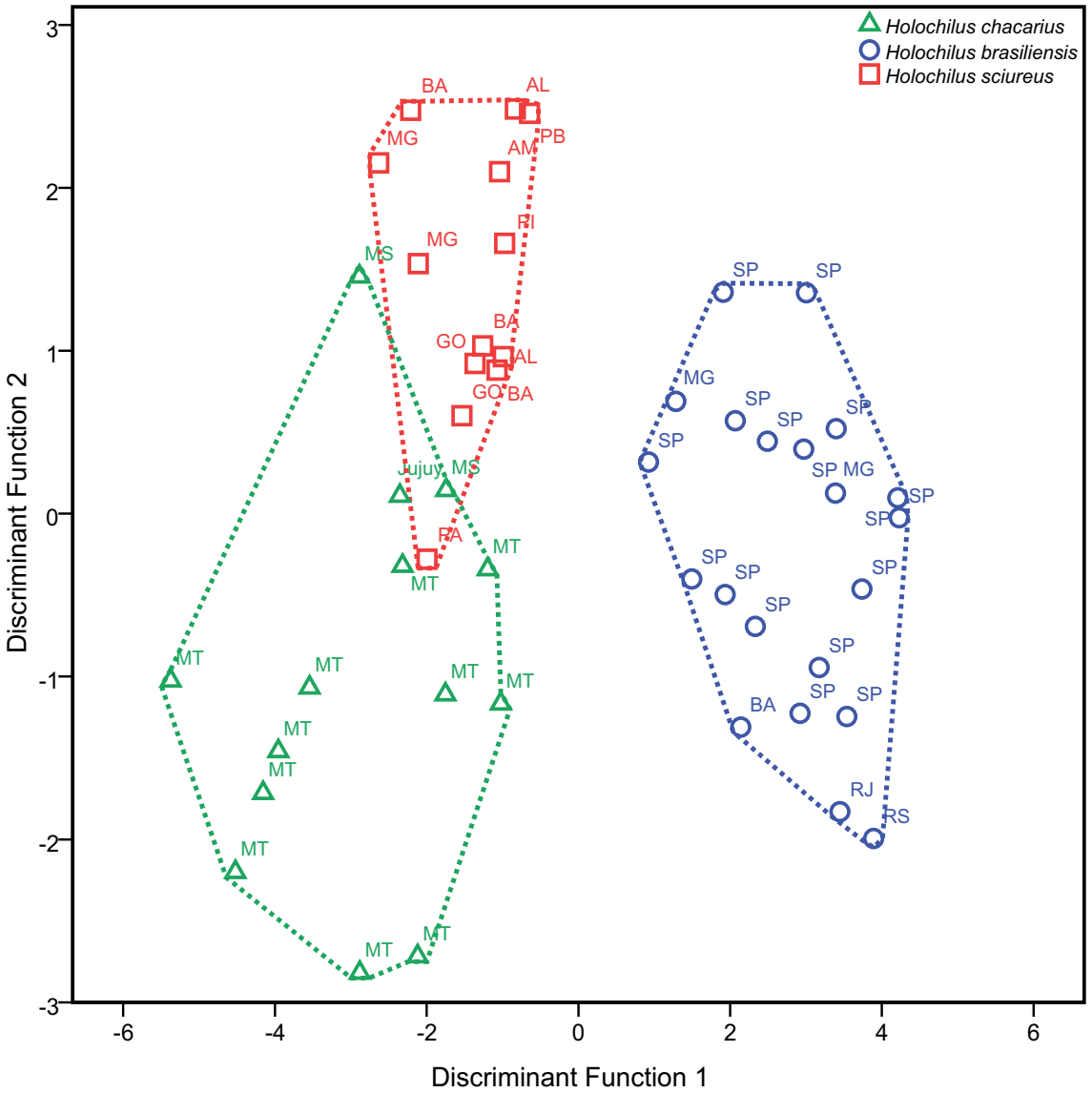

FIGURE 3: Distribution of the factorial scores in the first and second discriminant functions of the craniometrical variables (in decimal logarithm) of *Holochilus* males specimens.

species, but *H. sciureus* shows a slight separation from *H. brasiliensis* and *H. chacarius* (Fig. 3). Condyle-incisive length (CIL), length of the molar tooth row (MTR), breadth of the braincase (BB), length of the incisive foramina (LIF), length of nasals (LN), and breadth across the squamosal zygomatic processes (ZB) are the variables that contribute more, in decreasing order, to the second function.

A predict group membership (Table 5) shows that only *H. brasiliensis* is 100% (n = 21) correctly classified, while *H. chacarius* and *H. sciureus* are 85.7% (n = 11) and 92.3% (n = 12) correctly classified, respectively. In the cross-validated cases 90.5% (n = 19) of *H. brasiliensis,* 64.3% (n = 9) of *H. chacarius* and 69.2% (n = 18) of *H. sciureus* are correctly classified (Table 5).

Qualitative analysis

The morphometric evidence described above suggests the presence of a third species of *Holochilus* in Brazil. Indubitably, the specimens from western Brazil (Mato Grosso and Mato Grosso do Sul states) are related to *H. chacarius* as judged from their size, pelage color and geographical distribution pattern. Craniodental characteristics also proved to be informative in the identification of the current recognized

TABLE 5: Classification matrix for *Holochilus* spp. obtained by discriminant function analysis concerning the probabilities of classifying each species correctly into one of the three species.

			Classification Results [b,c]			
			Predicted Group Membership			
		Species	*H. chacarius*	*H. brasiliensis*	*H. sciureus*	Total
Original	**Count**	***H. chacarius***	12	0	2	14
		H. brasiliensis	0	21	0	21
		H. sciureus	1	0	12	13
	%	***H. chacarius***	85.7	0.0	14.3	100.0
		H. brasiliensis	0.0	100.0	0.0	100.0
		H. sciureus	7.7	0.0	92.3	100.0
Cross-validated[a]	**Count**	***H. chacarius***	9	0	5	14
		H. brasiliensis	0	19	2	21
		H. sciureus	4	0	18	13
	%	***H. chacarius***	64.3	0.0	35.7	100.0
		H. brasiliensis	0.0	90.5	9.5	100.0
		H. sciureus	30.8	0.0	69.2	100.0

[a.] Cross validation is done only for those cases in the analysis. In cross validation, each case is classified by the functions derived from all cases other than that case.
[b.] 93.8% of original grouped cases correctly classified.
[c.] 77.1% of cross-validated grouped cases correctly classified.

species. Remarkable contributions have already been made by others authors as described next.

After Hershkovitz (1955), who lumped 13 nominal species of *Holochilus* under *H. brasiliensis,* the most important contributions were the reports made by Massoia (1971, 1976, 1980, 1981). His reports were mostly related to comparisons between *H. brasiliensis* to one or another of the others currently recognized species (except for the recently described *H. lagigliai*), providing consistent diagnostic characters to distinguish them. Latter, following and expanding Massoia's work, significant contributions to diagnostic characters on *Holochilus* were made by Voss & Carleton (1993), Carleton & Olson (1999), and Machado *et al.* (2013), whom respectively compared this genus to *Lundomys, Noronhomys, Carletonomys* and *Reigomys* in order to recognize them as valid genus. Recently Pardiñas & Galliari (1998) and Pardiñas & Teta (2011) provided a substantial contribution to the knowledge on the fossil history of *Holochilus,* summing up all known characters for the species of this genus (except for the recently described *H. lagigliai*). All these mentioned works were the most significant references to the analysis of the specimens reported herein.

Currently it seems to be a general consensus that *Holochilus* spp. are easily distinguished based on cranial morphological traits into two groups: "*H. brasiliensis* group", characterized by skulls with longer dimensions (Table 1, Fig. 4), main molar cusps opposite or slightly alternated, proto/hypoconid with posterior faces oriented 45° inwards, presence or at least vestige of mesoloph on M1 and M2 (Fig. 5E) and ridges of the masseteric crest confluent at the level of the posterior face of m1 and continuing forward to the mental foramen (see Voss & Carleton, 1993: fig. 11; Voglino *et al.,* 2004: fig. 2); and "*H. sciureus* group", characterized by skulls with shorter dimensions (Table 1, Fig. 4), main molar cusps evidently alternated, proto/hypoconid with posterior faces transversally oriented, mesoloph on M1 and M2 absent (Fig. 5A and C) and ridges of the masseteric crest confluent at the level or slightly anterior to the mental foramen (see Voss & Carleton, 1993: fig. 11; Voglino *et al.,* 2004: fig. 2).

The majorities of the differences mentioned above consist on diagnostics characters that regard molar morphology. Most of them were summarized by Pardiñas & Teta (2011) and, since we consistently agree with several of the diagnostics characters cited by them (see Pardiñas & Teta, 2011: table 3), those characters are summarized here (Table 6). However, few of them are discussed here to clarify some issues on the identification of specimens of this genus.

Most of the specimens observed by Pardiñas & Teta (2011) were from Argentina and a few specimens from Brazil (three from Pirapora, Minas Gerais, and one from Barreiras, Bahia). Studying a higher number of specimens from Brazil, we consistently agree with the characters cited by the mentioned authors, but we realized that morphological variation within specimens examined by us were significant and it would be hard to distinguish few specimens of *H. sciureus* from *H. chacarius* looking only for molar differences. As can be observed in Pardiñas & Teta (2011: table 3),

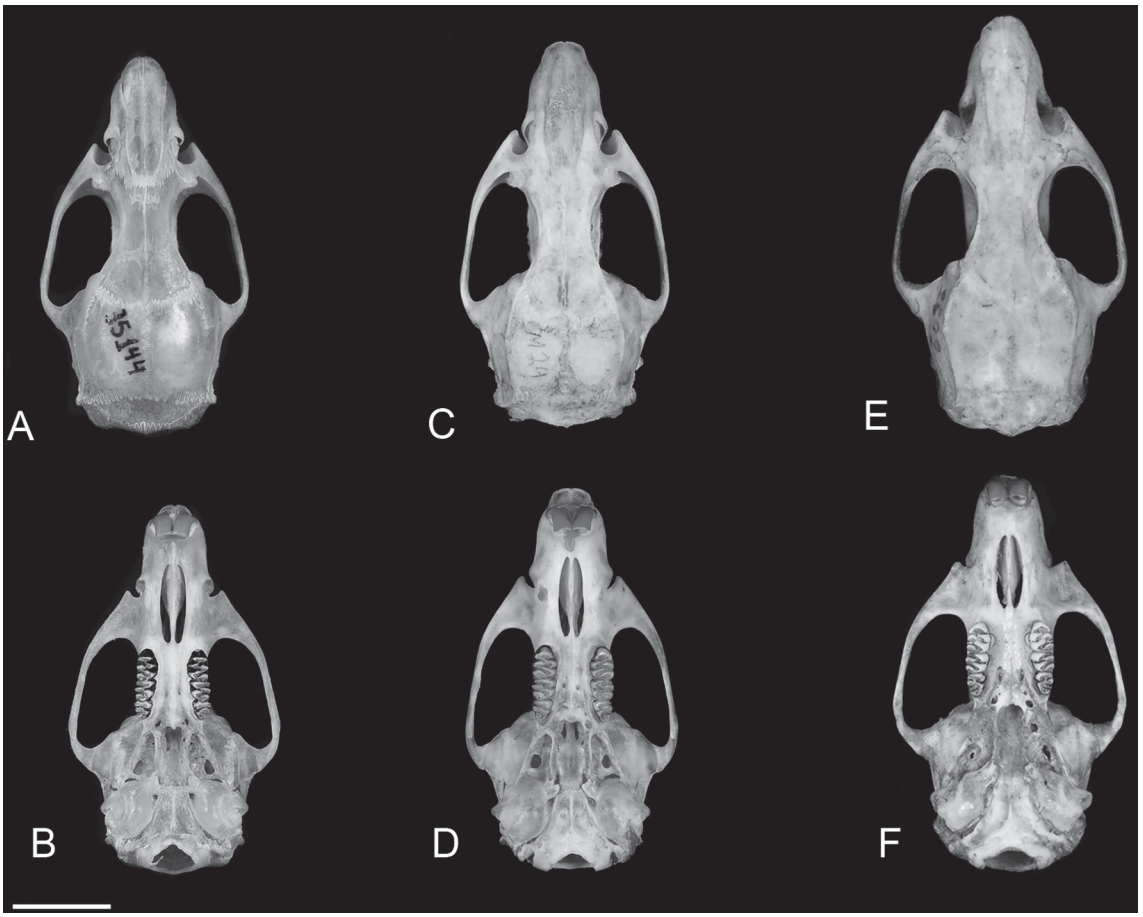

FIGURE 4: Dorsal and ventral view of skulls of *Holochilus* spp. **A:** *H. chacarius* (MZUSP 35144). **B:** *H. sciureus* (MZUSP 35269). **C:** *H. brasiliensis* (MZUSP 2692). Scale bar: 10 mm.

few differences were mentioned to distinguish *H. sciureus* from *H. chacarius,* been those restricted to: form of lophids (FL), form and size of the anteromedian fossetid (AF), and occlusal development of metaflexid (DM). We analyzed specimens that presented the mentioned differences (Fig. 5); but we also seen few specimens that presented characters referent to the other species (see Table 7 and Fig. 6).

For instance, although *H. sciureus* and *H. chacarius* present FL quite different to *H. brasiliensis,* we hardly agree that there are differences between *H. sciureus* and *H. chacarius* that can be easily detected for some specimens, although exist differences between these species. Following Pardiñas & Teta (2011) descriptions, most of the specimens of *sciureus* group analyzed by us presented FL similar to those of *H. chacarius* but also considerable amount presented the FL most similar to *H. sciureus.* It is worth mentioning that the specimen found by us that presents FL most remarkably similar to *H. sciureus,* which is described as distinctly compressed and with strongly acute outer margins (almost prismatic), actually belongs to an Argentinean specimen (Fig. 6A) identified by us as *H. chacarius.* As concerned to AF, Pardiñas & Galliari (1998) had anteriorly divided this character into tree types, each belonging to *H. sciureus, H. c. balnearum* and *H. c. chacarius,* who seemed to present an intermediate form of AF between the two others (see Pardiñas & Galliari, 1998: Table 2). Differently, Pardiñas & Teta (2011) did not recognized differences among subspecies of *H. chacarius* and recognized for this species the same AF reported by Pardiñas & Galliari (1998) to *H. c. balnearum.* Specimens analyzed by us proved to present all forms of AF mentioned by Pardiñas & Galliari (1998), therefore we were not able to distinguish quite easily those species or much less in subspecies level (see Fig. 5B, D and F; Fig. 6B, C and D for comparisons). Finally, we also find that DM is quite variable among those species and connection between metaflexid with the protoflexid, a characteristic anteriorly associated to *H. sciureus,* could be observed in all species of the genus considered here (Fig. 6B and D), including specimens of *H. brasiliensis.* Also, some specimens of *H. sciureus*

presented the anterior murid and, therefore, the connection between metaflexid with the protoflexid is absent.

All issues discussed above indicate that differences between the two species from *sciureus* group, made by previous authors, maybe are related to individual or to geographic variation found within these species, since distinctiveness between lower molar morphology can be sometimes quite difficult to observe.

It is worth mentioning that few specimens present good condition for the dental analyses due to the fact that most of them were much worn. Although *Holochilus* is a member of the tribe Oryzomyini, which usually present pentalophodont molars (generally associated to forested habitats), its species present simplification of molar oclusional surface through lost/reduction of dental structures, been now classified as a tetralophodont (generally associated to open/transitional areas) (see details on Voss & Carleton, 1993; Weksler, 2006; Machado *et al.* 2013). Therefore, the high degree of tooth wear is quite common for *Holochilus* populations due to its species diets, which usually include abrasives and fibrous items that might cause early tooth wear. This was especially true for

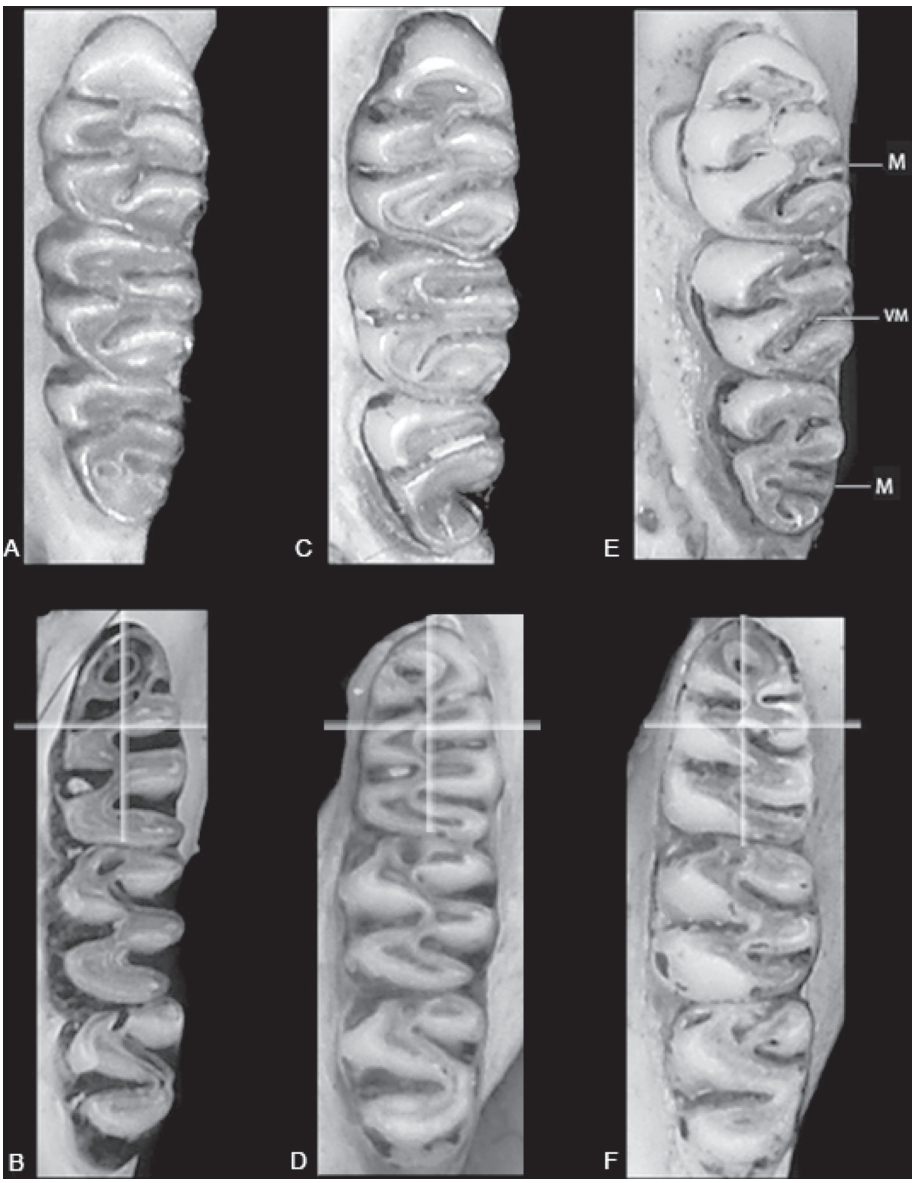

FIGURE 5: Upper and lower molars of *Holochilus* spp. *Holochilus chacarius:* **A:** MZUSP 35143. **B:** MZUSP 35144. *Holochilus sciureus:* **C:** MZUSP 7540; **D:** MZUSP 35269. *Holochilus brasiliensis:* **E** and **F:** MZUSP 2692. M: mesoloph. VM: vestigial mesoloph. The cross present on **B, D** and **F** were digitally made in order to a better comprehension on diagnostic characters of the lower molars.

TABLE 6: Diagnostic traits of three Brazilian *Holochilus* species showing qualitative variation among the external and cranial characteristics analyzed*.

	H. chacarius	***H. sciureus***	***H. brasiliensis***
General size	Smaller	Smaller to larger	Larger
Dorsal and lateral color	Light orange-brown, with light orangish lateral line	Tawny orange-brown, orangish wash of lateral line continuous from dorsum to venter	Orange-brow with dark patches of dark brown hairs mostly from back to rump, with orange lateral line gradually becoming paler toward the venter
Ventral color	Self-white, transition with dorsum well marked	Self-gray washed with orange, transition with dorsum not marked	Self-white or discretely gray based with white tips (except in trout and inguinal areas which are always self-white) washed with light-orange, transition with dorsum slightly marked
	Variation: sometimes with a light-gray base tipped with white stripe restricted to thorax and abdomen	*Variation:* sometimes without/ or only faintly orange wash. (mostly in juveniles).	*Variation:* sometimes light-orange wash very discrete
Ridges of the masseteric crest	Confluent at the level (or slightly above) of the mental foramen and at anterior part of m1	Confluent below the level of the mental foramen level and the middle part of m1	Confluent below the level of the mental foramen level and the posterior part of m1
Molar occlusal design	Main cusps alternating	Main cusps alternating	Main cusps essentially opposite
Form of lophids	Compressed and with acute outer margins, rarely prismatic	Compressed and with acute outer margins frequently prismatic	Not compressed, rounded outer margins
Position of the anteromedian fossetid	Labially displaced	Labially displaced	Central
Form and size of the anteromedian fossetid	Subcircular, small to medium	Transversally elongated, small to medium	Subcircular, medium to large
Occlusal development of metaflexid	Less developed, frequently not reaching the midline of the tooth	Well developed, reaching the midline of the tooth	Well developed, reaching the midline of the tooth
Form of the area in proto- and hipoconid	Subrectangular in outline	Subrectangular in outline	Subtriangular in outline
Orientation of proto- and hypoconid posterior faces	Transverse	Transverse	Typically 45°
Orientation of mesoflexid	Transverse	Transverse	Oblique
Mesoloph	Absent	Absent	Typically present, sometimes vestigial

* Modified from Voss & Carleton (1993), Voglino *et al.* (2004) and Pardiñas & Teta (2011).

H. sciureus specimens from semi-arid Caatinga areas, where xeric vegetation is much abrasive. Therefore, it is very hard to identify specimens from *sciureus* group to specific level based only on the form of the anteromedian fossetid or lophids. For that reason, although we include these characters at Table 6, they should be used with caution and never alone because we did not observed a distinctive difference between *H. sciureus* and *H. chacarius* that could not be frequently attributed to morphological variation for each species or if the phenotype observed is just related to tooth wear. Additional specimens referring to younger individuals with less degree of tooth wear, would probably solve these issues in the future.

According to Voss & Carleton (1993) another useful character is the point of confluence between the upper and lower ridges of the masseteric crest at the mandible, which is different among *Holochilus* species. However, no comparisons between the three species studied here are yet available. We observed that while in *H. chacarius* this point is horizontally at the level (or slightly above) of the mental foramen and vertically aligned to approximately the anterior part of m1, in *H. sciureus* and *H. brasiliensis* this point is below the level of the mental foramen level and aligned to the middle and posterior part of m1 respectively (Fig. 7).

Regarding pelage morphology, we realized that comparisons have been little explored to distinguish *Holochilus* species. In the present study, pelage morphology proved to posses important characters and, for most cases, species distinctiveness could be done

TABLE 7: Ranks of M1 in hemi-mandibles of *Holochilus* spp. by types of: form and size of the anteromedian fossetid (A) and occlusal development of metaflexid (B). A – fig. 6b; A' – fig. 6d; A" – fig. 7d. B – fig. 7b; B' – fig. 6b; B" – fig. 6d.

Táxon	A	A'	A"	Total (a)	B	B'	B"	Total (b)
H. chacarius	18	18	0	36	6	20	9	35
H. sciureus	9	13	0	17	3	1	17	21
H. brasiliensis	4	4	10	18	4	1	14	19

even in the field (Table 6). We observed that *Holochilus* species present orangish-brown dorsal pelage that differs in tone coloration between species and seems to be somewhat gradually darker as follows: *H. chacarius, H. brasiliensis* and *H. sciureus* (Fig. 1A, C and E). In *H. chacarius,* the dorsal pelage color tends to be more homogenous, while *H. sciureus* seems to have a slightly darker stripe along the midline of the dorsum and *H. brasiliensis* is somewhat streaked with darker hairs, except for the head area. Another useful difference is the presence of a lateral stripe, which shows hairs with gray basis and orangish-brow tip that also seems to present gradual intensity as follows: *H. chacarius, H. brasiliensis* and *H. sciureus* (Fig. 1B, D and F). In *H. chacarius,* this stripe is only slightly perceptible, while *H. sciureus* present a more notable orange stripe that is somewhat continuous with the ventral pelage and *H. brasiliensis* also presents a distinct lateral stripe but that gradually seems to became paler towards the venter.

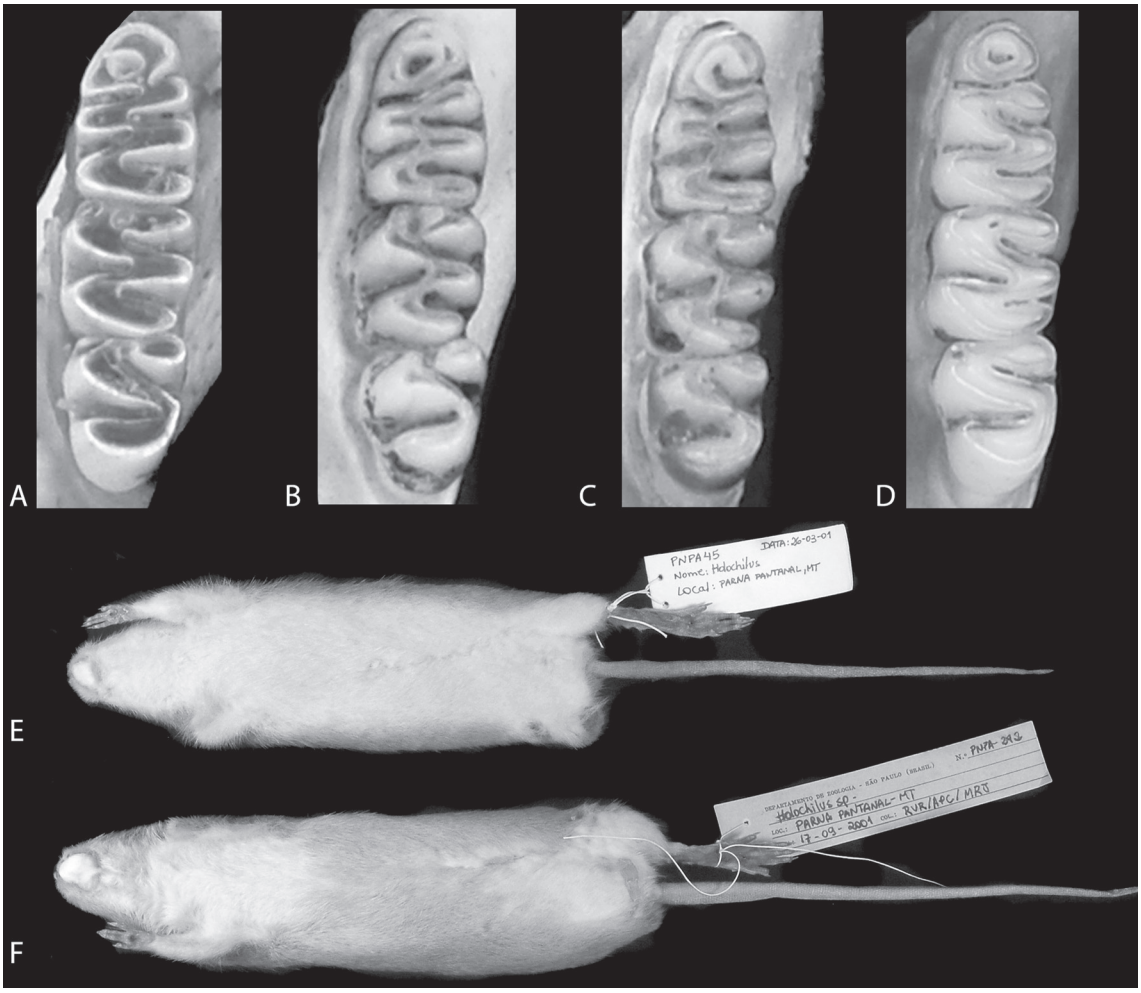

FIGURE 6: Lower molars of *Holochilus* spp. **A:** *H. chacarius* (MZUSP 21543). **B:** *H. sciureus* (MZUSP 35145). **C:** *H. sciureus* (MZUSP 35270). **D:** *H. brasiliensis* (MZUSP 10087). Ventral view of skin of *H. chacarius:* **E:** MZUSP 35145 and **F:** MZUSP 35142. Pictures are out of scale to didactic finalities.

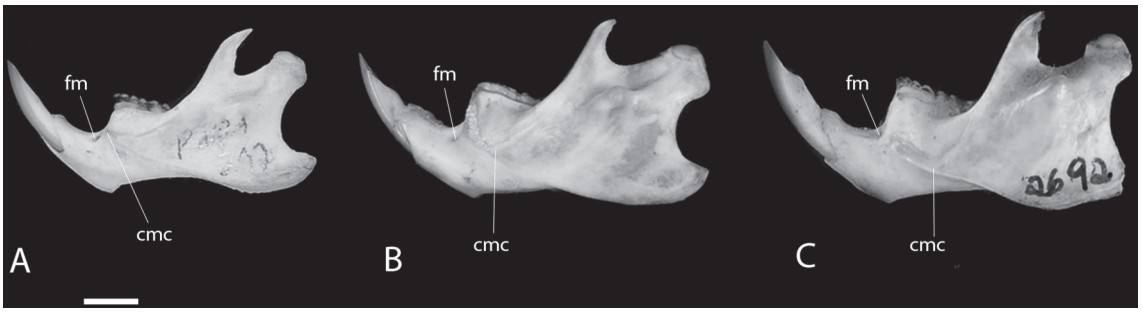

FIGURE 7: Lateral view of the mandible of *Holochilus* spp. **A:** *H. chacarius* (MZUSP 35145). **B:** *H. sciureus* (MZUSP 35269). **C:** *H. brasiliensis* (MZUSP 2692). Scale bar: 10 mm.

The most distinctive pelage character is the ventral coloration, which differs conspicuously among these species. While *H. sciureus* present grayish ventral pelage, *H. chacarius* and *H. brasiliensis* present whitish venters (Fig. 1B, D and F). Yet, in *H. chacarius* the orangish tones of ventral pelage are none existent and distinction between dorsum and venter is unambiguously perceptible, whereas in *H. brasiliensis* and *H. sciureus* the orange tones are constantly present and somewhat continuous toward the venter. However, in *H. sciureus,* this is notably much more distinct and the orange wash is continuous from lateral to venter, making transition between these areas almost unnoticeable. Regarding to *H. brasiliensis,* the orangish wash is much more discrete (light-orange), been frequently more evident at the shoulder region or faintly spread at the thorax and abdominal region (see Fig. 1D), toward a whitish venter. All mentioned pelage characters, as well as the cranial characters, are summarized in Table 6.

Some variation in ventral pelage coloration has been noticed among each species. In *H. sciureus* the orange wash was sometimes absent, but seems to be related only to age since most of these specimens were young, except for a single adult specimen (MN 3359). It is worth mentioning that specimens of *H. sciureus* from Bahia presented general pelage much brighter than typical *H. sciureus,* specially at the ventral pelage (MN 4147-48, 4166-67, 4171-72, 4175-76, 4178-80, 4202, 8255, 8338-39, 67401). However, smaller size and dental characters unambiguously classify them as *H. sciureus.* As related to *H. chacarius,* a few specimens from Mato Grosso present a light gray-based with white tipped hair associated to region between members (MZUSP 15336, 21216-17, 35145). However, the observed variation seems to have no taxonomic meaning since these specimens do not differ in any other aspect from typical *H. chacarius* from the same localities (Fig. 6E and F). Finally, the specimen from Cocalinho present a much vivid orange tone at the lateral area, but venter coloration and skull characters evidently classify it as *H. chacarius.*

The most remarkable variation in ventral morphology is related to *H. brasiliensis* from southeast areas (MZUSP 2788, 3179-90, 6366, 6542, 11213-14, 11226, 21219), whom presented whitish venter (with gray-based white-orangish tip fur) but the self-white fur was restricted to throat and inguinal areas. We suggest that this could prove to have taxonomic meaning in future analyses – see comparisons between *H. brasiliensis* subspecies (Massoia, 1976) – as they differ from specimens from southern localities (MZUSP 1027, 3179-80, 10083, 10087) which all present self-white ventral fur or, if gray-based, only ¼ of total hair length and only perceptible if pressed backwards. However, we only had few specimens available from areas that are crucial to resolve this issue and our major concern was to report *H. chacarius* for Brazil and compare it to species of *Holochilus* currently recognized by Musser & Carleton (2005).

It is worth mentioning that the reports of *H. sciureus* made by Bezerra *et al.* (2009) and by Rocha *et al.* (2011a) for Ilha do Bananal, Tocantins, should be reviewed for the reasons explained next. Both mentioned studies provided a detailed morphological description of the *Holochilus* specimens collected at Ilha do Bananal, mentioning a small body sized species that present whitish venter. Therefore, we hypothesized that these records could represent *H. chacarius.* We examined pictures of three specimens reported by Rocha *et al.* (2011a) and stated a high degree of morphological variation, which found no rematch with any other single locality analyzed by us or even in all the distribution range of *sciureus* species group. As we did not examine personally the skull of these specimens, we were not able to provide a more precise diagnostic; therefore, we could not attribute those specimens to any *Holochilus* species. However, these specimens clearly are from *sciureus* species group judging from body size (Rocha *et al.,* 2011a: 14) and could represent a contact area between *H. chacarius* and *H. sciureus,* representing a sympatric locality or

even hybrid individuals. Molecular and karyotipical data are essential to elucidate this issue.

Finally, it is essential to mention that *Holochilus lagigliai* was recently described for the west-central Argentina (Pardiñas *et al.,* 2013). This species was described based on a single specimen with skin and skull, and three additional right mandibles. In our present view, characters of the skull mentioned in the original description of this species are quite different from all specimens analyzed by us. Some characters of *H. lagigliai* draw attention to us, such as the broad braincase, short and broad rostrum, and dental morphology, which present mesoloph on upper molars (similar to *H. brasiliensis*), anteromedial fossetid laterally displaced (similar to *H. sciureus* group) and absence of hypoflexus and hypocone on third lower molar (exclusive of *H. lagigliai*). Yet, *H. lagigliai* locality is quite far away from the distribution range of *H. chacarius* and the other congeners. Therefore, although we consider a valid species, we did not compare *H. lagigliai* to the *Holochilus* specimens from Brazil because it seems very unlike that the former species might be present in this country and its morphological variation are yet poorly known.

We considered that the mentioned specimens from Araguaia, Tocantins, could represent *H. lagigliai.* However, we rejected this hypothesis based on body and cranial dimensions of those specimens (see Rocha *et al.,* 2011a: 14) compared to *H. lagigliai* (see Pardiñas *et al.,* 2013: 232), absence of mesoloph (see Rocha *et al.,* 2011a: 14), and presence of hypoflexus and hypocone on third lower molar (see Rocha *et al.,* 2011a: fig. 5).

Geographical distribution

The most recent contribution to the knowledge of the geographical distribution of this genus is provided by Prado & Percequillo (2013). Fortunately, these authors were very cautious while reporting records of *Holochilus* since no recent published revision of the genus exists. Their records were based only in museum databases and Hershkovitz (1955), without assigning specimens to any species in order to – at least to our view of Prado & Percequillo (2013) article – not cause further error of taxa assignments. However, despite Prado & Percequillo (2013) contribution, records of *Holochilus* in Brazil remain poorly discussed. Herein we provided a new map based on the analyses of the Brazilian *Holochilus* specimens studied by us (Fig. 8).

As previously noted, *H. chacarius* had already been mentioned for Brazil but no museum catalogued number has been provided. The only exception is Cáceres *et al.* (2008) which reported *H. chacarius* and *H. sciureus* for Mato Grosso do Sul state. However, the vouchers are only referent to *H. sciureus* while *H. chacarius* is only mentioned as a reference to Oliveira & Bonvicino (2006). These last authors report *H. chacarius* to Mato Grosso do Sul state but no detailed information on specimens is presented. Also, we analyzed specimens MN 1987, MZUSP 3780 and MZUSP 27430 reported as *H. sciureus* by Cáceres *et al.* (2008) and re-identified them as *H. chacarius;* therefore, the presence of *H. sciureus* was mistakenly attributed to Mato Grosso do Sul. It is worth to mention that Allen (1916) had already mentioned two species of *Holochilus* collected in the Roosevelt Expedition in Brazil: *H. amazonicus* (= *H. sciureus*) cited for Rio Solimões, Amazonas, and *H. vulpinus* from Urucúm, Mato Grosso do Sul, which probably refers to *H. chacarius* judgment by external measurements (see Allen, 1916: 571) and locality (MN 1987 and 1988 are *H. chacarius* – Present study), even though *vulpinus* is currently junior synonym of *H. brasiliensis* (Musser & Carleton, 2005). Recent list of mammals species from Brazil that include rodents, report the presence of *H. chacarius* exclusively for Mato do Grosso Sul state (*e.g.,* Bonvicino *et al.,* 2008; Oliveira & Bonvicino, 2006, 2011). Here we report that this species is also present at Mato Grosso state.

Sympatry records in Brazil are nonexistent for *Holochilus* spp. However, this should only be a matter of time before new inventories efforts are made in localities where these species nearly contact each other (Fig. 8).

Although sympatry between *H. sciureus* and *H. chacarius* have not yet been reported, it is very likely that these species distribution contact each other in central Bolivia (see Pardiñas *et al.,* 2013) and in center-west of Brazil (Fig. 8) – Mato Grosso/Pará limits (northeast and southwest limits of *H. chacarius* and *H. sciureus* distribution in Brazil respectively) and Mato Grosso/Mato Grosso do Sul/Goiás limits (east and southeast limits of *H. chacarius* and *H. sciureus* distribution in Brazil respectively). As mentioned earlier, the northernmost previously known record with museum catalogued specimens of *H. chacarius* was made for the center/south of Bolivia by Anderson (1997) – reported as *H. sciureus* but latter some specimens was reidentified by Pardiñas & Galliari (1998) – where also *H. sciureus* might be present. For that historical confusion, all records from Anderson (1997) should be reviewed.

Sympatric records between *H. chacarius* and *H. brasiliensis* in Argentina have been mentioned (Voglino *et al.,* 2004). However, since the distribu-

tion areas are somewhat far from each other in Brazil (Fig. 8), this is very unlikely to occur in this country or it is only restricted to a small area. On the other hand, *H. sciureus* and *H. brasiliensis* are very likely to occur simpatricaly since these species were reported here in very near localities in Minas Gerais. Another possible area of occurrence might be Bahia, where it is present the northern limit of *H. brasiliensis* distribution at Caravelas (17°45'S, 39°15'W), as previously noted by Massoia (1981), and the southeast limit of *H. sciureus* distribution at Jaguaquara (13°32'S, 39°58'W). These observations highlight the fact that only *H. sciureus* is known from Caatinga biome in northeastern Brazil, since we found no specimen of *H. brasiliensis* and morphological descriptions in literature (see qualitative analyses above) that could be assigned to this species.

Considering the previous Bolivian record from Beni (see Anderson, 1997), the present record not only provides a remarkable extension of 1,500 km toward the east of the distribution range of *H. chacarius,* but also represent an important record on a biogeographical standpoint. As expected, considering the distribution map observed in Bonvicino *et al.*

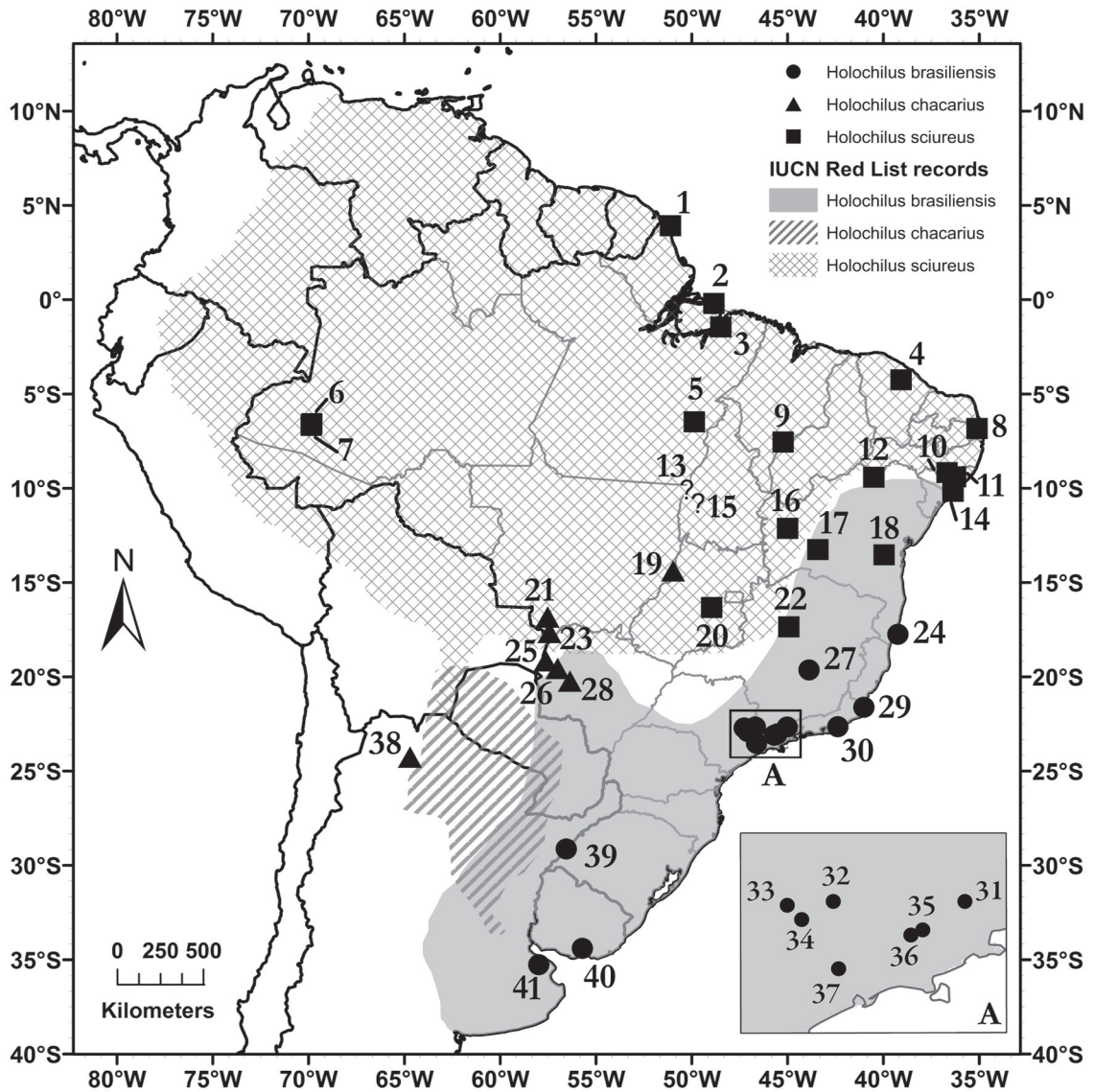

FIGURE 8: Map of the specimens of *Holochilus* spp. studied. Numbered points correspond to museums records of *H. brasiliensis* (**black circles**), *H. chacarius* (**black triangles**) and *H. sciureus* (**black squares**) listed in the gazetter (see Appendix II). The box **A** shows in details the museum records of the specimens from southeastern Brazil. The shades of gray are the geographic distribution of the *Holochillus* spp. suggested by the IUCN Red List (2013). **?:** Records from literature (Bezerra *et al.* 2009, Rocha *et al.,* 2011a, b) analyzed in the present work only by photos of skin.

(2008: 34), we report here the presence of this species to the Pantanal area of Brazil, but also the first record for the Cerrado. However, some consideration must be done regarding the presence of this species in the mentioned vegetation formations.

The record of *H. chacarius* in Cerrado is not totally unexpected, since the diversity of this biome have grown in the last few years, raising from 100 species (Redford & Fonseca, 1986) to 227 (Carmignotto *et al.,* 2012). In addition, *H. chacarius* is a semi-aquatic species that feed mainly on riverine vegetation (mostly grass); consequently its presence might be mostly related to the presence of water bodies and phytophysiognomies suitable to the species preferences, than to a determined biome itself. It is important to mention that Carmignotto *et al.* (2012) already considered this species as component of Cerrado fauna, though this decision was made based on their criteria that Pantanal and Cerrado represent a single zoogeographical unit.

Although it is evident that the new record of *H. chacarius* provided here much further into the Cerrado of central Brazil is quite relevant, it is also important to mention that all the records we include here to *H. chacarius* in Brazil are areas seasonally susceptive to floods, including the new record from Cocalinho (Carmignotto *pers. com.*). This locality resembles swampy areas from Pantanal, which is known as the biggest flooded area in South America and where *H. chacarius* has already been reported. Taking this fact into perspective, this data is consistent to Carmignotto *et al.* (2012) criteria, which treated the Pantanal and Cerrado as a single zoogeographical unit and mention a high number of species (25) shared between Cerrado and Chaco, where *H. chacarius* were already known to occur.

After all we discussed above, although the IUCN (2013) category of threatened species should not change with the present work, we propose some corrections on the distribution map of *Holochilus* spp. provided by the mentioned institution, because the current map reflect the historical errors of identity of the specimens of this genus (Fig. 8). Furthermore, the question mark indicates those *Holochilus* specimens from Ilha do Bananal, Tocantins, which presents uncertain identities since we could not evaluate properly with only pelage pictures of the specimens reported by Rocha *et al.* (2011a). This locality might represent a contact area between *H. chacarius* and *H. sciureus,* which could bring valuable genetic and morphological information to this genus.

According to our results, all literature records for *Holochilus* from Pantanal region should be recognized as *H. chacarius.* We found no evidence for the presence of another species of *Holochilus* in this biome and no other species are yet known for Mato Grosso state besides *H. chacarius,* although we considered that the presence of *H. sciureus* should be only a matter of time. Finally, since Bonvicino *et al.* (2008) and Paglia *et al.* (2012) report *H. vulpinus* for the Pampas area in Brazil, we believe that the validity of *vulpinus* is current under evaluation and this species should prove to be considered taxonomically valid in a near future, adding another species to Pampas biodiversity.

CONCLUSIONS

Several names as *amazonicus, balnearum, berbicensis, darwini, guianae, incarum, nanus, venezuelae* and *vulpinus* (see Hershkovitz, 1955: fig. 140; Musser & Carleton, 2005) are yet to be consistently evaluated and, as discussed above, species limits in *Holochilus* are poorly understood. It is expected that the geographic ranges of the currently recognized species to be somewhat smaller, since its seems likely that all current species of *Holochilus* are complex of two or more cryptic species, as already indicated by a highly variable karyotypes (see Gardner & Patton, 1976). However, specimens are still scarce for several areas of the broad distribution of *Holochilus* species, causing uncertainty of correspondence of karyotypical variants to definable morphologies, therefore it is difficult to determine from morphological characters with only the current material available. For that reason, the recognition of the current *Holochilus* species is only indicative rather than a precise diagnostic for the genus diversity since morphological cryptic species could be present.

As evidenced by the data provided here, *Holochilus* species inhabits more than one biome, consequently it puts in perspective that our knowledge on its species distribution is limited and the identification of specimens should be based mainly on diagnostic characters and not on geographic ranges, as seemed to be the case for the small forms of *Holochilus* in Brazil. As a result, many mistakes concerning the identification of *Holochilus* specimens were made in literature; therefore, a taxonomic revision for this genus is urgently needed.

RESUMO

Quatro espécies de Holochilus *(Cricetidae: Sigmodontinae) são atualmente reconhecidas. De acordo com*

a literatura, três espécies são registradas para o Brasil: H. brasiliensis, H. chacarius *e* H. sciureus. *Amostras provenientes do oeste do Brasil (Estados do Mato Grosso e Mato Grosso do Sul) são geralmente reportadas como* H. sciureus *e, quando referentes a espécimes do Pantanal, como* H. chacarius. *Entretanto, o estado taxonômico de espécimes dessa região ainda não foi propriamente avaliado através de análise morfológica detalhada e/ou espécimes testemunho. Cerca de 110 espécimes de* Holochilus *depositados em coleções brasileiras foram analisados qualitativa e quantitativamente (testes univariados e multivariados). Nossos resultados suportam a existência de três espécies (*H. sciureus, H. brasiliensis *e* H. chacarius*) no país, as quais possuem diferenças morfológicas e morfométricas significativas entre si, assim confirmando que as amostras do Mato Grosso e Mato Grosso do Sul, outrora erroneamente identificados como* H. sciureus, *pertencem à* H. chacarius. *Esta espécie difere de* H. sciureus *e* H. brasiliensis *por apresentar uma série de características da pelagem e do crânio, tais como: coloração da pelagem castanho claro no dorso, levemente alaranjada nas laterais e branca no ventre; crista massetérica confluente ao nível ou ligeiramente acima do forame mental e na parte anterior do m1; principais cúspides molares alternadas; lofideos compactados e com margens externas agudas (raramente prismática), fossetideo anteromediano labialmente deslocado e subcircular (pequeno a médio); metaflexideo menos desenvolvido (muitas vezes não atingindo a linha mediana do dente); protoconideo e hipoconideo subrectangulares e transversalmente orientados; mesoflexideo transversalmente orientado e mesolofo ausente. A distribuição geográfica atualizada de* Holochilus *no Brasil indica que* H. sciureus *está presente na porção norte do país, sendo a única espécie presente na Amazônia e na Caatinga e seu limite no Cerrado ocorre no centro de Goiás.* Holochilus brasiliensis *está restrito ao leste do Brasil, sendo seu limite norte na Mata Atlântica do sul da Bahia.* Holochilus chacarius *está presente nas áreas do Pantanal do Mato Grosso e Mato Grosso do Sul. Um novo registro estende a distribuição de* H. chacarius *para a porção de Cerrado no leste do Mato Grosso, comprovando sua presença neste bioma.*

Palavras-Chaves: *Holochilus chacarius; Holochilus sciureus; Holochilus brasiliensis;* Mato Grosso; Brasil.

ACKNOWLEDGMENTS

The authors would like to thank the curators Dr. Yuri R. Leite (UFES), Dr. Mario de Vivo (MZUSP), Dr. João Alves de Oliveira and Dr. Luiz Flamarion de Oliveira (MN) and museum staff Juliana Gualda-Barros (MZUSP) and Stela Franco (MN) for the access to the specimens of the mammal collection under their care. We are also grateful for MSc Joyce Rodrigues do Prado (ESALQ-USP) and MSc Guilherme Terra Garbino (MZUSP) for valuables comments and suggestion on manuscript. We also like to give a special thanks to Dione Seripierri, Marta Lucia Zamana and Cláudia M. Battagia for valuable effort in obtaining some references needed to the conception of this manuscript, and to Jeronymo Dalapicolla for kindly sending pictures of specimens from UFES. Part of this work was supported through grants from Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP 2010/03969-4) fellowship.

REFERENCES

Allen, J.A. 1916. Mammals collected on the Roosevelt Brazilian Expedition, with field notes by Leo E. Miller. *Bulletin of the American Museum of Natural History,* 35: 556-610.

Anderson, S. 1997. Mammals of Bolivia, taxonomy and distribution. *Bulletin of the American Museum of Natural History,* 231: 1-652.

Bezerra, A.M.R.; Carmignotto; A.P. & Rodrigues, F.H.G. 2009. Small non-volant mammals of an ecotone region between the Cerrado hotspot and Amazonian Rainforest, with comments on their taxonomy and distribution. *Zoological Studies,* 48: 861-874.

Bonvicino, C.R.; Oliveira, J.A. & D'Andrea, P.S. 2008. *Guia dos Roedores do Brasil, com chaves para gêneros baseadas em caracteres externos.* Rio de Janeiro, Centro Pan-Americano de Febre Aftosa – OPAS/OMS. 120 p.

Cáceres, N.C.; Carmignotto, A.P.; Fischer, E.A. & Santos, C.F. 2008. Mammals from Mato Grosso do Sul state, Brasil. *Check List,* 4: 321-335.

Cáceres, N.C.; Godoi, M.N.; Hannibal, W. & Ferreira, V.L. 2011. Effects of altitude and vegetation on small-mammal distribution in the Urucum Mountains, western Brazil. *Journal of Tropical Ecology,* 27: 279-287.

Carleton, M.D. & Olson, S.L. 1999. Americo Vespucci and the rat of Fernando de Noronha: a new genus and species of Rodentia (Muridae: Sigmodontinae) from a volcanic island off Brazil's continental shelf. *American Museum Novitates,* 3256: 1-59.

Carmignotto, A.P. 2005. *Pequenos mamíferos terrestres do bioma Cerrado: padrões faunísticos locais e regionais.* Ph.D. Thesis. São Paulo, Universidade de São Paulo.

Carmignotto, A.P.; Vivo, M. de & Langguth, A. 2012. Mammals of the Cerrado and Caatinga: distribution patterns of the tropical open biomes of Central South America. *In:* Patterson, B.D. & Costa, L.P. (Eds.). *Bones, clones, and biomes: the history and geography of recent Neotropical mammals.* Chicago, University of Chicago Press. p. 307-350.

Gardner, A.L. & Patton, J.L. 1976. Karyotypic variation in oryzomyni rodents (Cricetidae) with comments on chromosomal evolution in the neotropical cricetinae complex. *Louisiana State University Publications of the Museum of Zoology,* 49: 1-48.

Hershkovitz, P. 1955. South American marsh rats, genus *Holochilus,* with a summary of sigmodont rodents. *Fieldiana Zoology,* 37: 639-673.

IUCN – International Union for Conservation of Nature. 2013. *The IUCN Red List of Threatened Species.* Version 2013.2. www.iucnredlist.org. Downloaded on 21 November 2013.

Jolicoeur, P. & Mosimann, J.E. 1960. Size and shape variation in the painted turtle. A principal component analysis. *Growth,* 24: 339-354.

Machado, L.F.; Leite, Y.L.R.; Christoff, A.U. & Giugliano, L.G. 2013. Phylogeny and biogeography of tetralophodont rodents of the tribe Oryzomyini (Cricetidae: Sigmodontinae). *Zoological Scripta,* 43(2): 119-130.

Marques, R.V. 1988. O gênero *Holochilus* (Mammalia: Cricetidae) no Rio Grande do Sul: taxonomia e distribuição. *Revista Brasileira de Zoologia,* 4: 347-360.

Massoia, E. 1971. Caracteres y rasgos bioecológicos de *Holochilus brasiliensis chacarius* Thomas ("rata nutria") de la provincia de Formosa y comparaciones con *Holochilus brasiliensis vulpinus* (Brants) (Mammalia, Rodentia, Cricetidae). *Revista de Investigaciones Agropecuarias, INTA, Serie 1, Biología y Producción Animal,* 8: 13-40.

Massoia, E. 1976. Mammalia. *In:* Ringuelet, R. (Ed.). *Fauna de agua Dulce de la República Argentina.* Buenos Aires, Fundación Editorial Ciencia y Cultura. p. 1-128.

Massoia, E. 1980. El estado sistematico de cuatro espécies de cricetidos sudamericanos y comentários sobre otras especies congenrencas (Mammalia, Rodentia). *Ameghiniana,* 17: 280-287.

Massoia, E. 1981. El estado sistemático y zoogeografía de *Mus brasiliensis* Desmarest y *Holochilus sciureus* Wagner (Mammalia, Rodentia, Cricetidae). *Physis, Sección C,* 39: 31-34.

Moojen, J. 1952. *Os roedores do Brasil.* Rio de Janeiro, Instituto Nacional do Livro. 214 p. (Biblioteca Científica Brasileira, ser. A).

Musser, G.M. & Carleton, M.D. 2005. Superfamily Muroidea. *In:* Wilson, D.E. & Reeder, D.M. (Eds.). *Mammal Species of the World, Third Edition.* Baltimore, The Johns Hopkins University Press. p. 894-1531.

Oliveira, F.F. & Langguth, A. 2004. Pequenos Mamíferos (Didelphimorphia e Rodentia) de Paraíba e Pernambuco, Brasil. *Revista Nordestina de Biologia,* 18(2): 19-86.

Oliveira, J.A. & Bonvicino, C.R. 2006. Ordem Rodentia. *In:* Reis, N.R.; Peracchi, A.L.; Pedro, W.A. & Lima, I.P. (Eds.). *Mamíferos do Brasil.* Londrina, Pr., Universidade Estadual de Londrina. p. 347-406.

Oliveira, J.A.; Bonvicino, C.R. 2011. Ordem Rodentia. *In:* Reis, N.R.; Peracchi, A.L.; Pedro, W.A. & Lima, I.P. (Eds.). *Mamíferos do Brasil.* 2. ed. Londrina, Pr. ,Universidade Estadual de Londrina. p. 358-414.

Paglia, A.P.; Fonseca, G.A.B.; Rylands, A.B.; Herrmann, G.; Aguiar, L.M S.; Chiarello, A.G.; Leite, Y.L.R.; Costa, L.P.; Siciliano, S.; Kierulff, M.C.M.; Mendes, S.L.; Tavares, V.C.; Mittermeier, R.A. & Patton, J.L. 2012. Annotated Checklist of Brazilian Mammals. 2.ed. *Occasional Papers in Conservation Biology,* 6: 1-76.

Pardiñas, U.F.J. & Galliari, C.A. 1998. Sigmodontinos (Rodentia, Muridae) del Holoceno inferior de Bolivia. *Revista Española de Paleontología,* 13: 17-25.

Pardiñas, U.F.J. & Teta, P. 2011. Fossil history of the marsh rats of the genus *Holochilus* and *Lundomys. Estudios Geológicos,* 67(1): 111-129.

Pardiñas, U.F.J.; Teta, P.; Voglino, D. & Fernández, F.J. 2013. Enlarging rodent diversity in west-central Argentina: a new species of the genus *Holochilus* (Cricetidae, Sigmodontinae). *Journal of Mammalogy,* 94(1): 231-240.

Patton, J.L.; da Silva, M.N.F. & Malcolm, J.R. 2000. Mammals of the Rio Juruá and the evolutionary and ecological diversification of Amazonia. *Bulletin of the American Museum of Natural History,* 244: 1-306.

Paynter Jr., R.A. 1994. *Ornithological Gazetteer of Uruguay.* 2. ed. Massachusetts, Harvard University. 111 p.

Paynter Jr., R.A. 1995. *Ornithological Gazetteer of Argentina.* 2. ed. Massachusetts, Harvard University. 1045 p.

Paynter Jr., R.A. & Traylor Jr., M.A. 1991. *Ornithological Gazetteer of Brazil.* Massachusetts, Harvard University. 2 v.

Prado, J.R. & Percequillo, A.R. 2013. Geographic distribution of the genera of the Tribe Oryzomyini (Rodentia: Cricetidae: Sigmodontinae) in South America: patterns of distribution and diversity. *Arquivos de Zoologia,* São Paulo, 44(1): 1-120.

Redford, K.H. & Fonseca, G.A.B. da. 1986. "The role of gallery forests in the zoogeography of the Cerrado's non-volant mammalian fauna". *Biotropica,* 18: 126-35.

Rocha, R.G.; Ferreira; E.M.S.L.A.; Costa, B.A.; Martins, I.; Leite, Y.L.R.; Costa, L.P. & Fonseca, C.M. 2011a. Small mammals of the mid-Araguaia River in central Brazil, with the description of a new species of climbing rat, genus *Rhipidomys* (Rodentia: Sigmodontinae). *Zootaxa,* 2789: 1-34.

Rocha, R.G.; Ferreira, E.M.S.L.A.; Leite, Y.L.R.; Fonseca, C.M. & Costa, L.P. 2011b. Small mammals in the diet of Barn owls, *Tyto alba* (Aves: Strigiformes) along the mid-Araguaia River in central Brazil. *Zoologia,* Curitiba, 28(6): 709-716.

Voglino, D.; Pardiñas, U.F.J. & Teta, P. 2004. *Holochilus chacarius chacarius* (Rodentia, Cricetidae) en la provincia de Buenos Aires, Argentina. *Mastozoología Neotropical,* 11: 243-247.

Voss, R.S. & Carleton, M.D. 1993. A new genus for *Hesperomys molitor* Winge and *Holochilus magnus* Hershkovitz, with comments on phylogenetic relationships and oryzomyine monophyly. *American Museum Novitates,* 3085: 1-39.

Voss, R.S.; Lunde, D.P. & Simmons, N.B. 2001. The mammals of Paracou, French Guiana: A neotropical lowland rainforest fauna part 2. Nonvolant species. *Bulletin of the American Museum of Natural History,* 263: 1-236.

Weksler, M. 2006. Phylogenetic relationships of oryzomine rodents (Muroidea: Sigmodontinae): separate and combined analyses of morphological and molecular data. *Bulletin of the American Museum of Natural History,* 296: 1-149.

Aceito em: 05/10/2014
Impresso em: 31/03/2015

Publicado com o apoio financeiro do Programa de Apoio às Publicações Científicas Periódicas da USP

APPENDIX I

Specimens examined of *Holochilus* spp. – Collecting localities of the specimens are numbered from North to south, and numbers in bold correspond to numbered localities on the map (Fig. 1). Countries and states are listed in bold uppercase letters, followed by municipalities in bold, specific localities, latitude and longitude, and elevation (if available). Museum numbers are listed in parentheses and in brackets, respectively.

Holochilus brasiliensis

Brazil: MZUSP: **São Paulo:** *Taubaté (Bairro Barranco):* 11213; 11214; 11215; 11222; *Campinas:* 11226. *Cachoeira:* 2788. *São Paulo:* 824. *Monte Alegre:* 6542. **Rio de Janeiro:** *Atafona:* 21219. **Bahia:** *Caravelas:* 2692. **Rio Grande do Sul:** *Itaqui:* 3179; 3180.

Brazil: MN: **Minas Gerais:** *Lagoa Santa (Fazenda das Bicas):* 4096; 4097; **Rio de Janeiro:** *Imbaui, Silva Jardim:* 61802. **São Paulo:** *Taubaté (Gleba Paulo Japones, Bairro Barranco):* 67124; 67125; 67126; 67127; *Taubaté (Fazenda Kangutii, Bairro Remedio):* 24175; *Taubaté (Fazenda Antonio Tavares):* 24170; *São Paulo:* 67200; 67201; 67203; 67204; 67206; 67208; 67209; *Caçapava (Gleba José Nanié, Bairro Santa Luzia):* 24174; 67106; 67107; 67108. *Fazenda Santo Angelo, represa de Americana:* 24177.

Argentina: MZUSP: **Buenos Aires:** *La Plata:* 1027; *Buenos Aires:* 10087.

Uruguay: MZUSP: **Canelones:** *Banãdo Tropas Viejas:* 10083.

Holochilus chacarius

Brazil: MZUSP: **Mato Grosso do Sul:** *Passo da Lontra:* 27340; *Miranda:* 3780; **Mato Grosso:** *Ilha de Taiamã:* 13462; 13463; 19536; 19537; 19538; 19539; 19540; 21215; 21216; 21217; 21218; 24885; 25743; 25744; 25754; 26717. *Cocalinho:* 35143; Parque *Nacional do Pantanal:* 35142, 35144; 35145.

Brazil: MN: **Mato Grosso do Sul:** *F. Ucurum, Corumbá:* 1987; 1988.

Argentina, Jujuy: *Villa Carolina:* 21543.

Holochilus sciureus

Brazil: MZUSP: **Pernambuco:** *Sitio Caimbinhas, Bom Conselho:* 21220; 25218. **Alagoas:** *Manimbu* 7539; 7540; 7545; 7546; 7547; 7548. *Viçosa:* 21198. **Paraíba:** *Mamamguape:* 8419. **Ceará:** *Serra de Baturité:* 8702; 8703; 8704; 8705; 8706; 8707; 8708. **Pará:** *Belém:* 21211; 21214. *Canaã dos Carajás:* 35270. *Igarapé Tapebá:* 8598. **Amazônia:** *Igarapé grande:* 4492. *Eirunepé:* 4500. **Amapá:** *Vila Velha do Cassiporé:* 21212; 21213. **Piauí:** *Ribeirão Gonçalves:* 35269.

Brazil: MN: **Goiás:** *Anápolis:* 3359; 4381; 4361; 4362; 4341; 34181. **Minas Gerais:** *Pirapora:* 4205; 4207; 4209. MN: **Bahia:** *Fazenda do Horto, Juazeiro:* 8338; 8339. *Barreiras:* 4202; 4166; 4167; 4171; 4172; 4175; 4176; 4178; 4179; 4180; 8255. *Bom Jesus da Lapa (Ilha do Medo):* 4147; 4148. *Fazenda Trancadal, Jaquaquaia:* 67014.

APPENDIX II

Gazetteer of *Holochilus* localities

A list of the sampled localities of genus *Holochilus* and their respective geographical coordinates are given below. Note that the numbers preceding collecting localities are the same presented in figures 8. For each locality we also provide the altitude, source for geographical coordinates, collector, collecting date, museum[s] or collection[s], and species obtained in each locality. The gazetteer is arranged in alphabetical order, by country, state or province, and collecting locality.

Brasil: **1.** Vila Velha do Cassiporé, Amapá [03°55'N, 51°07'W]; **2.** Igarapé Taperebá, Pará [00°12'S, 48°51'W]; **3.** Belém, Pará [01°27'S, 48°29'W]; **4.** Serra de Baturité, Ceará [04°15'S, 39°05'W]; **5.** Canaã dos Carajás, Pará [06°29'S, 49°52'W]; **6.** Igarapé Grande, Amazonas [06°35'S, 69°50'W]; **7.** Eirunepé, Amazonas [06°40'S, 69°52'W]; **8.** Mamanguape, Paraíba [06°50'S, 35°07'W]; **9.** Ribeiro Gonçalves, Piauí [07°33'S, 45°14'W]; **10.** Bom Conselho, Pernambuco [09°10'S, 36°41'W]; **11.** Viçosa, Alagoas [09°24'S, 36°14'W], **12.** Juazeiro, Bahia [09°25'S, 40°30'W]; **13.** Fazenda Lago Verde, Tocantins [09°56'S, 50°07'W]; **14.** Manimbu, Alagoas [10°10'S, 36°22'W]; **15.** Parque Estadual do Cantão, Tocantins [10°45'S, 49°42'W]; **16.** Barreiras, Bahia [12°08'S, 45°00'W]; **17.** Bom Jesus da Lapa (Ilha do Medo), Bahia [13°15'S, 43°25'W]; **18.** Jaguaquara, Bahia [13°32'S, 39°58'W]; **19.** Cocalinho, Mato Grosso [14°23'S, 50°59'W]; **20.** Anápolis, Goiás [16°20'S, 48°58'W]; **21.** Ilha de Taiamã, Mato Grosso [16°50'S, 57°32'W]; **22.** Pirapora, Minas Gerais [17°21'S, 44°56'W]; **23.** Parque Nacional do Pantanal, Mato Grosso [17°39'S, 57°26'W]; **24.** Caravelas, Bahia [17°45'S, 39°15'W]; **25.** Corumbá, Mato Grosso do Sul [19°09'S, 57°38'W]; **26.** Passo da Lontra, Mato Grosso do Sul [19°34'S, 57°01'W]; **27.** Lagoa Santa, Minas Gerais [19°38'S, 43°53'W]; **28.** Miranda, Mato Grosso do Sul [20°14'S, 56°22'W]; **29.** Atafona, Rio de Janeiro [21°37'S, 41°01'W]; **30.** Imbaui, Silva Jardim, Rio de Janeiro [22°39'S, 42°23'W]; **31.** Cachoeira, São Paulo [22°40'S, 45°01'W]; **32.** Monte Alegre, São Paulo [22°40'S, 46°41'W]; **33.** represa de Americana, São Paulo [22°43'S, 47°16'W]; **34.** Campinas, São Paulo [22°54'S, 47°05'W]; **35.** Taubaté, São Paulo [23°02'S, 45°33'W]; **36.** Caçapava, São Paulo [23°06'S, 45°42'W]; **37.** São Paulo, São Paulo [23°32'S, 46°37'W]; **39.** Itaqui, Rio Grande do Sul [29°08'S, 56°33'W].

Argentina: **38.** Villa Carolina, Jujuy [24°16'S, 64°43'W]. **41: Buenos Aires, La Plata** [35°55'S, 57°57'W].

Uruguai: **40:** Bañado Tropas Viejas, Atlántida, Canelones [34°43'S, 55°54'W].