

## Occurrence, genetic diversity and zoonotic potential of *Blastocystis* sp. in forest musk deer (*Moschus berezovskii*) in Southwest China

Shanyu Chen<sup>1,a</sup>, Wanyu Meng<sup>1,a</sup>, Xianpeng Shi<sup>1,a</sup>, Yijun Chai<sup>1</sup>, Ziyao Zhou<sup>1</sup>, Haifeng Liu<sup>1</sup>, Zhijun Zhong<sup>1</sup>, Hualin Fu<sup>1</sup>, Suizhong Cao<sup>1</sup>, Xiaoping Ma<sup>1</sup>, Lihong Shen<sup>1</sup>, Lei Deng<sup>1,2,\*</sup>, and Guangneng Peng<sup>1,\*</sup>

<sup>1</sup> The Key Laboratory of Animal Disease and Human Health of Sichuan Province, College of Veterinary Medicine, Sichuan Agricultural University, Chengdu 611130, Sichuan, People's Republic of China

<sup>2</sup> Laboratory of Molecular and Cellular Parasitology, Healthy Longevity Translational Research Programme and Department of Microbiology and Immunology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore 117545, Singapore

Received 16 February 2022, Accepted 28 June 2022, Published online 14 July 2022

**Abstract** – *Blastocystis* sp. is a common anaerobic protist with controversial pathogenicity that can infect various animals and humans. However, there are no reports of *Blastocystis* sp. infections in forest musk deer (*Moschus berezovskii*). The present study was designed to examine the occurrence, subtype distribution and genetic characterization of *Blastocystis* sp. in forest musk deer in southwestern China, and to assess the potential for zoonotic transmission. A total of 504 fresh stool samples were collected from captive forest musk deer in four distinct areas of southwestern China. Overall, 14.7% of the forest musk deer (74/504) were found to be infected with *Blastocystis* sp. The highest occurrence of *Blastocystis* sp. was observed in Dujiangyan (27.5%), followed by Maerkang (23.3%). The occurrence of *Blastocystis* sp. was 7.9% and 4.1% in Shimian and Hanyuan, respectively. Significant differences in the occurrence of *Blastocystis* sp. among different areas were observed ( $p < 0.05$ ), while we did not observe significant differences among animals of different age and sex ( $p > 0.05$ ). Two known zoonotic subtypes (ST1 and ST5) and three animal-predominant subtypes (ST10, ST13, and ST14) were identified, of which ST10 was the most common (36/74, 48.6%). Our findings highlight that forest musk deer may be potential reservoirs of zoonotic human *Blastocystis* sp. infections.

**Key words:** *Blastocystis* sp., Zoonotic potential, Forest musk deer, Prevalence, China.

**Résumé** – **Présence, diversité génétique et potentiel zoonotique de *Blastocystis* sp. chez le cerf porte-musc (*Moschus berezovskii*) dans le sud-ouest de la Chine.** *Blastocystis* sp. est un protiste anaérobie commun, de pathogénicité controversée, et qui peut infecter divers animaux et les humains. Cependant, aucun cas d'infection par *Blastocystis* sp. n'a été rapporté chez le cerf porte-musc (*Moschus berezovskii*). La présente étude a été conçue pour examiner la présence, la distribution des sous-types et la caractérisation génétique de *Blastocystis* sp. chez le cerf porte-musc du sud-ouest de la Chine et pour évaluer son potentiel de transmission zoonotique. Au total, 504 échantillons de selles fraîches ont été prélevés sur des cerfs porte-musc captifs dans quatre régions distinctes du sud-ouest de la Chine. Dans l'ensemble, 14,7 % (74/504) des cerfs porte-musc se sont avérés infectés par *Blastocystis* sp. La plus forte occurrence de *Blastocystis* sp. a été observée à Dujiangyan (27,5 %), suivi de Maerkang (23,3 %). La présence de *Blastocystis* sp. était respectivement de 7,9 % et 4,1 % à Shimian et Hanyuan. Des différences significatives dans la présence de *Blastocystis* sp. entre les différentes zones ont été observées ( $p < 0,05$ ), alors que nous n'avons pas observé de différences significatives entre les animaux d'âge et de sexe différents ( $p > 0,05$ ). Deux sous-types zoonotiques connus (ST1 et ST5) et trois sous-types à prédominance animale (ST10, ST13 et ST14) ont été identifiés, dont ST10 était le sous-type le plus courant (36/74, 48,6 %). Nos découvertes mettent en évidence que le cerf porte-musc forestier peut être un réservoir potentiel d'infections à *Blastocystis* sp.

Edited by: Emmanuel Liénard

\*Corresponding authors: pgn.sicau@163.com; cd1931023@163.com

<sup>a</sup> These authors contributed equally to this work.

## Introduction

*Blastocystis* sp. belongs to the phylum Stramenopiles and is a common unicellular intestinal parasite of various animals. Generally, *Blastocystis* sp. is transmitted via the fecal-oral route, which is the primary mode of transmission [22, 23, 44]. Several studies have shown that humans are susceptible to zoonotic *Blastocystis* sp. [31, 50]. Epidemiological surveys estimate that the parasite has colonized between one and two billion people worldwide [34]. However, the pathogenicity of *Blastocystis* sp. is still uncertain, although some studies have demonstrated possible associations of the parasite to a variety of gastrointestinal disorders, such as irritable bowel syndrome (IBS) and inflammatory bowel disease (IBD) [7, 13, 19]. In contrast, some studies demonstrated that *Blastocystis* sp. is a common commensal microorganism in the human gut, associated with increased diversity of gut microbiota [4, 6, 46].

Based on analysis of the small subunit (SSU) rRNA gene of *Blastocystis* sp., at least 28 subtypes (ST1–ST17, ST21, ST23–ST32) have been confirmed in humans and in a variety of animals worldwide [17, 27, 28, 42]. Among them, ST1 to ST9 and ST12 are known to infect humans, while ST1 to ST4 account for more than 90% of human *Blastocystis* sp. infections [25, 43]. Interestingly, the prevalence of different subtypes seems to vary greatly among different regions and countries [10], and different subtypes demonstrate remarkably diverse biological characteristics, such as pathogenicity, drug resistance, and effects on microbiota [1, 32, 53].

The forest musk deer (*Moschus berezovskii*) is a small ruminant unique to Asia and belongs to the Moschidae family [12]. Musk deer (*Moschus* spp.) are an endangered species currently considered class I-protected animals in China. The forest musk deer is the largest musk deer species in China, mainly distributed in Guizhou and Sichuan province [16, 51]. It has been determined that forest musk deer can harbor several zoonotic intestinal pathogens (e.g., *Enterocytozoon bieneusi* and *Giardia duodenalis*) and have the ability to transmit these organisms to humans [39]. However, there are no studies focusing on the isolation of *Blastocystis* sp. from forest musk deer, and whether it is an infection reservoir for other animals and humans remains unclear. In this study, we explored the prevalence and subtype distribution characteristics of *Blastocystis* sp. in forest musk deer for the first time, emphasizing the potential threat of zoonotic transmission.

## Materials and methods

### Ethics statement

This study was performed in accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals of the Ministry of Health, China. As only fecal samples collected after spontaneous defecation of forest musk deer were analyzed, this study did not require full Animal Ethics Committee approval in accordance with Chinese law. No animals were harmed during the sampling process. Permission was obtained from farm owners and managers before collection of fecal specimens.



**Figure 1.** Locations of the sampled sites (filled triangle) in Sichuan Province, Southwestern China.

### Sample collection

A total of 504 fecal samples from captive forest musk deer was collected from four areas of Sichuan province between August and September 2020 (Fig. 1), with 139 samples collected in Shimian (29°16' N, 102°20' E) at an altitude of 2572 m, 144 in Hanyuan (29°29' N, 102°37' E) at an altitude of 1076 m, 131 in Dujiangyan (31°01' N, 103°35' E) at an altitude of 739 m, and 90 in Maerkang (31°53' N, 102°07' E) at an altitude of 2526 m (Table 1). The forest musk deer breeding farms were cleaned the night before sampling, and each individual was kept in a separate enclosure so that the fresh feces of each individual could be collected the following morning. All fecal samples were collected by laboratory staff or farmers trained in sample collection, and strict controls were implemented to minimize potential contamination between samples. Approximately 5–10 g of fresh fecal samples were collected using sterile disposal latex gloves after defecation of the forest musk deer, stored in individual plastic bags, with gender, age, and number recorded. During the sample collection process, only the middle layer of feces was collected to avoid contamination. All samples were immediately stored in liquid nitrogen for transportation back to the laboratory and later stored at –80 °C until processing. The animals that had been sampled exhibited no obvious clinical signs.

### DNA extraction

Fecal samples were sieved and washed with distilled water three times by centrifugation at 3000× g for 10 min. Genomic DNA was extracted using a QIAamp Fast DNA Stool Mini Kit (Qiagen, Germany), according to the manufacturer's instructions. Both negative and positive control stools were included.

**Table 1.** Factors associated with the prevalence of *Blastocystis* in forest musk deer in China.

Factors	No. of positive/overall	Prevalence (95% CI)	OR (95% CI)	P value
Locations				
Shimian	11/139	7.9 (3.4–12.4)	Reference	Reference
Hanyuan	6/144	4.1 (0.9–7.4)	0.5 (0.2–1.4)	0.192
Dujiangyan	36/131	27.5 (19.8–35.1)	4.4 (2.1–9.1)	0
Maerkang	21/90	23.3 (14.6–32.1)	3.5 (1.6–7.8)	0.002
Sex				
Female	29/243	11.9 (7.9–16.0)	Reference	Reference
Male	45/261	17.2 (12.7–21.8)	1.5 (0.9–2.5)	0.094
Age (years)				
≤ 1.5	19/119	16.0 (9.3–22.5)	Reference	Reference
> 1.5	55/385	14.3 (10.8–17.8)	0.9 (0.5–1.5)	0.651
Total	74/504	14.7 (11.6–17.8)		

The DNA was eluted in 200 µL of buffer and stored at −20 °C until use, and the quality of the DNA was verified using NanoDrop (Thermo Fisher Scientific, Carlsbad, CA, USA).

### PCR amplification

PCR amplification of the barcode region (a fragment of ~600 bp) of the SSU rRNA gene was used to screen all DNA preparations to identify *Blastocystis* sp. The cycling parameters and primers were the same as previously described by Scicluna et al. [35]. Taq PCR Master Mix (Sangon Biotech Co., Ltd., Shanghai, China) was used for all PCRs. All PCR tests included positive and negative controls and were performed in triplicate. The PCR products were subjected to 1.5% agarose gel electrophoresis and visualized by staining with SYBR Safe DNA Gel Stain (Thermo Fisher Scientific).

### Nucleotide sequencing and analysis

A QIAQuick Gel Extraction Kit (Qiagen) was used to purify PCR products from agarose gel, according to the manufacturer's instructions. The expected product size was ~600 bp. All positive PCR products were bidirectionally sequenced at the BioSune Biotechnology Company (Shanghai, China); A BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, Waltham, MA, USA) was used. The consensus sequences obtained in our study were submitted to BLAST searches (<http://www.ncbi.nlm.nih.gov/blast/>), then aligned and analyzed. Reference sequences were downloaded from the GenBank database (<http://www.ncbi.nlm.nih.gov/>); we then used Clustal X 2.0 (<http://www.clustal.org/>) to identify the subtypes of *Blastocystis* sp. The nucleotide sequences generated in this study were deposited in GenBank with the accession numbers OK445532–OK445537, and OK445663–OK445665.

### Partial phylogenetic analysis

To evaluate the genetic relationship among the sequences of *Blastocystis* sp. genotypes obtained in this study and those identified previously, MEGA 6 software (<http://www.megasoftware.net/>) was used to construct a neighbor-joining tree for partial phylogenetic analysis. The Kimura 2-parameter model

was used to calculate the evolutionary distances. Undefined positions were removed from the alignment before partial phylogenetic analysis, and the alignment was trimmed by MEGA 6. Finally, we assessed the reliability of the trees by Bootstrap analysis (with 1000 replicates).

### Statistical analysis

Variations in the prevalence of *Blastocystis* sp. (y1) in forest musk deer according to geographical location (x1), sex (x2), and age (x3) were analyzed by binary logit model using SPSS 22 (<https://www.ibm.com/analytics/spss-statistics-software>). P-value < 0.05 represented statistical significance (Table 1).

## Results

### Prevalence of *Blastocystis* sp. in forest musk deer

A total of 504 fecal samples (144 Hanyuan, 139 Shimian, 131 Dujiangyan, and 90 Maerkang) were screened by PCR amplification to identify *Blastocystis* sp. The overall prevalence of *Blastocystis* sp. in forest musk deer was 14.7% (74/504). The highest prevalence was in Dujiangyan at 27.5% (36/131), followed by Maerkang 23.3% (21/90), Shimian 7.9% (11/139), and Hanyuan 4.1% (6/144) (Table 1). There were significant differences in prevalence between the four areas ( $p < 0.05$ ). The prevalence of *Blastocystis* sp. among females and males were 48.2% and 51.9%, respectively but there was no significant difference between them ( $ps > 0.05$ ). Similarly, the differences in prevalence of *Blastocystis* sp. among forest musk deer of different ages were not statistically significant ( $p > 0.05$ ).

### Subtype distributions of *Blastocystis* sp. in forest musk deer

Five subtypes of *Blastocystis* sp. were identified from 74 positive samples, including two potentially zoonotic STs (ST1, ST5) and three animal-specific STs (ST10, ST13, ST14). Although Sanger sequencing cannot identify the subtypes involved in mixed infection and only identified the dominant

**Table 2.** Subtype distributions from different locations.

<i>Blastocystis</i> sp. STs (accession number)	Shimian	Hanyuan	Dujiangyan	Maerkang
ST1 (OK445532)	2			
ST1 (OK445533)	2			
ST5 (OK445534)			13	5
ST10 (OK445535)	2	3	17	8
ST10 (OK445536)				2
ST10 (OK445537)			2	2
ST13 (OK445663)	2	1		
ST13 (OK445664)	3	2		2
ST14 (OK445665)			4	2
Total	11	6	36	21

subtypes in the samples, we believe that there were no mixed infections because there were no ambiguous peaks in the electropherograms. ST10 (36/74) was the dominant subtype found in the forest musk deer examined, followed by ST5 (18/74), ST13 (10/74), ST14 (6/74) and ST1 (4/74) (Table 2). Interestingly, ST1 was found only in forest musk deer in Shimian.

### Genetic characteristics of *Blastocystis* sp. subtypes

Analysis of the SSU rRNA gene revealed that four sequences of ST1 isolate contained two representative sequences, the sequences OK445532 ( $n = 2$ ) and OK445533 ( $n = 2$ ). They have 99.82% and 99.65% similarity to the ST1 sequence isolated from humans (MK782501), with one and two nucleotide substitutions, respectively. ST5 sequences ( $n = 18$ ) showed 100% identity to that of alpaca in China (MN382283). ST10 isolates contained three representative sequences, the sequences OK445536 ( $n = 2$ ) and OK445537 ( $n = 4$ ) were identical to sheep from Iran (MW426240) and cattle from Malaysia (MG831508), respectively. The remaining sequence OK445535 ( $n = 30$ ) showed 99.82% similarity to sika deer from China (MK930355) and white-tailed deer from the USA (MZ267679) with one nucleotide substitution. ST13 isolates ( $n = 10$ ) contained two representative sequences, the sequence OK445663 ( $n = 3$ ) showed 100% identity to a sequence that was isolated from crested deer in China (MT889741), and the sequence OK445664 ( $n = 7$ ) showed 99.82% similarity to a sambar sequence from South Korea (MT114848) with one nucleotide substitution. ST14 sequences ( $n = 6$ ) exhibited 100% identity to that of sheep in China (MT672788) and the Czech Republic (MT039559).

### Partial phylogenetic analysis of *Blastocystis* sp.

Nine representative sequences were obtained from the 74 *Blastocystis* sp. isolates in this study. These newly identified sequences showed high similarity to reference sequences of *Blastocystis* sp. in GenBank, and belong to ST1, ST5, ST10, ST13 and ST14. The ST1 found in this study clustered together with sequences originating from humans and cattle. ST5 is grouped with sequences that are mainly from sheep and alpaca. ST10 clustered with sequences from sheep, sika deer, alpaca, cattle, and white-tailed deer, while ST13 grouped with sequences isolated from crested deer, Tibetan antelope, reindeer and

sambar. ST14 formed a clade with sequences from sheep (Fig. 2).

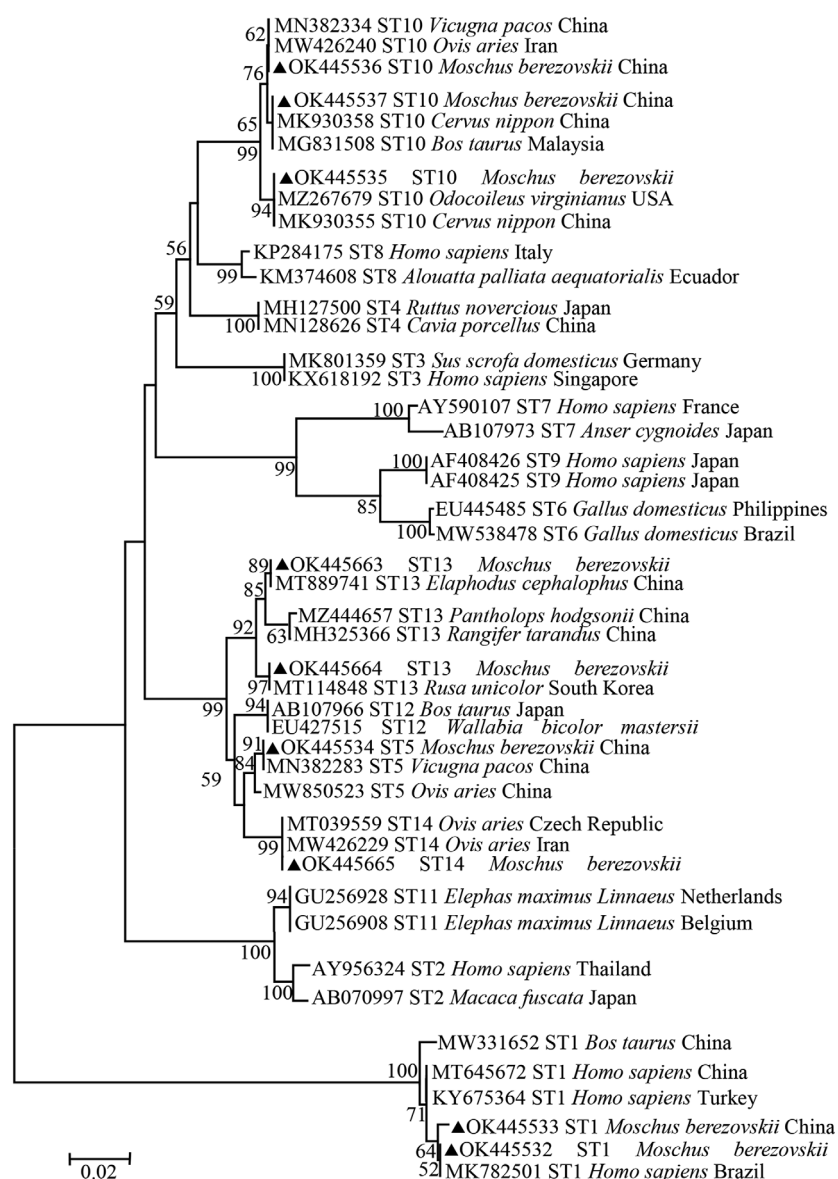
## Discussion

*Blastocystis* sp. is one of the most common parasites and is distributed globally. Epidemiological studies of *Blastocystis* sp. in wild ruminants, such as takin, bushbuck, red deer, fallow deer, white-lipped deer, giraffe and reindeer, have been reported, but there is no research on captive forest musk deer [18]. The prevalence of *Blastocystis* sp. in forest musk deer examined in this study was 14.7% (74/504), which is lower than that previously found in wild takin in China (57.1%, 28/49) [56], wild Père David's deer in China (56.3%, 72/128) [29], wild Korean water deer in Korea (40.8%, 51/125) [21], farmed Alpine musk deer in northwestern China (39.8%, 80/201) [49], farmed goats in Malaysia (30.9%, 73/236) [45], and farmed camels in Libya (24%, 47/196) [3]. However, the prevalence is higher than that observed in farmed sika deer in northeastern China (14.6%, 12/82) [47], wild reindeer in China (6.73%, 7/104) [48], farmed sika deer in northern China (0.8%, 6/760) [30], and farmed goats in Nepal (0.75%, 3/400) [15]. The reason for the different prevalence of *Blastocystis* sp. may be due to the captive conditions, management methods, the size of the examined samples, the animal species and different countries.

In this study, we found no statistical differences in the prevalence of *Blastocystis* sp. among females and males, nor between age groups ( $p > 0.05$ ). However, the infection prevalence in forest musk deer examined was significantly different depending on the geographical area of origin in Sichuan Province ( $p < 0.05$ ), with the highest prevalence (27.5%) in Dujiangyan and the lowest (4.1%) in Hanyuan. Previous reports have observed similar differences in the prevalence of this protist in cattle between different regions of China [57]. The different *Blastocystis* sp. prevalence may be related to the farm management methods and sanitary conditions in distinct regions. The higher prevalence in Dujiangyan and Maerkang is due to the lack of good immunization programs and deworming, as well as relatively poor hygiene conditions.

Five *Blastocystis* sp. subtypes (ST1, ST5, ST10, ST13 and ST14) were identified in 74 *Blastocystis* sp.-positive fecal samples from forest musk deer (Table 2). Maloney et al. used PCR and next generation amplicon sequencing to determine the occurrence and subtypes of *Blastocystis* sp. in white-tailed





**Figure 2.** Partial Phylogenetic relationships among nucleotide sequences of *Blastocystis* sp. partial small subunit ribosomal RNA (SSU rRNA) genes. The neighbor-joining method was used to construct the trees by the Kimura-2-parameter model. The numbers on the branches are percent bootstrap support values from 1000 replicates; only values of more than 50% are shown on the tree. Each sequence is identified by its accession number, subtype, host origin, and country. Sequences marked with black triangles are representative sequences identified in this study.

deer (*Odocoileus virginianus*) [27]. Ten previously reported subtypes (ST1, ST3, ST4, ST10, ST14, ST21, and ST23–ST26) and two novel subtypes (ST30 and ST31) were identified [27]. However, except for ST1, ST10 and ST14, none of the other subtypes found in white-tailed deer (*Odocoileus virginianus*) were identified in this study. Similarly, Ni et al used amplification of the SSU rDNA gene to confirm the presence of *Blastocystis* sp. infection in Père David's deer (*Elaphurus davidianus*) in the National Nature Reserve of Shishou, Hubei Province of China [29]. Five known subtypes, which consisted of one zoonotic subtype (ST10) and four ruminant-specific subtypes (ST21, ST23, ST25, and ST26), were identified [29]. However, except for ST10, the other subtypes found in Père David's deer were not found in forest musk deer in this study.

In China, ST10 is the main subtype in animal infection [26, 33, 38, 56, 57], followed by ST5 [37], and the results of the present study are in line with this conclusion. Zoonotic STs can be transmitted between humans and animals, and some animal-origin STs are linked to human infections [24, 36, 58]. One potentially zoonotic subtype identified in this study, ST1, has been reported as one of the most widespread subtypes in humans [50]. Previous studies have shown that the *Blastocystis* sp. subtypes ST1, ST2 and ST3 were commonly identified in primate hosts [11, 31, 55]. ST1 is also found in ruminants around the world, such as sika deer in China [9] and white-tailed deer in the United States [27]. Subtype ST1 was found in forest musk deer in this study (Table 2). Interestingly, the ST1 subtype variant detected in this study from Shimian farm

forest musk deer showed high similarity with known sequences from humans in China (MT645672), emphasizing that these STs have the potential for zoonotic transmission. ST5, another zoonotic subtype detected in this study, is the dominant subtype infecting hoofed animals like pigs and cattle worldwide [2, 5, 37, 50, 54]. Additionally, ST5 has also been detected in humans with animal contact history, demonstrating that this subtype has zoonotic transmission risk [41, 52]. For instance, ST5 has been detected in both pigs and humans in Jiangxi province, where children and pigs sometimes share common outdoor areas [52].

In contrast, although ST10 has rarely been detected in humans, it is very prevalent in Artiodactyla [3, 18, 50], such as waterbuck in Bangladesh [24], takin, yak, bushbuck, eland and reindeer in China [48, 56], and roe deer in Denmark [40]. The results of this study indicate that ST10 was the most prevalent *Blastocystis* sp. ST in forest musk deer, which is consistent with the previously reported dominance of ST10 in Artiodactyla [56]. Moreover, as ST10 was identified in all four forest musk deer farms, ST10 distribution is not limited to certain geographic locations and has a wider range than reported [3]. Surprisingly, ST13, relatively rare in this study, was also detected in a mouse deer in the United Kingdom [3] and Java mouse-deer in France [8]. So far, ST14 has mostly been detected in Artiodactyla such as sheep, camels, mouflon and cattle [3, 14, 26, 56]. A recent study reported that ST10 and ST14 can also infect humans [20].

## Conclusions

This study determined the occurrence, subtype distribution and genetic characteristics of *Blastocystis* sp. for the first time in captive forest musk deer in China. The data showed that five subtypes of *Blastocystis* sp. including two zoonotic subtypes (ST1 and ST5) can infect forest musk deer. These findings provide fundamental data for monitoring and exploring the transmission routes of *Blastocystis* sp. between forest musk deer and humans.

## Abbreviations

PCR	Polymerase chain reaction;
STs	Subtypes;
SSU rRNA	Small subunit ribosomal RNA;
IBS	Irritable bowel syndrome;
IBD	Inflammatory bowel disease;
ORs	Odds ratios.

**Acknowledgements.** We are very grateful to Liuchao You, Lian You, Han Dai and Xin Yu for their contribution to the comments on the manuscript, as well as to the assistance of the farm staff in sampling process and the help of Lijuan Liu, Yu Zeng and Wenqing Zhang in sample processing.

## Authors' contributions

Conceptualization, LD and GP; Data curation, ZZ (Ziyao Zhou), SC (Suizhong Cao), XM and LS; Formal analysis, SC (Shanyu Chen); Investigation, WM; Resources, XS, YC, HL, ZZ (Zhijun Zhong) and HF; Software, SC (Shanyu Chen); Supervision, GP; Writing – original draft, SC (Shanyu Chen);

Writing – review & editing, ZZ (Zhijun Zhong), HF, LD and GP. All authors have read and approved the manuscript.

## Funding

This work was funded by the Special Fund for Forestry Reform and Development of Wolong Special Administrative Region of Sichuan Province (510000-02-064387).

## Availability of data and materials

The nucleotide sequences generated in the present study have been deposited in GenBank (<https://www.ncbi.nlm.nih.gov/>) under accession numbers OK445532–OK445537, and OK445663–OK445665. The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

## Competing interests

The authors declare that they have no competing interests.

## References

- Ajjampur SS, Tan KS. 2016. Pathogenic mechanisms in *Blastocystis* spp. – Interpreting results from in vitro and in vivo studies. *Parasitology International*, 65(6 Pt B), 772–779.
- Alfellani MA, Stensvold CR, Vidal-Lapiedra A, Onuoha ES, Fagbenro-Beyioku AF, Clark CG. 2013. Variable geographic distribution of *Blastocystis* subtypes and its potential implications. *Acta Tropica*, 126(1), 11–18.
- Alfellani MA, Taner-Mulla D, Jacob AS, Imeede CA, Yoshikawa H, Stensvold CR, Clark CG. 2013. Genetic diversity of *Blastocystis* in livestock and zoo animals. *Protist*, 164(4), 497–509.
- Audebert C, Even G, Cian A, Group BI, Loywick A, Merlin S, Viscogliosi E, Chabé M. 2016. Colonization with the enteric protozoa *Blastocystis* is associated with increased diversity of human gut bacterial microbiota. *Scientific Reports*, 6, 25255.
- Badparva E, Sadraee J, Kheirandish F. 2015. Genetic diversity of *Blastocystis* isolated from cattle in khorramabad, Iran. *Jundishapur Journal of Microbiology*, 8(3), e14810.
- Beghini F, Pasolli E, Truong TD, Putignani L, Cacciò SM, Segata N. 2017. Large-scale comparative metagenomics of *Blastocystis*, a common member of the human gut microbiome. *ISME Journal*, 11(12), 2848–2863.
- Boorom KF, Smith H, Nimri L, Viscogliosi E, Spanakos G, Parkar U, Li LH, Zhou XN, Ok UZ, Leelayoova S, Jones MS. 2008. Oh my aching gut: irritable bowel syndrome, *Blastocystis*, and asymptomatic infection. *Parasites & Vectors*, 1(1), 40.
- Cian A, El Safadi D, Osman M, Moriniere R, Gantois N, Benamrouz-Vanneste S, Delgado-Viscogliosi P, Guyot K, Li LL, Monchy S, Noël C, Poirier P, Nourrisson C, Wawrzyniak I, Delbac F, Bosc S, Chabé M, Petit T, Certad G, Viscogliosi E. 2017. Molecular epidemiology of *Blastocystis* sp. in various animal groups from two French zoos and evaluation of potential zoonotic risk. *PloS One*, 12(1), e0169659.
- Deng L, Yao J, Chen S, He T, Chai Y, Zhou Z, Shi X, Liu H, Zhong Z, Fu H, Peng G. 2021. First identification and molecular subtyping of *Blastocystis* sp. in zoo animals in southwestern China. *Parasites & Vectors*, 14 (1), 11.

10. Clark CG, van der Giezen M, Alfellani MA, Stensvold CR. 2013. Recent developments in *Blastocystis* research. *Advances in Parasitology*, 82, 1–32.
11. Deng L, Yao JX, Liu HF, Zhou ZY, Chai YJ, Wang WY, Zhong ZJ, Deng JL, Ren ZH, Fu HL, Yan X, Yue CJ, Peng GN. 2019. First report of *Blastocystis* in giant pandas, red pandas, and various bird species in Sichuan province, southwestern China. *International Journal for Parasitology, Parasites and Wildlife*, 9, 298–304.
12. Deng XZ, Luo Q, Li YM. 2014. Musk artificial breeding technology, 2014. *Animal husbandry*, 15, 53–54.
13. Dogruman-AI F, Kustimur S, Yoshikawa H, Tuncer C, Simsek Z, Tanyuksel M, Araz E, Boorum K. 2009. *Blastocystis* subtypes in irritable bowel syndrome and inflammatory bowel disease in Ankara, Turkey. *Memórias do Instituto Oswaldo Cruz*, 104(5), 724–727.
14. Fayer R, Santin M, Macarasin D. 2012. Detection of concurrent infection of dairy cattle with *Blastocystis*, *Cryptosporidium*, *Giardia*, and *Enterocytozoon* by molecular and microscopic methods. *Parasitology Research*, 111(3), 1349–1355.
15. Ghimire TR, Bhattarai N. 2019. A survey of gastrointestinal parasites of goats in a goat market in Kathmandu, Nepal. *Journal of Parasitic Diseases*, 43(4), 686–695.
16. Green MJB. 1985. Aspects of the ecology of the Himalayan Musk Deer. The University of Cambridge: Cambridge.
17. Higuera A, Herrera G, Jimenez P, García-Corredor D, Pulido-Medellín M, Bulla-Castañeda DM, Pinilla JC, Moreno-Pérez DA, Maloney JG, Santín M, Ramírez JD. 2021. Identification of multiple *Blastocystis* subtypes in domestic animals from Colombia using amplicon-based next generation sequencing. *Frontiers in Veterinary Science*, 24(8), 732129.
18. Hublin JSY, Maloney JG, Santin M. 2021. *Blastocystis* in domesticated and wild mammals and birds. *Research in Veterinary Science*, 135, 260–282.
19. Jimenez-Gonzalez DE, Martinez-Flores WA, Reyes-Gordillo J, Ramirez-Miranda ME, Arroyo-Escalante S, Romero-Valdovinos M, Stark D, Souza-Saldivar V, Martinez-Hernandez F, Flisser A, Olivo-Diaz A, Maravilla P. 2012. *Blastocystis* infection is associated with irritable bowel syndrome in a Mexican patient population. *Parasitology Research*, 110(3), 1269–1275.
20. Khaled S, Gantois N, Ly AT, Senghor S, Even G, Dautel E, Dejager R, Sawant M, Baydoun M, Benamrouz-Vanneste S, Chabé M, Ndiaye S, Schacht AM, Certad G, Riveau G, Viscogliosi E. 2020. Prevalence and subtype distribution of *Blastocystis* sp. in Senegalese school children. *Microorganisms*, 8(9), 1408.
21. Kim KT, Noh G, Lee H, Kim SH, Jeong H, Kim Y, Jheong WH, Oem JK, Kim TH, Kwon OD, Kwak D. 2020. Genetic diversity and zoonotic potential of *Blastocystis* in Korean Water Deer, *Hydropotes inermis argyropus*. *Pathogens*, 9(11), 955.
22. Leelayoova S, Siripattanapipong S, Thathaisong U, Naaglor T, Taamasri P, Piyaraj P, Mungthin M. 2008. Drinking water: a possible source of *Blastocystis* spp. subtype 1 infection in schoolchildren of a rural community in central Thailand. *American Journal of Tropical Medicine and Hygiene*, 79(3), 401–406.
23. Li IL, Tan TC, Karmacharya BM, Govind SK. 2012. *Blastocystis* sp.: waterborne zoonotic organism, a possibility? *Parasites & Vectors*, 5, 130.
24. Li J, Karim MR, Li D, Rahaman Sumon SMM, Siddiki SHMF, Rume FI, Sun R, Jia Y, Zhang L. 2019. Molecular characterization of *Blastocystis* sp. in captive wildlife in Bangladesh National Zoo: non-human primates with high prevalence and zoonotic significance. *International Journal for Parasitology, Parasites and Wildlife*, 10, 314–320.
25. Li LH, Zhang XP, Lv S, Zhang L, Yoshikawa H, Wu Z, Steinmann P, Utzinger J, Tong XM, Chen SH. 2007. Cross-sectional surveys and subtype classification of human *Blastocystis* isolates from four epidemiological settings in China. *Parasitology Research*, 102, 83–90.
26. Li WC, Wang K, Gu Y. 2018. Occurrence of *Blastocystis* sp. and *Pentatrichomonas hominis* in sheep and goats in China. *Parasites & Vectors*, 11(1), 93.
27. Maloney JG, Jang Y, Molokin A, George NS, Santin M. 2021. Wide genetic diversity of *Blastocystis* in white-tailed deer (*Odocoileus virginianus*) from Maryland, USA. *Microorganisms*, 9(6), 1343.
28. Maloney JG, Santin M. 2021. Mind the Gap: New full-length sequences of *Blastocystis* subtypes generated via Oxford nanopore minion sequencing allow for comparisons between full-length and partial sequences of the small subunit of the ribosomal RNA Gene. *Microorganisms*, 9(5), 997.
29. Ni F, Yu F, Yang X, An Z, Ge Y, Liu X, Qi M. 2022. Identification and genetic characterization of *Blastocystis* subtypes in Père David's deer (*Elaphurus davidianus*) from Shishou, China. *Veterinary Research Communications*, 19. Advance online publication. <https://doi.org/10.1007/s11259-022-09905-8>.
30. Ni HB, Gong QL, Zhang NZ, Zhao Q, Tao WF, Qiu HY, Fei YC, Zhang XX. 2021. Molecular detection of *Blastocystis* in black bears and sika deer in northern China. *Parasitology Research*, 120(4), 1481–1487.
31. Parkar U, Traub RJ, Vitali S, Elliot A, Levecke B, Robertson I, Geurden T, Steele J, Drake B, Thompson RC. 2010. Molecular characterization of *Blastocystis* isolates from zoo animals and their animal keepers. *Veterinary Parasitology*, 169(1–2), 8–17.
32. Rajamanikam A, Hooi HS, Kudva M, Samudi C, Kumar S. 2019. Resistance towards metronidazole in *Blastocystis* sp.: A pathogenic consequence. *PloS One*, 14(2), e0212542.
33. Ren M, Song JK, Yang F, Zou M, Wang PX, Wang D, Zhang HJ, Zhao GH, Lin Q. 2019. First genotyping of *Blastocystis* in yaks from Qinghai Province, northwestern China. *Parasites & Vectors*, 12(1), 171.
34. Scanlan PD, Stensvold CR. 2013. *Blastocystis*: getting to grips with our guileful guest. *Trends in Parasitology*, 29(11), 523–529.
35. Scicluna SM, Tawari B, Clark CG. 2006. DNA Barcoding of *Blastocystis*. *Protist*, 157, 77–85.
36. Sharif Y, Abbasi F, Shahabi S, Zarai A, Mikaeili F, Sarkari B. 2020. Comparative genotyping of *Blastocystis* infecting cattle and human in the south of Iran. *Comparative Immunology, Microbiology and Infectious Diseases*, 72, 101529.
37. Song JK, Hu RS, Fan XC, Wang SS, Zhang HJ, Zhao GH. 2017. Molecular characterization of *Blastocystis* from pigs in Shaanxi province of China. *Acta Tropica*, 173, 130–135.
38. Song JK, Yin YL, Yuan YJ, Tang H, Ren GJ, Zhang HJ, Li ZX, Zhang YM, Zhao GH. 2017. First genotyping of *Blastocystis* sp. in dairy, meat, and cashmere goats in northwestern China. *Acta Tropica*, 176, 277–282.
39. Song Y, Li W, Liu H, Zhong Z, Luo Y, Wei Y, Fu W, Ren Z, Zhou Z, Deng L, Cheng J, Peng G. 2018. First report of *Giardia duodenalis* and *Enterocytozoon bieneusi* in forest musk deer (*Moschus berezovskii*) in China. *Parasites & Vectors*, 11(1), 204.
40. Stensvold CR, Alfellani MA, Nørskov-Lauritsen S, Prip K, Victory EL, Maddox C, Nielsen HV, Clark CG. 2009. Subtype distribution of *Blastocystis* isolates from synanthropic and zoo animals and identification of a new subtype. *International Journal for Parasitology*, 39(4), 473–479.
41. Stensvold CR, Clark CG. 2016. Current status of *Blastocystis*: A personal view. *Parasitology International*, 65(6 Pt B), 763–771.

42. Stensvold CR, Clark CG. 2020. Pre-empting Pandora's Box: *Blastocystis* subtypes revisited. *Trends in Parasitology*, 36(3), 229–232.
43. Stensvold CR, Tan KSW, Clark CG. 2020. *Blastocystis*. *Trends in Parasitology*, 36(3), 315–316.
44. Tan KSW. 2008. New insights on classification, identification, and clinical relevance of *Blastocystis* spp. *Clinical Microbiology Reviews*, 21(4), 639–665.
45. Tan TC, Tan PC, Sharma R, Sugnaseelan S, Suresh KG. 2013. Genetic diversity of caprine *Blastocystis* from Peninsular Malaysia. *Parasitology Research*, 112(1), 85–89.
46. Tito RY, Chaffron S, Caenepeel C, Lima-Mendez G, Wang J, Vieira-Silva S, Falony G, Hildebrand F, Darzi Y, Rymenans L, Verspecht C, Bork P, Vermeire S, Joossens M, Raes J. 2019. Population-level analysis of *Blastocystis* subtype prevalence and variation in the human gut microbiota. *Gut*, 68, 1180–1189.
47. Wang J, Gong B, Liu X, Zhao W, Bu T, Zhang W, Liu A, Yang F. 2018. Distribution and genetic diversity of *Blastocystis* subtypes in various mammal and bird species in northeastern China. *Parasites & Vectors*, 11(1), 522.
48. Wang J, Gong B, Yang F, Zhang W, Zheng Y, Liu A. 2018. Subtype distribution and genetic characterizations of *Blastocystis* in pigs, cattle, sheep and goats in northeastern China's Heilongjiang Province. *Infection, Genetics and Evolution*, 57, 171–176.
49. Wang Q, Liu X, Li Y, Xin L, Zhou X, Yu F, Zhao A, Qi M. 2022. Genetic diversity of *Blastocystis* subtypes in the Alpine musk deer (*Moschus chrysogaster*) in Gansu province, north-western China. *Journal of Eukaryotic Microbiology*, e12910.
50. Wang W, Owen H, Traub RJ, Cuttill L, Inpankaew T, Bielefeldtohm H. 2014. Molecular epidemiology of *Blastocystis* in pigs and their in-contact humans in Southeast Queensland, Australia, and Cambodia. *Veterinary Parasitology*, 203(3–4), 264–269.
51. Yang Q, Meng X, Xia L, Feng Z. 2003. Conservation status and causes of decline of musk deer (*Moschus* spp.) in China. *Biological Conservation*, 109(3), 333–342.
52. Yan Y, Su S, Ye J, Lai X, Lai R, Liao H, Chen G, Zhang R, Hou Z, Luo X. 2007. *Blastocystis* sp. subtype 5: a possibly zoonotic genotype. *Parasitology Research*, 101(6), 1527–1532.
53. Yason JA, Liang YR, Png CW, Zhang Y, Tan KSW. 2019. Interactions between a pathogenic *Blastocystis* subtype and gut microbiota: in vitro and in vivo studies. *Microbiome*, 7(1), 30.
54. Yoshikawa H, Abe N, Wu Z. 2004. PCR-based identification of zoonotic isolates of *Blastocystis* from mammals and birds. *Microbiology*, 150(Pt 5), 1147–1151.
55. Yoshikawa H, Wu Z, Pandey K, Pandey BD, Sherchand JB, Yanagi T, Kanbara H. 2009. Molecular characterization of *Blastocystis* isolates from children and rhesus monkeys in Kathmandu, Nepal. *Veterinary Parasitology*, 160(3–4), 295–300.
56. Zhao GH, Hu XF, Liu TL, Hu RS, Yu ZQ, Yang WB, Wu YL, Yu SK, Song JK. 2017. Molecular characterization of *Blastocystis* sp. in captive wild animals in Qinling Mountains. *Parasitology Research*, 116(8), 2327–2333.
57. Zhu W, Tao W, Gong B, Yang H, Li Y, Song M, Lu Y, Li W. 2017. First report of *Blastocystis* infections in cattle in China. *Veterinary Parasitology*, 246, 38–42.
58. Zhu W, Wei Z, Li Q, Lin Y, Yang H, Li W. 2020. Prevalence and subtype diversity of *Blastocystis* in human and nonhuman primates in North China. *Parasitology Research*, 119(8), 2719–2725.

**Cite this article as:** Chen S, Meng W, Shi X, Chai Y, Zhou Z, Liu H, Zhong Z, Fu H, Cao S, Ma X, Shen L, Deng L & Peng G. 2022. Occurrence, genetic diversity and zoonotic potential of *Blastocystis* sp. in forest musk deer (*Moschus berezovskii*) in Southwest China. *Parasite* 29, 34.



An international open-access, peer-reviewed, online journal publishing high quality papers on all aspects of human and animal parasitology

Reviews, articles and short notes may be submitted. Fields include, but are not limited to: general, medical and veterinary parasitology; morphology, including ultrastructure; parasite systematics, including entomology, acarology, helminthology and protistology, and molecular analyses; molecular biology and biochemistry; immunology of parasitic diseases; host-parasite relationships; ecology and life history of parasites; epidemiology; therapeutics; new diagnostic tools.

All papers in Parasite are published in English. Manuscripts should have a broad interest and must not have been published or submitted elsewhere. No limit is imposed on the length of manuscripts.

**Parasite** (open-access) continues **Parasite** (print and online editions, 1994–2012) and **Annales de Parasitologie Humaine et Comparée** (1923–1993) and is the official journal of the Société Française de Parasitologie.

Editor-in-Chief:  
Jean-Lou Justine, Paris

Submit your manuscript at  
<http://parasite.edmgr.com/>