ZOOLOGIA 32(5): 371–379, October 2015
http://dx.doi.org/10.1590/S1984-46702015000500006

Cytogenetics and DNA barcoding of the Round-eared bats, *Tonatia* (Chiroptera: Phyllostomidae): a new karyotype for *Tonatia bidens*

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ABSTRACT. There are two species of Neotropical Round-eared bats, *Tonatia bidens* Spix, 1823 and *Tonatia saurophila* Koopman & Williams, 1951, which present highly similar morphological characteristics that can lead to errors of identification. Specimens originally identified as *T. bidens* have recently been reclassified as *T. saurophila*, and the only karyotype documented previously for these species was 2n = 16, FN = 20. In the present study, specimens of *Tonatia* collected in the municipality of Barra do Garças, in the Brazilian state of Mato Grosso, were analyzed morphologically, using conventional cytogenetic techniques (C-banding, Ag-NOR, and CMA_3), and through sequences of the mitochondrial cytochrome c oxidase subunit I (COI) gene. In the specimens morphologically identified as *T. bidens*, the diploid number (2n) was 26, and the fundamental number (FN), 38, while in *T. saurophila*, 2n = 16 and FN = 20, which is the karyotype also described previously for *T. bidens*. The dendograms obtained with sequences of the COI marker resulted in the formation of two distinct groups between *T. bidens* and *T. saurophila*, consistent with the two species, with a high sequence divergence value (14.22%). Distinct clades were also observed between *T. bidens* and the other phyllostomines analyzed in this study, with *T. bidens* also close to *Phyllostomus hastatus* (14.18% of sequence divergence).

KEY WORDS. Chromosomes; COI gene; karyotypes; Phyllostominae; taxonomy.

The Phyllostomidae is endemic to the New World and one of the most morphologically diverse chiropteran family, with 203 known species arranged in 60 genera (Solari & Martinez-Arias 2014). The exact number of subfamilies remains unclear, with between seven and 11 according to the phylogenetic approach adopted. The molecular evidence suggests that some subfamilies, such as the Phyllostominae, are paraphyletic, a classification supported by the chromosomal data (Baker et al. 2003, Pieczarka et al. 2013, Ribas et al. 2015). However, the morphological evidence supports the classification in seven subfamilies, which includes a number of phyllostomine taxa that are not supported by the molecular evidence (Wetterer et al. 2000, Simmons 2005).

Baker et al. (2003) arranged the Phyllostominae in three tribes – Macrophyllini, which includes *Trachops* Gray, 1827and *Macrophyllum* Gray 1838; Phyllostomini composed by *Tonatia* Gray, 1847, *Lophostoma* d'Orbigny, 1836, *Mimon* Gray 1847, *Phyllostomus* Lacépède, 1799; and *Phylloderma* Peters, 1865, and Vampyrini, with two genera, *Chrotopterus* Peters, 1865 and *Vampyrum* Rafinesque, 1815. *Tonatia* originally included the species now assigned to *Lophostoma*, but is now composed of only two species, *Tonatia bidens* Spix, 1823 and *Tonatia saurophila* Koopman & Williams, 1951. This alteration was proposed by Lee-Jr et al. (2002) based on sequences of mitochondrial DNA, which revealed that *T. bidens* and *T. saurophila* formed a well-defined clade clearly separated from the other five species included originally in the genus, which did not correspond to a monophyletic group.

The type locality of the Greater round-eared bat, *Tonatia bidens*, is close to the São Francisco River in the Brazilian state of Bahia (Williams et al. 1995). The species is found throughout southern and central Brazil, ranging west and south into Bolivia, Paraguay, and northern Argentina (Williams et al. 1995, Paca et al. 2012, Reis et al. 2013). The type locality of the Stripe-headed round-eared bat, *T. saurophila*, is Wallingford Roadside Cave in Balaclava, St. Elizabeth Parish, Jamaica, although the species is known from this site only from fossils (Williams et al. 1995). This species ranges from southern Mexico (Chiapas)

through Belize, Colombia, Venezuela, Trinidad, the Guianas, Surinam, Ecuador, Peru, Bolivia, and Brazil, as far south as Argentina (Barquez & Diaz 2001, Clarke & Downie 2001, Simmons 2005, McDonough et al. 2011, Saavedra-Rodríguez & Rojas-Díaz 2011). In Brazil, *T. saurophila* is found predominantly in the north, with a limited distribution in the central and southern parts of the country (Reis et al. 2013).

Tonatia bidens and *T. saurophila* present highly similar morphological characteristics, which may lead to the erroneous identification of species. Williams et al. (1995) recognized specimens originally identified as *T. bidens* to be in fact *T. saurophila*. These authors emphasized the light-colored stripe on the top of the head of *T. bidens* as the most useful diagnostic feature for the differentiation of the two species. In addition to these traits, a number of other characters are also useful for diagnosis, including measurements of the post orbital constriction, the secondary process on the mastoid, and the gap between the cingula of the lower canines.

The cytological data available on *Tonatia* refer to the karyotype of *T. bidens* as having a diploid number (2n) of 16 and a fundamental number (FN) of 20 (Baker 1970, Baker & Hsu 1970, Patton & Baker 1978, Baker & Bickham 1980, Santos et al. 2002). However, nearly all these specimens were recognized as *T. saurophila* following the review of Williams et al. (1995).

In recent years, a partial sequence of the mitochondrial cytochrome c oxidase subunit I (COI) gene has been used as a DNA barcode for the identification of species (Hebert et al. 2003). While studies of COI in bats are incipient in general, the gene has been sequenced in more than 160 Neotropical species (Clare et al. 2007, 2011, Nesi et al. 2011, Hernandez-Da'vila et al. 2012).

In the present study, we diagnosed the karyotypes of specimens of *T. bidens* and *T. saurophila* captured in the Cerrado savanna of central Brazil, and used morphometric and COI sequences data to assess the identity of the species analyzed.

MATERIAL AND METHODS

Two female specimens of *T. bidens* and one female *T. saurophila* were collected from natural populations in the municipality of Barra do Garças, in eastern Mato Grosso, Brazil. The *T. bidens* specimens were collected in the Serra Azul State Park, a 11,000 ha area of Cerrado savanna, with diverse habitat types. One of the specimens was captured in well-preserved gallery forest neighboring an abandoned pasture, at an altitude of 533 m (15°50'17.5"S, 52°14'56.0"W). The second specimen was captured in an area of scrubby savanna, surrounded by more well-preserved vegetation, at an altitude of 728 m (15°49'36.2"S, 52°13'50.1"W). The specimen of *T. saurophila* was collected from a gallery forest on a private property surrounded by well-preserved vegetation (15°20'36.6"S, 52°12'59.0"W).

The bats were captured in 9 x 3 m mist-nets, and specimen collection was authorized by the Brazilian federal environment institute (IBAMA) through special license IBAMA-SISBIO #18276-1. The specimens were initially identified based on the specialized literature (Vizotto & Taddei 1973, Gardner 2007, Reis et al. 2013) and the identifications were confirmed by Dr. Marlon Zortéa from the Animal Biodiversity Laboratory of the Universidade Federal de Goiás at Jataí, Goiás, Brazil. External and cranial measurements were taken following the protocol of Vizotto & Taddei (1973) with a manual caliper (0.05 mm precision), and presented in mm. Once processed, the specimens were deposited as vouchers in the scientific collection of the Genetics Laboratory of Universidade do Estado de Mato Grosso (UNEMAT) at Nova Xavantina, under catalog numbers RM 338 and RM 356 (*T. bidens*), and RM 354 (*T. saurophila*).

Samples for cytogenetic analyses were extracted directly from the bone marrow of *T. bidens* e *T. saurophila* using an approach modified from Morielle-Versute et al. (1996). Chromosome morphology was observed through conventional Giemsa staining (Guerra 1988), C-banding was based on the procedure described by Sumner (1973), and Ag-NOR staining followed Varella-Garcia & Taddei (1989).

Base-specific fluorochrome CMA_3 staining adapted from Schweizer (1980) was used to highlight the regions rich in C and G. The chromosomes were measured and classified according to the position of the centromere, with four classes – metacentric, submetacentric, subtelocentric, and acrocentric, following Levan et al. (1964). An Olympus BX51 microscope was used to acquire the images and the karyotypes were mounted in Adobe Photoshop 7.0.

Samples of DNA were obtained from tissue fragments extracted from specimens RM 338 (*T. bidens*) and RM 354 (*T. saurophila*), as well as those of other phyllostomines collected in the Cerrado of Mato Grosso (*Lophostoma silvicolum* d'Orbigny, 1836: specimens RM 95, RM 105, RM 106 and RM 160 and *Phyllostomus discolor* Wagner, 1843: specimens RM 100, RM 122 and RM 245). The DNA was extracted following the protocol of Aljanabi & Martinez (1997) and the COI primers $FishF_2$ and $FishR_2$ (Ward et al. 2005) were used to amplify the COI gene. The PCR reaction contained a final volume of 12.5 µl, including 9.08 µl of autoclaved milli-Q water, 1.25 µl of 10 x PCR buffer, 0.375 µl of $MgCl_2$ (50 mM), 0.25 µl of each primer (10 µM), 0.20 µl of dNTP (2.5 mM), 0.10 µl of Taq polymerase (5 U/µl), and 1.0 µl of genomic DNA (10ng/µl). The reactions were conducted in a graded Eppendorf thermocycler. The cycle consisted of an initial step of 5 min at 94°C, followed by 30 cycles of 45 s at 94°C, 1 min at 50°C, and 1 min at 72°C, and a final extension of 5 min at 72°C. The PCR products were run in 1.5% agarose gel solubilized in 0.5 x TBE buffer and visualized under ultraviolet light. The PCR product of the COI gene was sequenced by a specialized company using the Sanger technique in an ABI 3500 *Genetic Analyzer*. The sequences were deposited in the National Center for Biotechnology Information (GenBank, http://www.ncbi.nlm.nih.gov) under the numbers KR921585 to KR921593.

To improve our data, sequences of *Desmodus rotundus* (E. Geoffroy, 1810) and *Diphylla ecaudata* Spix, 1823, from GenBank, were added to the analyses as the outgroup, as well as 30 other specimens representing 10 genera of Phyllostominae in the ingroup: *T. saurophila*, *Lophostoma schulzi* (Genoways & Williams, 1980), *Lophostoma carrikeri* (J.A. Allen, 1910), *Lophostoma brasiliense* Peters, 1867, *Lophostoma evotis* (*Davis* & Carter, 1978), *Chrotopterus auritus* (Peters, 1856), *Micronycteris nicefori* (Sanborn, 1949), *Macrophyllum macrophyllum* (Schinz,1821), *Mimon crenulatum* (E. Geoffroy, 1803), *Phylloderma stenops* Peters, 1865, *Phyllostomus hastatus* (Pallas, 1767), *Trachops cirrhosus* (Spix, 1823), and *Vampyrum spectrum* (Linnaeus, 1758).

The sequences were added using Blastn (default parameters) by scoring ⩾ 90% identities with our sequences, aligned using the webPRANK alignment server with default settings (Löytynoja & Goldman 2005) and edited manually in MEGA 6.0 (Tamura et al. 2013). MEGA6 was used to identify the best-fitting model of nucleotide substitution using the second-order Akaike Information Criteria (AICc) (Akaike 1974, Sugiura 1978). Branches corresponding to partitions reproduced in less than 50% bootstrap replicates were collapsed (Felsenstein 1985). The evolutionary history was inferred by using the Neighbor-Joining (NJ) and Maximum lilelihood (ML) methods. For the NJ, the evolutionary distances were computed using Tamura-Nei method (Tamura & Nei 1993) and the rate variation among sites was modeled with a gamma distribution. For the ML, the evolutionary distances were computed using the Hasegawa-Kishino-Yano model (Hasegawa et al. 1985), a discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories) and the rate variation model allowed for some sites to be evolutionarily invariable. For both NJ and ML analyses, the codon positions included were $1^{st}+2^{nd}+3^{rd}$+Non-coding. All ambiguous positions were removed for each sequence pair. There were a total of 591 positions in the final dataset. All evolutionary analyses were conducted in MEGA6.

RESULTS

The morphometric analyses revealed a high degree of similarity between the species in body size, with external and cranial dimensions typical of the respective species (Table 1). In addition to these measurements, which overlapped in the two species, qualitative diagnostic features were also evaluated for the differentiation of the specimens. The two species were distinguished by the following traits: (i) the presence of a white stripe on the top of the head of *T. saurophila*, which was absent from *T. bidens*, (ii) presence of a narrow opening between the lower canines and relatively narrow lower incisors in *T. bidens*, in contrast with a wider space and broader incisors in *T. saurophila*, (iii) presence of a broader post-orbital constriction in the cranium of *T. bidens* in comparison with *T. saurophila*, (iv) agglomeration in the second lower premolar, which is obscured by the cingula of the first and third premolars in *T. bidens*, whereas in *T. saurophila*, the second lower premolar is more robust and is not obscured by the neighboring premolars (Figs. 1-4).

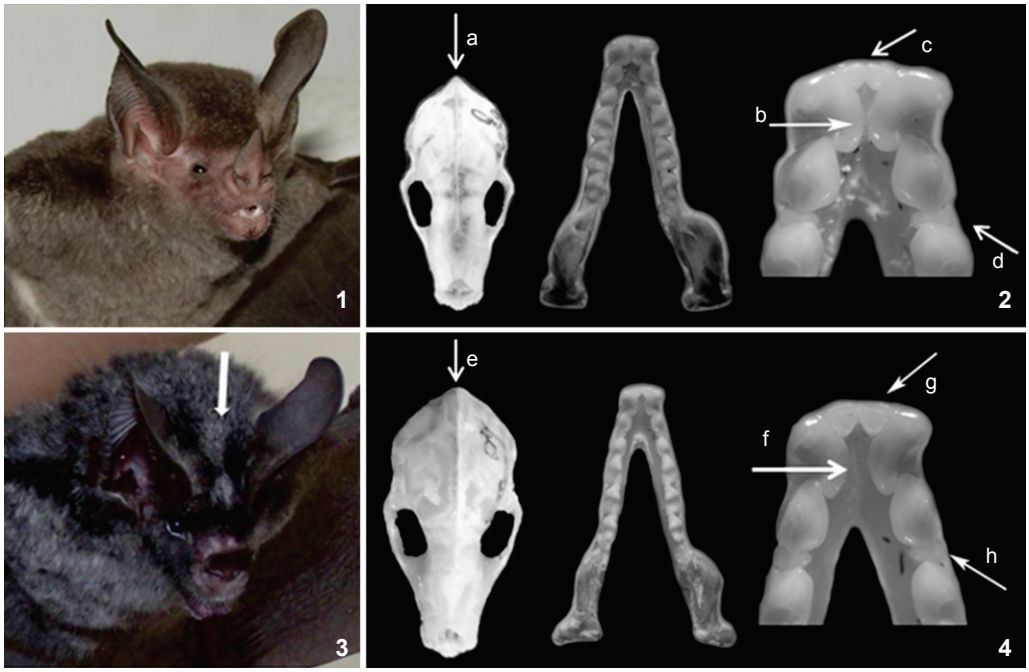
Table 1. External and cranial measurements (mm) of specimens of *Tonatia bidens* and *T. saurophila* captured in the Cerrado savanna of central Brazil.

Measurements (mm)	*T. bidens* (RM 338)	*T. bidens* (RM 356)	*T. saurophila* (RM 354)
Forearm	56.0	53.5	59.1
Greatest length of skull	27.1	27.0	28.5
Condylobasal length	25.4	25.6	26.4
Zygomatic breadth	13.8	12.8	14.9
Postorbital constriction breadth	5.7	5.7	5.3
Braincase breadth	10.9	10.7	11.5
Mastoid breadth	13.0	12.8	13.4
Maxillary toothrow length	9.6	9.5	9.9
Width across molars	8.5	8.5	9.1
Width across canines	5.8	5.7	5.7
Weight (g)	30.0	27.0	37.0

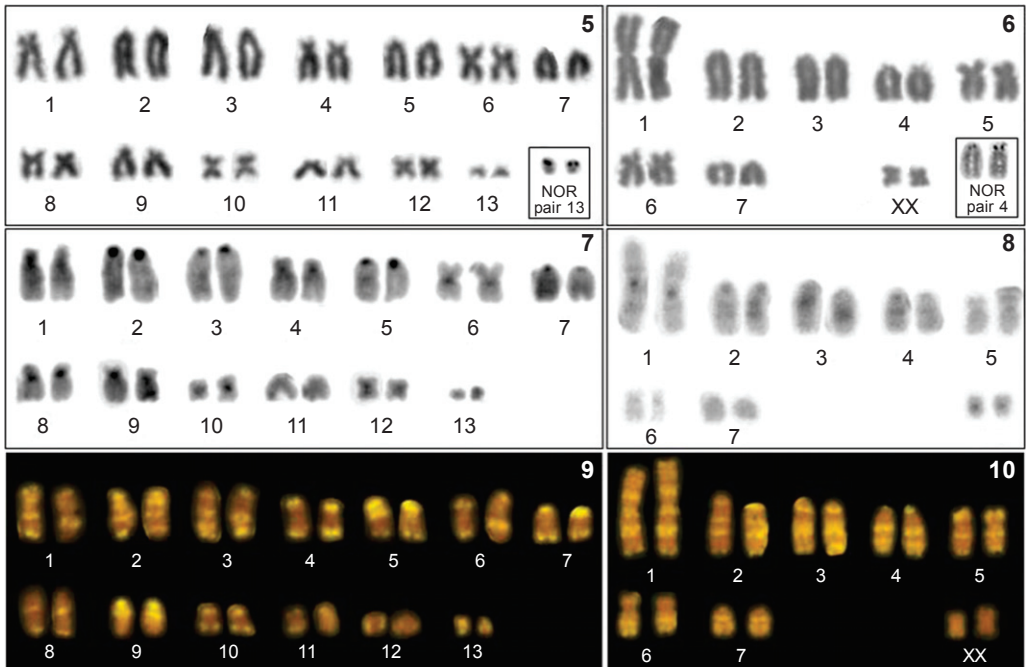
Tonatia bidens presented 2n = 26. The karyotype consisted of three pairs of metacentric chromosomes (6, 10 and 12), two submetacentric pairs (4 and 8), three subtelocentric pairs (1, 9 and 11), and five acrocentric pairs (2, 3, 5, 7 and 13). Although it was not possible to identify the sexual chromosomes directly, the FN is probably equal to 38 due to the fact that most phyllostomids and phyllostomines present a conserved biarmed X (Patton & Baker 1978, Ribas et al. 2015). A nucleolar organizer region (Ag-NOR) was identified in one pair of acrocentric chromosomes (pair 13), while the C-banding indicated the presence of regions of constitutive heterochromatin in the pericentromeric regions of all the chromosomes (Figs. 5-7).

The karyotype of *T. saurophila* was 2n = 16 and FN = 20. This set of autosomal chromosomes included one metacentric pair (1), two submetacentric pairs (5 and 6) and four acrocentric pairs (2, 3, 4 and 7). The X chromosomes are the smallest submetacentric pair in the karyotype (Patton & Baker 1978, Ribas et al. 2015). The Ag-NORs were observed in the acrocentric pair 4. The C-banding identified regions of constitutive heterochromatin in the centromeric regions of pairs 1, 2, 6 and X, and in the interstitial regions in pairs 3, 4 and 5 (Figs. 8-10).

The DNA barcode obtained here for *T. bidens* was the first of its kind for the species. The NJ and ML analyses produced different topologies; however, *T. bidens* and *T. saurophila* appear as sister taxa in both trees with a higher value bootstraps in the ML (71%) (Figs. 11 and 12). The comparison of the sequence divergence values obtained for *T. bidens* and *T. saurophila* with those of other Phyllostominae from Mato Grosso and GenBank, indicated that they belong to two distinct species, and *T. bidens* is closer to *T. saurophila* (14.22%) and to *P. hastatus* (14.18%) (Table 2).



Figures 1-4. (1) *T. bidens* (RM 338); (2) cranium (left), mandible (center) and lower dentition of (right) *T. bidens*: (a) broad post-orbital constriction, (b) narrow opening separating the lower canines, (c) narrow lower incisors, and d) agglomeration of the second lower premolar; (3) *T. saurophila* (RM 354), showing the distinct white stripe on the top of the head (arrow); (4) cranium (left), mandible (center) and lower dentition (right) of *T. saurophila*: (e) post-orbital constriction less evident, (f) lower canines separated by a relatively wide space, (g) relatively broad lower incisors, and (h) second lower premolar more robust and less agglomerated.



Figures 5-10. Representative karyotypes with Giemsa, Ag-NOR staining, C-banding and base-specific fluorochrome CMA_3 staining of *T. bidens* (5-7) and *T. saurophila* (8-10).

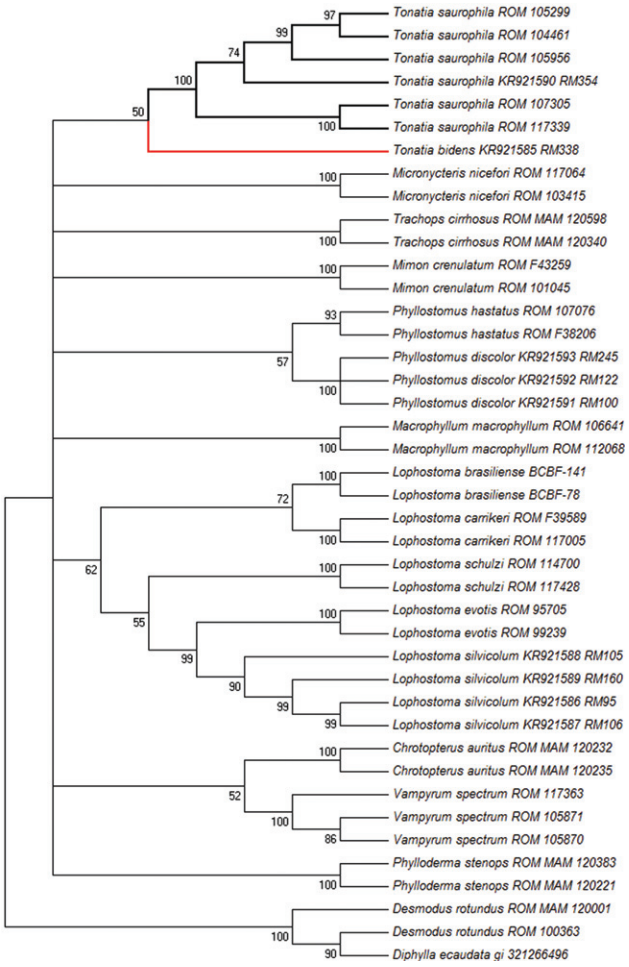


Figure 11. Neighbor-joining (NJ) dendogram describing relationships among genera of bats in Phyllostominae and calculated from COI sequence data (the species of *Tonatia* are highlighted). The best model for the evolutionary distance was computed using Tamura-Nei method. The sequences under the numbers KR921585 to KR921593 belong to specimens collected in the Cerrado of Mato Grosso.

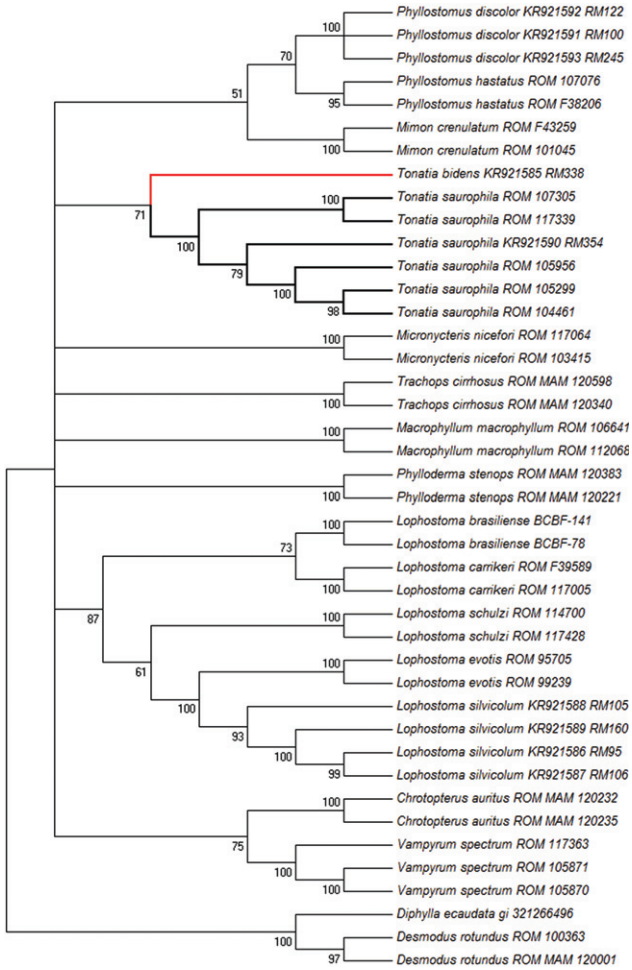


Figure 12. Maximum likelihood (ML) dendogram describing relationships among genera of bats in Phyllostominae and calculated from COI sequence data (the species of *Tonatia* are highlighted). The best model for the evolutionary distance was computed using the Hasegawa-Kishino-Yano model. The sequences under the numbers KR921585 to KR921593 belong to specimens collected in the Cerrado of Mato Grosso.

DISCUSSION

Few data are available on *Tonatia* from the wild, and most authors have considered these bats to be rare (Esbérard & Bergallo 2004). While a large number of inventories have been conducted in Brazil, records of this genus and cytogenetic data (Santos et al. 2002, Ribas et al. 2015) are scant. We considered the external morphological characteristics and cranial measurements presented by Williams et al. (1995) for the identification of the specimens analyzed in the present study. Karyotyping indicated 2n = 26 and FN = 38 for *T. bidens*, and 2n = 16 and FN = 20 for *T. saurophila*. Previous studies (Baker 1970, 1979, Baker & Hsu 1970, Gardner 1977, Patton & Baker 1978, Baker & Bickham 1980, Honeycutt et al. 1980) assigned a karyotype of 2n = 16 (FN = 20) to *T. bidens*, however, these were recognized as *T. saurophila* after the findings of Williams et al. (1995).

In chiropterans, chromosomal conservatism is so intense in some genera that different species may present the same chromosomal constitution, and even the same G-banding pattern (Baker 1970, Morielle-Versute et al. 1996, Faria & Morielle-Versute 2006). In some genera, however, considerable variation is found in the diploid and fundamental numbers (Varella-Garcia & Taddei 1989, Silva et al. 2005). The marked diploid number variation found in the *Tonatia* species is not unique

Table 2. Sequence divergence values (%) computed using the Tamura-Nei model, among the *Phyllostominae* analyzed. *Desmodus rotundus* and *Diphylla ecaudata* were used as outgroup for comparison.

	D. rotundus	*D. ecaudata*	*L. silvicolum*	*L. evotis*	*L. schulzi*	*L. brasiliense*	*L. carrikeri*	*P. discolor*	*P. hastatus*	*M. crenulatum*	*T. saurophila*	*T. bidens*	*M. nicefori*	*T. cirrhosus*	*M. macrophyllum*	*P. stenops*	*C. auritus*	*V. spectrum*
D. rotundus	–	1.05	2.47	2.43	2.32	2.38	2.33	2.46	2.07	2.53	2.26	2.45	2.37	2.84	2.53	2.46	2.58	2.31
D.ecaudata	5.84	–	2.65	2.64	2.61	2.70	2.64	2.70	2.33	2.69	2.56	2.69	2.60	3.10	2.73	2.68	2.81	2.75
L. silvicolum	22.87	27.51	–	0.94	1.71	1.66	1.90	1.99	1.67	2.11	2.20	2.17	2.24	2.21	2.01	2.07	2.35	2.30
L. evotis	22.52	27.28	5.40	–	1.80	1.79	1.79	2.24	1.82	2.18	1.99	2.45	2.15	2.54	2.19	2.28	2.41	2.44
L. schulzi	23.23	28.95	12.39	14.10	–	2.12	1.76	2.26	1.94	2.29	2.26	2.46	2.44	2.38	2.32	2.39	2.58	2.12
L. brasiliense	23.42	28.98	13.12	14.95	16.72	–	1.72	2.04	1.79	2.12	2.02	2.48	2.44	2.29	2.02	2.60	2.47	2.64
L. carrikeri	23.50	29.40	15.58	14.84	15.07	13.43	–	2.49	1.75	1.98	1.92	2.33	2.42	2.43	2.12	2.61	2.64	2.14
P. discolor	24.82	30.24	18.59	21.01	20.76	18.52	23.19	–	1.53	1.96	2.12	2.23	2.25	2.33	2.36	2.76	2.26	2.25
P. hastatus	17.91	22.78	13.01	14.48	14.88	14.53	13.10	10.36	–	1.63	1.86	1.87	2.10	1.93	1.82	2.16	2.15	2.04
M. crenulatum	26.05	30.85	19.52	20.54	20.84	20.07	18.91	18.65	11.74	–	1.87	2.13	2.27	2.29	2.08	2.66	2.59	2.55
T. saurophila	22.51	27.89	18.41	17.11	19.34	16.99	15.97	19.25	12.67	16.44	–	1.65	2.06	2.09	2.05	2.31	2.33	2.15
T. bidens	25.59	31.25	18.52	21.39	20.29	21.88	20.94	19.84	14.18	18.97	14.22	–	2.04	2.40	2.24	2.63	2.53	2.36
M. nicefori	24.36	30.04	20.72	20.54	21.08	21.79	22.68	20.94	17.45	20.20	19.16	18.47	–	2.32	2.29	2.50	2.42	2.53
T. cirrhosus	26.51	32.09	20.09	23.41	21.76	21.53	23.32	22.80	14.62	21.38	17.72	21.54	19.51	–	2.12	2.51	2.38	2.60
M. macrophyllum	23.95	28.23	18.50	20.24	20.74	17.79	19.74	22.38	14.97	19.30	18.27	20.12	21.09	19.06	–	2.20	2.19	2.49
P. stenops	25.59	30.33	18.55	21.73	22.27	24.17	24.41	25.05	16.68	26.24	21.10	26.44	26.40	25.74	21.62	–	2.69	2.61
C. auritus	26.15	31.35	21.41	22.11	24.74	23.47	24.76	21.75	19.53	26.33	23.23	24.82	22.95	24.49	22.48	27.05	–	2.38
V. spectrum	23.10	30.01	20.23	22.00	18.73	23.74	19.92	20.99	16.82	22.90	20.90	23.26	23.63	24.59	22.84	26.20	21.15	–

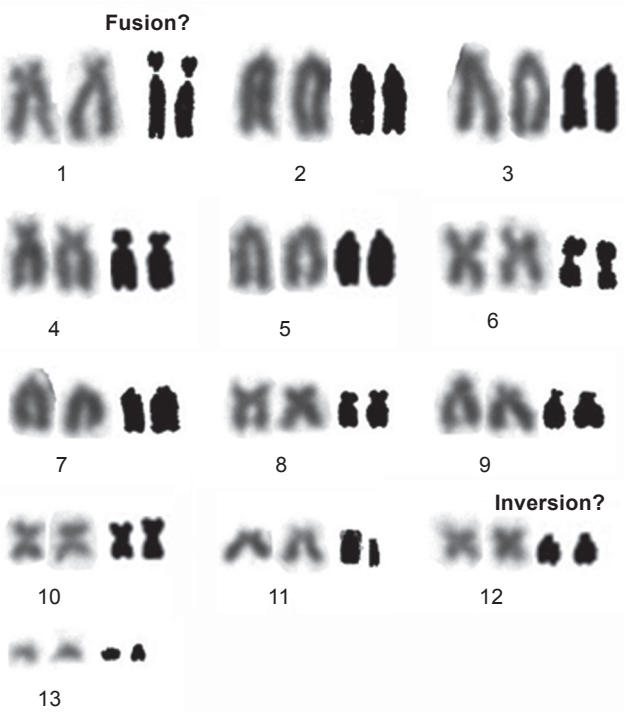


Figure 13. Chromosomal comparison between *T. bidens* collected in the Brazilian state of Mato Grosso (left) and *L. schulzi* (Honeycutt et al. 1980).

among congeners within Phyllostomidae. The number of chromosomes can vary from a single extra pair in different *Mimon* species to seven extra pairs in *Micronycteris* Gray, 1847 (Baker & Hsu 1970, Patton & Baker 1978, Baker 1979, Baker et al. 1981, Ribas et al. 2013).

The karyotype of *T. bidens* recorded in the present study not only contrasted considerably with that of *T. saurophila*, but also with those of most other bat species. A number of other species have a diploid number of 26, although their chromosomal morphology is quite distinct, being reflected in different fundamental numbers (Gardner 1977, Baker 1967, Baker & Jordan 1970, Baker et al. 1981, Varella-Garcia et al. 1989).

When compared to the karyotypes of the species of *Lophostoma*, which were previously considered to be members of *Tonatia*, *T. saurophila* (2n = 16, FN = 20) was considered to be the most divergent, and no interspecific homologies in the G banding could be identified (Patton & Baker 1978). Baker & Bickham (1980) concluded that the karyotypic differences found in the *Tonatia* species were the result of a number of different rearrangements, which occurred primarily in the 2n = 16 karyotype, considered to be the most derived of all. However the karyotype of *T. bidens* presented here (2n = 26, FN = 38) is highly similar to that described for *L. schulzi* (2n = 28, FN = 36) (Honeycutt et al. 1980), which apparently differ by only a few chromosomal rearrangements (Fig. 13).

The Ag-NORs of *T. saurophila* identified in the present study were found on the same pair of medium-sized acrocentric chromosomes (pair 4) identified in previous studies (Santos et al. 2002, Martins et al. 2010, Ribas et al. 2015). The Ag-NORs described here for the new karyotype of *T. bidens* were observed on the smallest pair of acrocentric chromosomes (pair 13). The constitutive heterochromatin found in the pericentromeric region of the chromosomes of *T. bidens* and in the pericentromeric and interstitial regions of some of the chromosomes of *T. saurophila* was consistent with the pattern observed in other studies of phyllostomine bats (Varella-Garcia et al. 1989, Morielle-Versute et al. 1996, Santos et al. 2001, Silva et al. 2005, Barros et al. 2009, Gomes et al. 2010).

In the DNA barcoding analyses, the *T. saurophila* specimen from the present study grouped decisively with those from GenBank, forming a distinct clade in relation to the *T. bidens* specimen, emphasizing the differentiation of the two species. Samples of a number of different species of phyllostomines were also included here for comparison, using Hoffmann et al. (2008) as the reference for the choice of the taxa. In this work, the authors performed independent Bayesian and maximum likelihood analyses with the complete cytochrome-b gene, 12S-16S rRNA and RAG-2 sequences. The authors also confirmed the status of *T. bidens* and *T. saurophila* as sister taxa, albeit the ML phylogram obtained presented a higher resolution topology, with nodes most strongly supported by a bootstrap $\geqslant 80\%$, higher than the value found in this study. In addition, genetic distance values obtained here do not allow us to state that the specimen 2n = 26 is indeed a *Tonatia*, since the values show *T. bidens* also close to *P. hastatus*.

Overall, although the specimens of *Tonatia* captured in the Cerrado savanna of Mato Grosso were highly similar in general morphology, they presented a number of diagnostic traits in external and cranial morphology, which allowed them to be distinguished systematically, based on the proposal of Williams et al. 1995, and identified as *T. bidens* (RM 338 and RM 356) and *T. saurophila* (RM 354). The constitution of the karyotype of *T. bidens* was also completely different from that of *T. saurophila* and most other phyllostomid species, providing an additional diagnostic parameter for the differentiation of the species. The fact that this karyotype (2n = 26, FN = 38) is closely similar to that of *L. schulzi* raised the possibility that the species found in the Cerrado of Mato Grosso may in fact be an undescribed form of *Lophostoma* or other Phyllostominae from western Brazil. In addition, values obtained in the molecular analyses (COI) indicate that *T. bidens* and *T. saurophila* are distinct species, phylogenetically related, but the genetic distance values do not provide enough support to state that they belong to the same genus. Thus, further analysis including other *T. bidens* individuals and sequences of other mitochondrial and nuclear genes may provide us more robust molecular information that will allow us to confirm the taxonomic status of the specimens identified as *T. bidens*.

ACKNOWLEDGEMENTS

The authors thank the Fundação de Amparo à Pesquisa do Estado de Mato Grosso, Conselho Nacional de Desenvolvimento Científico e Tecnológico and the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior for financial support; Dr. Marlon Zórtea for assistance in identifying specimens; the biologists Sérgio Oliveira, Loana Souza and Hellen Silva for their help in fieldwork.

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Submitted: 25 February 2015
Received in revised form: 5 August 2015
Accepted: 21 September 2015
Editorial responsibility: Maria Wilhelmina Pil