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GENETICS, EVOLUTION, AND PHYLOGENY - ORIGINAL PAPER

Identification of *Cryptosporidium* bat genotypes XVI–XVIII in bats from Brazil

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Abstract

Cryptosporidiosis is an emergent zoonotic disease caused by the globally distributed protozoa *Cryptosporidium* spp. Although several *Cryptosporidium* studies related to humans and many animal species have been published, there are still limited studies on the epidemiology of *Cryptosporidium* infection in bats. The aim of this study was to determine the occurrence of *Cryptosporidium* spp. and to perform the molecular characterization of *Cryptosporidium* species and genotypes in fecal samples from bats in an urban area of the municipality of Araçatuba, state of São Paulo, Brazil. Nested PCR targeting the 18S rRNA, actin, and HSP-70 genes was performed to screen 141 fecal samples from bats and detected *Cryptosporidium* spp. in 16.3% (23/141) of the samples. Bidirectional sequencing identified three novel *Cryptosporidium* bat genotypes (XVI, XVII, and XVIII) and a new genotype (18SH) genetically similar to *Cryptosporidium avium* in six species of bats. This is the first report on the occurrence and molecular characterization of *Cryptosporidium* spp. in Brazilian bats. Zoonotic *Cryptosporidium* species were not found in fecal samples from bats living in an urban area in the municipality of Araçatuba, state of São Paulo, Brazil.

Keywords Chiropteran · Brazil · Cryptosporidial infection · Molecular characterization

Introduction

The order Chiroptera is the second most diverse among mammalian orders, with 1198 species described worldwide (Kasso and Balakrishnan 2013; IUCN 2018). In Brazil, Bordignon et al. (2017) reported the occurrence of 180 bat species. Bats act as pollinators and seed dispersers of hundreds of plant species (Bredt et al. 2012), and insectivorous bats play an important role as insect predators (Peracchi et al. 2011).

Cryptosporidial infection has been reported in humans and in domestic and wild animals, mainly as clinical or subclinical infections in the gastrointestinal tract (Santín 2012). Human infections with several *Cryptosporidium* species and genotypes have also been reported as zoonotic and originated from domestic and wild animals (Chalmers and Giles 2010; Ryan et al. 2016). Although little information concerning the zoonotic potential of bat *Cryptosporidium* species or genotypes is available, bats may be potential reservoirs of zoonotic microorganisms (Allocati et al. 2016), including *Cryptosporidium parvum* and *Cryptosporidium hominis* (Kváč et al. 2015; Schiller et al. 2016).

Currently, there are nearly 38 *Cryptosporidium* species classified in amphibians, birds, fishes, mammals, and reptiles. In addition, several *Cryptosporidium* genotypes have been described based on molecular analyses (Feng et al. 2018). Epidemiological studies performed up to date show a great genetic diversity of the genus *Cryptosporidium* in bats and describe 15 *Cryptosporidium* genotypes in 12 species of bats (Wang et al. 2013; Kváč et al. 2015; Murakoshi et al. 2016; Schiller et al. 2016; Murakoshi et al. 2018; Li et al. 2019).

The literature on taxonomy and the prevalence of cryptosporidial infection in chiropteran is scarce. In addition, there is no information on the clinicopathological features of *Cryptosporidium* infection in bats. Reports on the occurrence of *Cryptosporidium* sp. in fecal samples of bats were first performed by Dubey et al. (1998), Morgan et al. (1998), Morgan et al. (1999), and Ziegler et al. (2007); however, *Cryptosporidium* species or genotypes were not determined in these reports.

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The first description of the genetic characterization of bat *Cryptosporidium* isolates was performed in China (Wang et al. 2013), with the designation of bat genotypes I and II in a Chinese rufous horseshoe bat (*Rhinolophus sinicus*) and Stoliczka's trident bat (*Aselliscus stoliczkanus*), respectively. Kvác et al. (2015) described, in the USA and Czech Republic, bat genotype III in a large brown bat (*Eptesicus fuscus*) and bat genotype IV in a common pipistrelle (*Pipistrellus pipistrellus*). In the Philippines, Murakoshi et al. (2016) described bat genotype II in a Leschenault's rousette (*Rousettus leschenaultia*) and greater musky fruit bat (*Ptenochirus jagori*), bat genotype V in a cave nectar bat (*Eonycteris spelaea)*, bat genotype VI in a lesser short-nosed fruit bat (*Cynopterus brachyotis*), and bat genotype VII in a Philippine forest horseshoe bat (*Rhinolophus inops*). Schiller et al. (2016) described bat genotypes VIII, IX, X, and XI in gray-headed flying fox (*Pteropus poliocephalus*) in Australia, and bat genotype XII was described in a northern bat (*Eptesicus nilssonii*) (Murakoshi et al. 2018) in Japan. The most recent study related to bat *Cryptosporidium* (Li et al. 2019) designated two genotypes identified in straw-colored fruit bats (*Eidolon helvum*) from Nigeria as bat genotypes XIV and XV. Li et al. (2019) have also designated bat genotype XIII in substitution with bat genotype II (LC089978) previously described by Murakoshi et al. (2016).

The present study aimed to detect and perform the molecular characterization of *Cryptosporidium* spp. in fecal samples from bats in an urban area of the municipality of Araçatuba, state of São Paulo, Brazil.

Materials and methods

Fecal samples

This study was authorized by the Ethics Committee on Animal Use (CEUA) of the São Paulo State University (UNESP), School of Veterinary Medicine, Araçatuba, process no. 00507-2016. The capture of bats was authorized by the Brazilian Institute for the Environment and Renewable Natural Resources (IBAMA), permanent license no. 27346-1.

Bats were captured in forest fragments in an urban area in the city of Araçatuba, state of São Paulo, Brazil, using fog networks armed through vegetation at dusk. The captured bats were placed in cotton bags (one for each bat) for approximately 60 min, and the bat species were then identified by morphological characteristics. The fecal samples excreted inside the cotton bags were collected. The bats were released at the same site of capture.

Each fecal sample was collected using a disposable wooden spatula, transferred to a 2-mL microtube, and stored at 4 °C. Samples were fragmented and homogenized in a 2-mL microtube containing phosphate-buffered saline and 0.1% Tween 20, sieved using a disposable plastic sieve, and centrifuged at 10.000*g* for 5 min. Fecal sediments were frozen at − 20 °C for DNA extraction and amplification by nested PCR.

Molecular characterization

The extraction of DNA was performed using the ZR Fecal DNA MiniPrep™ (Zymo Research) following the manufacturer's guidelines.

Three nested PCR protocols were performed to amplify fragments of the actin (Sulaiman et al. 2002), 18S rRNA (Xiao et al. 2000), and HSP-70 (Morgan et al. 2001) genes, following the author's protocols, using the JumpStart™ Taq ReadyMix™ (Sigma-Aldrich). As positive and negative controls, genomic DNA of *C. parvum* and ultrapure water were used, respectively. The amplified fragments were subjected to electrophoresis on 1.5% agarose stained with GelRed™ (Biotium) and purified using the PureLink™ PCR Purification Kit (Thermo Fisher Scientific) or the Illustra ExoProStar 1-Step™ (GE Healthcare Life Sciences).

Molecular cloning was performed when nested PCRs resulted in a low DNA yield, viewed as faint DNA bands by electrophoresis, using the CloneJET™ PCR Cloning Kit following the manufacturer's instructions (Thermo Fisher Scientific). Plasmid DNA was purified with the GenElute™ Five-Minute Plasmid Miniprep Kit (Sigma-Aldrich).

DNA sequence analysis

Genetic sequencing was performed using the ABI Prism™ Dye Terminator Cycling Sequence kit (Applied Biosystems) in an Automatic Sequencer ABI 3730XL (Applied Biosystems).

DNA sequences were assembled with Codoncode Aligner version 7.1.1 software (CodonCode Corporation). The homology of amplified products to sequences from GenBank was assessed using BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi) searches. Homologous sequences were aligned with consensus sequences using Clustal W (Thompson et al. 1997) and BioEdit Sequence Alignment Editor (Hall 1999).

Phylogenetic analyses were conducted in MEGA7 (Kumar et al. 2016) using maximum likelihood analysis based on the Tamura 3-parameter model (Tamura 1992) and the general time reversible model (Nei and Kumar 2000) for 18S rRNA and actin genes, respectively. Initial tree(s) for the heuristic search were obtained automatically by applying neighbor-join and BioNJ algorithms to a matrix of pairwise distances estimated using the maximum composite likelihood (MCL) approach and then selecting the topology with superior log-likelihood value. Substitution models and optional parameter sets were chosen using the model selection option in MEGA7. Trees were rooted with sequences from *Plasmodium falciparum* JQ627151 and EF472536 for 18S rRNA and actin genes, respectively.

Nucleotide sequences generated in this study were submitted to the GenBank database under the accession numbers MH553322-MH553335.

Statistical analysis

Prevalence rates with 95% confidence intervals were calculated using Wilson (score) intervals (Sergeant 2017).

Results

A total of 141 fecal samples were collected from eight genera and 11 species of bats. Nested PCR targeting the 18S rRNA gene of *Cryptosporidium* spp. identified 23/141 (16.3%; confidence interval 11.1–23.3) positive samples. Six out of 11 species of bats examined were positive for *Cryptosporidium* spp. Nested PCR targeting the actin and HSP-70 genes performed in 18S rRNA-positive samples resulted in 9/23 (39.1%) and 1/23 (4.34%) positive samples, respectively (Table 1).

All 18S rRNA and actin nested PCR products were cloned, and up to five clones were sequenced for each sample. Sequencing of the HSP-70 nested PCR amplicon from one sample and 18S rRNA and actin clone sequences from each gene identified four novel genotypes, namely, bat genotype XVI in flat-faced fruit-eating bats (*Artibeus planirostris*), great fruit-eating bat (*Artibeus lituratus*), and white-lined broad-nosed bat (*Platyrrhinus lineatus*), bat genotype XVII in a flat-faced fruit-eating bat and dark fruit-eating bat *(Artibeus obscurus*), bat genotype XVIII in a Seba's short-tailed bat (*Carollia perspicillata*), and genotype 18SH in a flat-faced fruit-eating bat (Table 1).

Minor intraclonal variation was observed in two out of five 18S rRNA clone sequences from isolate OP19 of bat genotype XVI (99.8% intraclonal genetic similarity), two out of four clone sequences from isolate OP130 of bat genotype XVII (99.7% intraclonal genetic similarity), and four out of five clone sequences from isolate OP88 of bat genotype XVIII (99.7–99.8% intraclonal genetic similarity). One clone sequence from isolate OP105 was designated genotype 18SH and shared an intraclonal genetic similarity of 96% to the other four clone sequences corresponding to bat genotype XVI and 99.2% genetic similarity (5 nucleotide substitutions) to *Cryptosporidium avium*. Clone sequences from other samples did not present intraclonal nucleotide variation.

The genetic similarities among bat genotype XVI and bat genotypes XVII and XVIII at the 18S rRNA gene were 97.7% and 98.2%, respectively, and between bat genotype XVII and bat genotype XVIII was 96.5%. Furthermore, genetic similarities among published sequences from *Cryptosporidium* species and genotypes at the 18S rRNA gene and sequences from bat genotypes XVI were 90.8% (bat genotype II) to 98.9% (*C. suis*); for bat genotype XVII, 89.8% (bat genotype II) to 97.5% (*C. suis*); and for bat genotype XVIII, 91.3% (bat genotype II) to 99.3% (skunk-like genotype).

The genetic similarity between bat genotypes XVI and XVII at the actin locus was 81.7%. The actin locus genetic similarity to *Cryptosporidium* sequences published in GenBank ranged from 77.6% (bat genotype XII) to 84.9% (*C. apodemi*) and 71% (*C. serpentis*) to 80.1% (*C. canis*), respectively. Minor intraclonal variation (2 nucleotide substitutions) was observed in one out of two actin clone sequences from bat genotype XVI (OP19) and one out of two actin clone sequences from bat genotype XVII (OP130). Direct sequencing and cloning of the actin gene nested PCR amplicons from bat genotype XVIII were not successful.

HSP-70 amplicon sequencing was successful for one sample positive for bat genotype XVIII (OP88), revealing genetic similarity of 76% (*C. serpentis*) to 89.9% (skunk-like genotype). Since the HSP-70 nested PCR amplicon sequencing of sample OP88 resulted in a clean sequence, cloning was not performed for this sample. Direct amplicon sequencing and cloning were not successful for sample OP90, which was positive for *Cryptosporidium* spp. by nested PCR targeting of the HSP-70 gene.

Analysis of the 18S rRNA gene sequences showed the phylogenetic grouping of the bat genotypes XVI and XVII as sister taxa in a clade comprising *Cryptosporidium occultus*, *Cryptosporidium suis*, and *Cryptosporidium ubiquitum*, although with low bootstrap values in maximum likelihood analysis (Fig. 1).

The grouping of bat genotypes XVI and XVII as sister taxa in a clade that includes bat genotype XIII and *Cryptosporidium felis* was observed in the phylogenetic tree of actin gene sequences, with high bootstrap support (Fig. 2).

Bat genotype XVIII 18S rRNA clone sequences clustered in a separate clade with *Cryptosporidium* sp. 80ANT from southern elephant seal and skunk-like genotype, also with low bootstrap support (Fig. 1).

Discussion

This study describes a prevalence rate of 16.3% *Cryptosporidium* spp. in bats from an urban area in Brazil. In addition, four novel *Cryptosporidium* genotypes, namely, bat genotypes XVI, XVII, and XVIII, and genotype 18SH, are described in fecal samples from bats.

In comparison with other mammalian species, cryptosporidiosis in bats has only recently attracted the attention of researchers and research efforts have been directed towards the screening and molecular characterization of *Cryptosporidium* spp. in feces. The few studies related to the epidemiology of cryptosporidial infection in bats revealed prevalence rates of 2.8% (Kváč et al. 2015), 7.2% (Ziegler et al. 2007), 7.7% (Wang et al. 2013), 8.9% (Murakoshi

Table 1 Identification of *Cryptosporidium* genotypes in bat fecal samples using nested PCR and genetic sequencing

Feeding habits	Host		% positive (no. positive/no. sampled)	Sample identification	Nested PCR[a]			Genotype		
	Common name	Species			18S rRNA	Actin	HSP-70	18S rRNA	Actin	HSP-70
Frugivorous	Flat-faced fruit-eating bat	*Artibeus planirostris*	19.7 (15/76)	OP2	+	–	–	XVI	–	–
				OP10	+	–	–	–	–	–
				OP19	+	+	–	XVI	XVI	–
				OP25	+	–	–	–	–	–
				OP40	+	–	–	–	–	–
				OP50	+	–	–	–	–	–
				OP53	+	+	–	XVI	XVI	–
				OP56	+	–	–	–	–	–
				OP57	+	–	–	–	–	–
				OP62	+	–	–	–	–	–
				OP90	+	+	+	XVI	XVI	–
				OP105	+	+	–	XVI, 18SH	XVI	–
				OP113	+	–	–	–	–	–
				OP124	+	+	–	XVI	–	–
				OP130	+	+	–	XVII	XVII	–
	Great fruit-eating bat	*Artibeus lituratus*	14.3 (2/14)	OP87	+	+	–	XVI	XVI	–
				OP95	+	–	–	–	–	–
	Dark fruit-eating bat	*Artibeus obscurus*	16.7 (1/6)	OP108	+	+	–	XVII	XVII	–
	Seba's short-tailed bat	*Carollia perspicillata*	8.3 (1/12)	OP88	+	+	+	XVIII	–	XVIII
	White-lined broad-nosed bat	*Platyrrhinus lineatus*	13 (3/23)	OP118	+	–	–	–	–	–
				OP134	+	–	–	–	–	–
				OP139	+	–	–	XVI	–	–
Insectivorous	Little yellow-shouldered bat	*Sturnira lilium*	0 (0/1)	–	–	–	–	–	–	–
	–	*Eptesicus* sp.	0 (0/2)	–	–	–	–	–	–	–
	Black myotis	*Myotis nigricans*	33.3 (1/3)	OP81	+	–	–	–	–	–
Nectarivorous	Pallas's long-tongued bat	*Glossophaga soricina*	0 (0/2)	–	–	–	–	–	–	–
Onivorous	Pale spear-nosed bat	*Phyllostomus discolor*	0 (0/1)	–	–	–	–	–	–	–
	Greater spear-nosed bat	*Phyllostomus hastatus*	0 (0/1)	–	–	–	–	–	–	–
Total			16.3 (23/141)							

[a] Nested PCR targeting actin and HSP-70 genes were performed in samples previously positive by 18S rRNA nested PCR

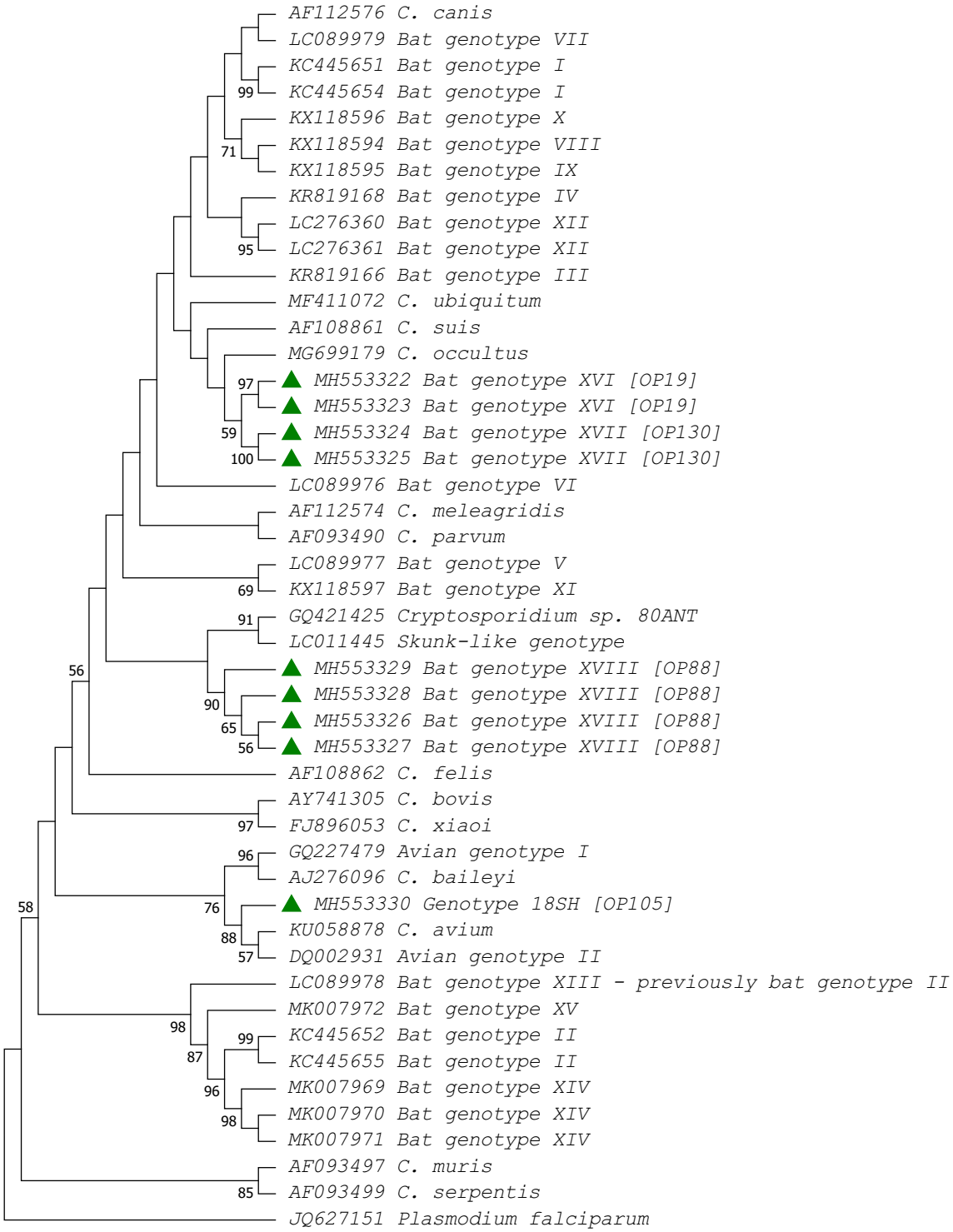

Fig. 1 Phylogenetic trees of the 18SrRNA gene sequences (617 base positions in the final dataset) from *Cryptosporidium* bat genotypes from this manuscript (green triangle) and selected *Cryptosporidium* species according to the maximum likelihood analysis based on the Tamura 3-parameter model. A discrete gamma distribution was used to model evolutionary rate differences among sites (5 categories (+*G*, parameter = 0.2074)). Numbers on the left of the supported nodes indicate the bootstrap values (1000 replicates). Only bootstrap values > 50% are shown. The tree was rooted with a sequence from *Plasmodium falciparum*. Sequences MH553322-MH553323 (bat genotype XVI), MH553324-MH553325 (bat genotype XVII), and MH553326-MH553329 (bat genotype XVIII) are originated from clones with minor nucleotide intraclonal variation

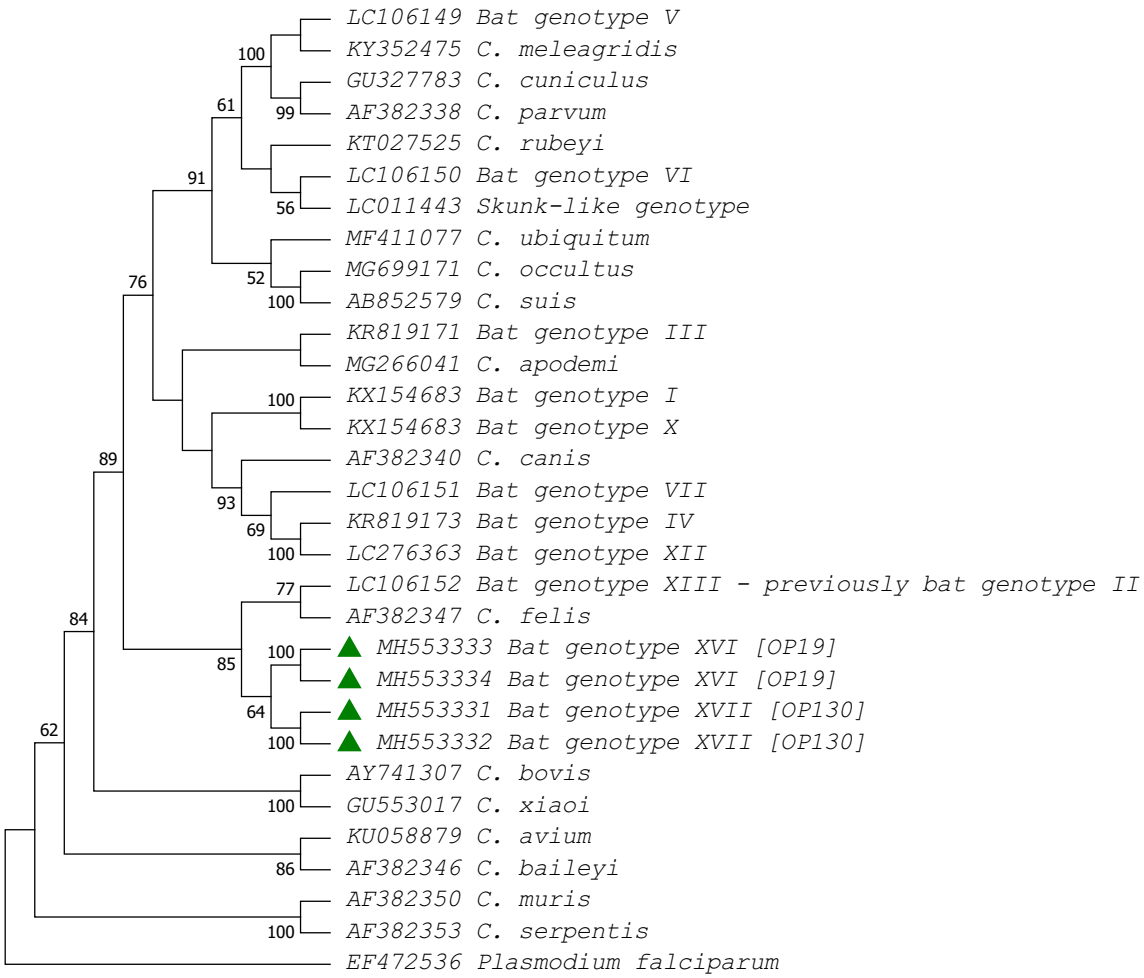

Fig. 2 Phylogenetic tree of the actin gene sequences (717 base positions in the final dataset) from *Cryptosporidium* bat genotypes from this manuscript (green triangle) and selected *Cryptosporidium* species according to the maximum likelihood analysis based on the general time-reversible model. A discrete gamma distribution was used to model evolutionary rate differences among sites (5 categories (+*G*, parameter = 0.4521)). The rate variation model allowed for some sites to be evolutionarily invariable (+*I*), 29.43% sites. Numbers on the left of the supported nodes indicate the bootstrap values (1000 replicates). Only bootstrap values > 50% are shown. The tree was rooted with a sequence from *Plasmodium falciparum*. Sequences MH553331-MH553332 (bat genotype XVII) and MH553333-MH553334 (bat genotype XVI) are originated from clones with minor nucleotide intraclonal variation

et al. 2016), and 5.5% (Li et al. 2019), which are lower than the prevalence rate in Brazilian bat species from this report.

Our results demonstrated that bat genotype XVI was the most prevalent in bats in an urban area of Araçatuba municipality, with identification in seven samples from flat-faced fruit-eating bats (5), great fruit-eating bats (1), and white-lined broad-nosed bats (1). Bat genotypes XVII and XVIII were detected at lower rates in two samples from a flat-faced fruit-eating bat (1) and dark fruit-eating bat (1) and in one sample from a Seba's short-tailed bat, respectively. Five out of seven samples positive for bat genotype XVI originated from a flat-faced fruit-eating bat; however, since the convenience sampling adopted in this study resulted in very different sample sizes among the species of bats and bat feeding habits, it was not possible to draw conclusions concerning statistical significance related to the prevalence rates among bat species and feeding habits and host specificity of bat genotypes.

Sequencing of bat genotype clones showed minor intraclonal variation in the 18S rRNA sequences from bat genotypes XVI (OP19), XVII (OP130), and XVIII (OP88). Sequences from the other samples positive for bat genotypes XVI and XVII (Table 1) were 100% genetically similar to sequences MH553322 and MH553324, respectively. In addition, the 18S rRNA clones from bat genotype XVIII (OP88) had four clonal sequences presenting minor genetic variation and the genera *Cryptosporidium* is known to have two paralog copies of the 18S rRNA gene (Le Blancq et al. 1997). Intraclonal divergence has been reported in the 18S rRNA sequences from *Cryptosporidium* bat genotypes VIII–XI (Schiller et al. 2016) and could be the result of coamplification of paralog copies of the 18S rRNA gene (Le Blancq et al. 1997). Although we can cannot rule out the presence of a mixed infection with coamplification of sequences from different genotypes, or even the occurrence of point mutations during PCR and genetic

cloning (Qiu et al. 2001), the genetic similarity of 81.7% between bat genotypes XVI and XVII at the actin locus and the minor intra-genotype variation between actin sequences suggests that 18S rRNA clone sequences from bat genotypes XVI and XVII could represent 18S rRNA gene paralog copies. Furthermore, minor nucleotide divergences among four bat genotype XVIII (OP88) clone sequences could also be related to rapid birth-and-death evolution of the apicomplexan genome (Rooney 2004), as suggested by Ikarashi et al. (2013) for *Cryptosporidium andersoni* clone sequences.

Minor intraclonal variation in the actin sequences was detected in one clone out of two from bat genotype XVI (isolate OP130) and in one clone out of two from bat genotype XVII (isolate OP19). Analyses of both sequenced strands did not reveal evidence of errors in genetic sequencing. Since intraclonal variation was observed in clones originating from the same isolate and the actin gene is a single copy gene (Kim et al. 1992), minor nucleotide variations could be the result of point mutations during PCR and genetic cloning (Qiu et al. 2001).

Phylogenetic relationships among *Cryptosporidium* bat genotypes XVI and XVII and *Cryptosporidium* species and genotypes from various hosts were not consistent with each other. Bat genotype XVI grouped in the clade that includes *C. occultus*, *C. suis*, and *C. ubiquitum*, and bat genotype XVII grouped in the clade comprising bat genotype XIII and *Cryptosporidium felis*. Bat genotype XVIII sequences grouped in the same clade that included the skunk-like genotype from feral racoon and *Cryptosporidium* sp. 80ANT from the southern elephant seal in the 18S rRNA gene tree, although with low bootstrap support. Inconsistencies in branching within phylogenetic trees between the 18S rRNA and actin gene sequences were attributed by Xiao et al. (2002) to differences in the rate and nature of genetic variations between these genes. There are no published HSP-70 gene sequences from *Cryptosporidium* bat genotypes in genetic databases, so it was not possible to compare the HSP-70 sequence of bat genotype XVIII with other bat *Cryptosporidium* HSP-70 sequences.

New genotype 18SH identified in one clone sequence from the 18S rRNA gene is genetically related to *C. avium*. This genotype was detected in one out of five clones from isolate OP105, in conjunction with four clone sequences of bat genotype XVI. Genotype 18SH has 96.1% and 99.2% genetic similarity to bat genotype XVI and *C. avium*, respectively. Paralog 18S rRNA copies can be coamplified and detected simultaneously during *Cryptosporidium* amplicon sequencing. Most *Cryptosporidium* 18S rRNA paralogs share a sister group relationship and high genetic similarity (~ 98–99%) (Le Blancq et al. 1997; Xiao et al. 1999; Morgan et al. 2001; Sevá et al. 2011; Ikarashi et al. 2013), although paralog copies with higher genetic diversity have been described in *Cryptosporidium* genotypes from chipmunks (Stenger et al. 2015) and common brushtail possums (Hill et al. 2008). Genotype 18SH did not share a sister group relationship with any genotype identified in this study, and it was not coamplified with bat genotype XVI sequences in any of the other six samples positive for bat genotype XVI (Table 1). In addition, although genetic similarity or phylogenetic analyses do not provide information related to host specificity, we suggest that genotype 18SH could actually be a novel genotype of avian origin mechanically carried in fecal samples from frugivorous bat species because genotype 18SH grouped in a clade that comprises only avian *Cryptosporidium* species and genotypes presenting tissue tropism to the bursa of Fabricius, cloaca, and trachea (Nakamura and Meireles 2015), such as *Cryptosporidium baileyi*, *C. avium*, and avian genotypes I and II (Fig. 1).

Cryptosporidium hominis and *C. parvum* have been described in fecal samples from bats (Kváč et al. 2015; Schiller et al. 2016), and Schiller et al. (2016) suggested that flying foxes are potential vectors for zooanthroponotic *C. hominis* in Australia. The results from our study and from other reports of cryptosporidial infection in bats from other countries (Wang et al. 2013; Kváč et al. 2015; Murakoshi et al. 2016; Murakoshi et al. 2018; Li et al. 2019) did not find evidence that bats from the wild or from urban environments are important reservoir hosts of zoonotic *Cryptosporidium* species or genotypes known to date. In contrast, it seems that bats are infected mainly by host-adapted *Cryptosporidium* species or genotypes.

Including the samples from this report, 18 genotypes have been described in 14 bat species examined to date (Wang et al. 2013; Kváč et al. 2015; Murakoshi et al. 2016; Schiller et al. 2016; Murakoshi et al. 2018; Li et al. 2019). As also noted by Schiller et al. (2016) and Li et al. (2019), owing to the small number of bat species screened for *Cryptosporidium* spp. to date and considering that the order Chiroptera is the second most diverse among mammalian orders, it would not be surprising to describe several other *Cryptosporidium* species/genotypes in bats.

Conclusion

Brazilian bats are natural hosts of three novel *Cryptosporidium* bat genotypes. Six bat species in an urban area in Brazil are described as new hosts of *Cryptosporidium*. There was a higher prevalence of bat genotype XVI. Bat genotypes XVII and XVIII, and a new genotype genetically related to avian *Cryptosporidium* were detected at lower positivity rates. Zoonotic *Cryptosporidium* species were not found in fecal samples from bats living in an urban area in the municipality of Araçatuba, state of São Paulo, Brazil.

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Compliance with ethical standards

This study was authorized by the Ethics Committee on Animal Use (CEUA) of the São Paulo State University (UNESP), School of Veterinary Medicine, Araçatuba, process no. 00507-2016. The capture of bats was authorized by the Brazilian Institute for the Environment and Renewable Natural Resources (IBAMA), permanent license no. 27346-1.

Conflict of interest The authors declare that they have no conflict of interest.

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