Pakistan J. Zool., vol. 53(6), pp 2167-2173, 2021 DOI: https://dx.doi.org/10.17582/journal.pjz/20200616020657

A New Record of *Hepatozoon* sp. Miller, 1908 (Apicomplexa: Adelerina) Infecting the Snake *Naja atra* (Squamata: Dipsadidae) in China

Nianhong Huang, Yan Wu, Yuanyuan Li* and Jinhong Zhao*

Department of Parasitology, Wannan Medical College, Wuhu, Anhui, China.

ABSTRACT

Species of the genus *Hepatozoon* are the most common haemoparasites infecting snakes. High parasitemia of *Hepatozoon* can negatively impact snake fitness, growth rate and reproductive output. Given that snakes are a valuable source of traditional Chinese medicinal materials and food consumption, stresses the importance of conducting a study on snakes and its parasites. The present study investigated three kinds of snakes, *Elaphe carinata*, *Naja atra* and *Ptyas mucosus* from Wuhu, Anhui province, China. The results show that *Hepatozoon* sp. were only detected and characterized found in the snakes *Elaphe carinata* and *Naja atra.* Mature gamonts were generally long and elliptic, both ends obtuse and one end slightly pointed under the blood smear examinations. Morphological comparison of parasitized and non-parasitized erythrocytes showed that most analyzed features were significantly different for both linear and area dimensions. The sequences of 18S rRNA gene of *Hepatozoon* sp. were amplified, cloned and demonstrated the homology and the variation. Phylogenetic analysis based on the 18S rRNA gene analyzed in the present study together with other published *Hepatozoon* species showed that there is no significant difference in the geographical distribution and limited host specificity. This is the first time to report the infection of *Hepatozoon* sp. in *Naja atra* for the endemic species in China and increases the number of known host species. Morphological and molecular analysis of *Hepatozoon* sp. establishes a basis for identification of the genera *Hepatozoon,* increasing protection and prevention of parasitic diseases of snakes.

Article Information
Received 16 June 2020
Revised 30 July 2020
Accepted 08 August 2020
Available online 15 September 2021

Authors' Contribution
NH analysed the data and wrote the manuscript. YW performed the experiments. YL helped in performing the analysis with constructive discussions. JZ conceived and designed the experiments, performed the data analyses. YL and JZ approved the final draft.

Key words
***Hepatozoon, Naja atra*, Morphology, Phylogenetic analysis**

INTRODUCTION

Species of the genus *Hepatozoon* are the most common intracellular haemoparasites found in reptiles, amphibians and mammals (Mitchell, 2011; Wozniak *et al*., 1996). The life cycles of *Hepatozoon* species is characterized by heteroxenous, with tissue merogony and gametogony occurring in vertebrate host, while a sexual cycle and sporogony occur in invertebrate host (Soares *et al*., 2017). *Hepatozoonosis* is a protozoan disease caused by polypide of Heamogregarinidae and *Hepatozoon*, transmitting by ticks (Baneth *et al*., 2003; Xiao *et al*., 2019). Study on snakes have indicated diverse influences of *Hepatozoon* on the hosts, ranging from reports of only trivial consequences for host fitness, to severe effects on host growth rate and reproductive output (Madsen *et al*., 2005). Studies of these parasites are therefore necessary, not only to better characterize this component of biodiversity, but also to assess if parasites may pose a risk to host populations (Pedersen *et al*., 2007).

To date, more than 300 *Hepatozoon* species have been identified, among them more than 120 species were described from snakes (Han *et al*., 2015). Cook *et al*. (2018) described two new species of *Hepatozoon* parasitizing species of *Philothamnus* (Ophidia: Colubridae) from South Africa (Cook *et al*., 2018). Snakes (*Crotalus durissus*, *Epicrates crassus* and *Boa constrictor*) from Brazil were found positive for *Hepatozoon* sp. with the positive rate of 12.78% (20/157) (Ungari *et al*., 2018). However, only three reports about *Hepatozoon* sp. have been previously reported in snakes from China (Han *et al*., 2015; Xiao *et al*., 2019), even though it has been widely recognized around the world. *Elaphe* is one of the main genera of rat snakes. *Hepatozoon chinensis* was first found in *Elaphe carinata,* with the infection rate of 100% (Han *et al*., 2015); *Naja* is one of the main genera of cobra snakes. Nevertheless, there are not yet any reports or information about the infection of *Hepatozoon* with *Naja atra* currently. The present study investigated three kinds of snakes, *Elaphe carinata*, *Naja atra* and *Ptyas mucosus.* As a result, we only detected and characterized the *Hepatozoon* sp. found in snakes *Elaphe carinata* and *Naja atra.* For further molecular analyses, the 18S rRNA gene of *Hepatozoon* was sequenced and used to analyze the phylogenetic relationships among different hosts and geographical distribution. To our knowledge,

* Corresponding author: 414299385@qq.com; jhjhzhao@aliyun.com
0030-9923/2021/0006-21 $ 9.00/0

this is the first report of *Hepatozoon* sp. infection in *Naja atra* which is an endemic species in China.

MATERIALS AND METHODS

Sampling and slide examination

Thirteen snakes were captured from various snake farms of Wuhu, Anhui province, China during March and December 2019, including 4 *Elaphe carinata*, 5 *Naja atra* and 4 *Ptyas mucosus*. Snakes were collected by hand, identified, and digitally photographed. Tail tips were removed and blood from the tail was used to make blood smears for microscopic examination. Blood smears were prepared immediately upon collection and three smears were made per snake. The smears were air dried, stained with 10% Giemsa for 30 min, and then examined for haemoparasites using an Olympus BX51 microscope (Olympus, Japan) at × 1000 magnification with a digital camera.

The morphology and morphometry of the gamonts, and the changes in infected erythrocytes caused by the parasites were analyzed using a computer image analysis system and measured using Olympus stream software (Olympus, Japan). The analyzed variables included: area, length and width of the parasite and the parasite nucleus; area, length and width of infected and uninfected erythrocytes and their nuclei. There were 8 infected erythrocytes and 20 uninfected erythrocytes were examined for every host.

All recorded data were analyzed by SPSS statistical software (SPSS for Windows 16.0, SPSS Inc., Chicago, IL, USA). Differences among individuals were tested by one-way ANOVA and Duncan's test. Differences between normal and infected erythrocytes were compared using *t*-tests. Values of $P<0.05$, indicating significant differences, and values of $P<0.01$, indicating highly significant differences.

Molecular detection

DNA was extracted from blood samples using TIAName Blood DNA kit (TIANGEN, Beijing, China), following the manufacturer's instructions. Detection of the presence of *Hepatozoon* species was initially made using PCR reactions with the primers HEMO1 and HEMO2 (Han *et al*., 2015), targeting part of the 18S rRNA gene. Samples were then also used in a further PCR reaction using the primers HepF300 and HepR900 (Han *et al*., 2015), targeting another part of the 18S rRNA gene (Table I).

The PCR reactions were run in a 25µl reaction mixture containing 12.5µl of Premix Taq, 1.0µl of each primer, 8.5µl ddH_2O and 2.0µl of DNA. The reaction mix was heated to 94℃ for 1 min, and then amplification was performed through 35 cycles at 94 °C for 30 sec, 56 °C for 30 min, and 56 °C for 2 min, followed by a final 10 min extension at 72 °C. Negative and positive controls were run with each reaction and then were detected by 2% agarose gel electrophoresis. Positive PCR products were purified, cloned and sequenced by a commercial sequencing facility (General Biosystems Co., Ltd., Anhui, China).

Phylogenetic analysis

Consensus sequences of 18S rRNA were created by combining the sequences of the two partially-overlapping regions from the product of PCR. The sequences were subjected to the basic local alignment search tool (BLAST) sequence similarity search to identify the most similar available sequences. 18S rRNA sequences of named or unnamed *Hepatozoon* species were retrieved from GenBank using Clustal X2.1 software implemented in BioEdit. Phylogenetic relationships were estimated using the Neighbor-joining method (NJ) with Mega 7 and conducted 1000 replicate heuristic searches.

Table I. Primer sequences (Han *et al.*, 2015).

Name	Sequence (5'-3')
HEMO1	TATTGGTTTTAAGAACTAATTTTATGATTG
HEMO2	CTTCTCCTTCCTTTAAGTGATAAGGTT CAC
HepF300	GTTTCTGACCTATCAGCTTTCGACG
HepR900	CAAATCTAAGAATTTCACCTCTGAC

RESULTS

Morphological characteristics

The present study detected and characterized the *Hepatozoon* sp. in 13 snakes (4 *Elaphe carinata,* 5 *Naja atra* and 4 *Ptyas mucosus*). Among the 13 snakes, 1 sample was positive to *Hepatozoon* with an infection rate of 25.0% (1/4) in *Elaphe carinata*, and 2 samples were positive to *Hepatozoon* with an infection rate of 40.0% (2/5) in *Naja atra*. *Hepatozoon* sp. gamonts were all found in the positive blood smears from *Elaphe carinata* and *Naja atra*. However, no *Hepatozoon* sp. gamonts were found in the smears of *Ptyas mucosus.*

Mature gamonts were generally long and elliptic, both ends obtuse or one end slightly pointed. Some of them bend slightly to one side in the shape of a kidney. Nucleus of gamonts were centrally located or extended into the quarter. Some infected erythrocytes became slightly hypertrophic and nucleus of infected erythrocytes were flatter than uninfected erythrocytes (Fig. 1). There was a total of 24 infected erythrocytes and gamonts and 60 uninfected erythrocytes were measured for three snakes. The average

morphometric measurement of gamonts were: parasite whole cell (area = 39.66 ± 6.89 μm², n=24; length = 13.42 ± 0.87 μm, n=24; width = 2.57 ± 0.38 μm, n=24); nucleus (area = 4.83 ± 0.46 μm², n=24; length = 3.75 + 0.59 μm, n=24; width = 1.55 ± 0.20 μm, n=24) (Table II). Infected erythrocytes swelled slightly; they were measured a length of 16.24 ± 1.28 μm (14.44-19.34, n=24), width of 9.48 ± 0.91 μm (8.23-11.74, n=24) and an area of 124.42 ± 16.51 μm² (98.87-155.52, n=24). Uninfected erythrocytes were measured for a length of 15.18 ± 0.93 μm (13.02-17.21, n=60) and a width of 9.58 ± 0.94 μm (7.30-11.48, n=60) with an area of 114.68 ± 15.69 μm² (83.82-144.70, n=60). The length and area of infected erythrocytes were all greater than those of uninfected erythrocytes (P<0.05), but the width was similar (P=0.07) (Table III). The nuclei of infected erythrocytes were usually forced to one side of the host cell and were irregular. They measured a length of 7.46 ± 0.78 μm (6.11-8.90, n=24) and a width of 3.33 ± 0.82 μm (2.22-4.69, n=24) with an area of 25.11 ± 3.40 μm² (20.23-33.31, n=24). The nuclei of uninfected erythrocytes measured a length of 6.70 ± 0.84 μm (4.71-9.06, n=60) and a width of 4.72 ± 0.69 μm (3.15-6.10, n=60) with an area of 27.06 ± 6.18 μm² (15.14-50.38, n=60). The length of nuclei in infected erythrocytes was greater than those in uninfected erythrocytes (P<0.01), the width of nuclei in infected erythrocytes was smaller than those in uninfected erythrocytes (P<0.01), but the areas were similar (P = 0.205).

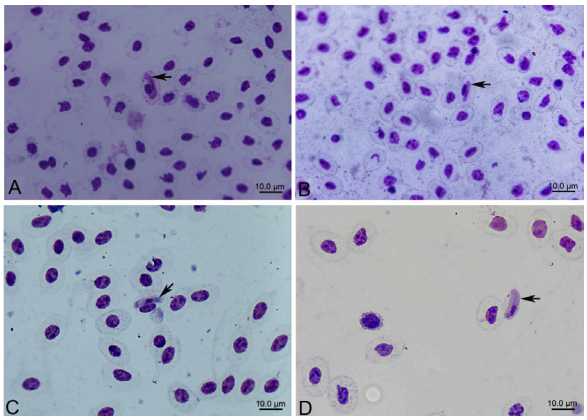

Fig. 1. *Hepatozoon* sp. gamonts from *Elaphe carinata* and *Naja atra.* Blood smears stained by Giemsa (×1000). (A) the gamont folded back hook-wise at one end, nucleus of the gamont located in the central, infected erythrocytes became slightly hypertrophic; (B) nucleus of the gamont extended into the quarter, nucleus of infected erythrocytes was flatter than uninfected erythrocytes and infected erythrocytes became slightly hypertrophic; (C) the gamont's both ends were obtuse and bend slightly to one side in the shape of a kidney, nucleus of the gamont located in the center, nucleus of infected erythrocytes was forced to one side of the host cell and flatter than in the uninfected erythrocytes; (D) gamont was outside of the erythrocyte and nucleus of the gamont extended into the quarter. Arrow indicates gamont of *Hepatozoon* sp.

Table II. Comparative analysis of *Hepatozoon* sp. gamonts in 3 specimens of naturally-infected snakes from Wuhu, China.

Specimen	LE	WE	AE	LEN	WEN	AEN
EC1	12.63±0.98^{b}	2.50±0.74^{ab}	36.15±6.25^{b}	3.23±0.37^{b}	1.70±0.28^{b}	6.22±1.08^{a}
NA2	15.60±1.45^{a}	3.32±0.40^{a}	57.9±7.67^{a}	5.27±1.30^{a}	2.05±0.33^{a}	10.01±0.97^{a}
NA3	12.91±1.86^{b}	2.25±0.83^{b}	36.15±15.31^{b}	3.38±1.17^{b}	1.18±0.15^{c}	6.10±0.68^{a}

EC1, *Elaphe carinata* sample; NA2 and NA3, *Naja atra* sample; LE, length of gamont; WE, width of gamont; AE, area of gamont; LEN, length of gamont nucleus; WEN, width of gamont nucleus; AEN, area of gamont nucleus. Column data marked with the same letter shows no significant difference (P>0.05); with the different letter shows significant difference (P<0.05). All values represent mean ± standard deviation.

Table III. Comparative analysis of normal and *Hepatozoon*-parasitized erythrocytes in 3 naturally-infected specimens of snakes from Wuhu, China.

Specimen		LE	WE	AE	LEN	WEN	AEN
EC1	PE	15.94±1.04^{a}	9.21±0.48^{a}	118.96±15.09^{a}	7.49±0.64^{a}	3.60±0.62^{a}	24.30±2.89^{a}
	NE	14.70±0.98^{b}	9.08±0.77^{a}	105.55±13.44^{b}	6.30±0.92^{b}	4.42±0.49^{b}	24.05±4.30^{a}
NA2	PE	16.70±2.25^{a}	10.00±1.34^{a}	129.67±9.88^{a}	7.94±0.60^{a}	3.91±0.81^{a}	28.55±3.89^{a}
	NE	15.40±0.79^{b}	9.46±0.98^{a}	114.60±14.92^{b}	7.00±0.82^{b}	4.91±0.71^{b}	29.07±8.06^{a}
NA3	PE	16.23±0.95^{a}	9.43±0.93^{a}	125.89±14.10^{a}	7.20±0.91^{a}	2.84±0.73^{a}	24.01±2.61^{a}
	NE	15.45±0.86^{b}	10.19±0.74^{b}	123.89±5.95^{a}	6.79±0.63^{a}	4.82±0.75^{b}	27.92±4.57^{b}

For abbreviations and statistical details, see Table II.

Molecular characteristics

The 18S rRNA gene of *Hepatozoon* sp. was all amplified from the 13 snakes blood samples (4 *Elaphe carinata,* 5 *Naja atra* and 4 *Ptyas mucosus*) by PCR. Only specimens that appeared infected when the blood smears were analyzed gave real positive PCR results from one *Elaphe carinata* and two *Naja atra*. The molecular result of PCR was consistent with the morphological result of blood smears. The positive PCR results for two gene regions, in turn, gave rise to aligned sequences of 1,416 bp of the 18S rRNA gene. Three sequences, one *Elaphe carinata* and two *Naja atra*, were 100% identical to each other. The sequences were submitted to GenBank (accession number MT114683) and were subjected to the basic local alignment search tool (BLAST) sequence similarity search to identify the most similar available sequences.

Phylogenetic analysis

The sequences generated in this study (MT114683) were aligned with related sequences retrieved from GenBank (Table IV), nucleotide sequence analysis which demonstrated the homology and the variation between them. The results showed that the homology of *Hepatozoon*

Table IV. Related species used in the phylogenetic analysis.

Species	Host	Geographical origin	GenBank accession number
Hepatozoon sp.	*Clethrionomys glareolus*	Spain	AY600625
			AY600626
Hepatozoon felis	*Felis catus*	Spain	AY620232
			AY628681
Hepatozoon ayorgbor	*Python regius*	Ghana	EF157822
Hepatozoon sp.	*Abrothrix olivaceus*	Chile	FJ719815
	Abrothrix sanborni	Chile	FJ719816
	Abrothrix olivaceus	Chile	FJ719818
	Abrothrix sanborni	Chile	FJ719819
	Lycognathophis seychellensis	Seychelles	HQ292773
			HQ292774
	Quedenfeldtia moerens	Morocco	HQ734789
	Podarcis vaucheri	Morocco	HQ734795
	Myodes glareolus	Hungary	JX644996
			JX644997
			JX644998
	Elaphe carinata	China	KF939622
			KF939626
	Caiman yacare	Brazil	KJ413132
			KJ413133
Hepatozoon canis	*Vulpes vulpes*	Czech republic	KU893123
	Canis familiaris	Czech republic	KU893126
Hepatozoon felis	*Asiatic lion*	India	KX017290
Hepatozoon caimani	*Caiman crocodilus*	Brazil	MF435048
Hepatozoon canis	*Canis lupus familiaris*	Australia	MG062866
Hepatozoon sp.	*Gallotia galloti*	Spain	MG787244
	Gallotia caesaris	Spain	MG787247
Hepatozoon canis	*Canis lupus familiaris*	Israel	MK091087
Adelina bambarooniae	*Dermolepida albohirtum*	Australia	AF494058

sp. gene in this study with the reported *Hepatozoon* genes sequence in GenBank was 95.1%~99.7%. New sequences in this study have the highest sequence homology 99.7% compared with the reported *Hepatozoon* sp. isolated from Morocco (HQ734795) in GenBank. The homology of *Hepatozoon* sp. gene in this study with the reported *Hepatozoon* sp. gene sequence also isolated from China (KF939622, KF939626) was 98.7% and 99.1%, respectively.

Phylogenetic relationships were estimated using Neighbor-joining method (NJ) in this study. 18S rRNA sequences of 29 *Hepatozoon* (Family: Hepatozoidae) species and outgroup species from the Family Adeleidae (*Adelina bambarooniae*) were used for the phylogenetic analysis. The phylogenetic results showed that the grouping of the *Hepatozoon* lineages could be divided into two clades. One clade was constituted by *Hepatozoon* lineages found in caimans, lizards, snakes and murids. The other clade was constituted by *Hepatozoon* lineages found in lizards, felines and canines (Fig. 2). The *Hepatozoon* sp. obtained in this study found in snakes was determined to be most closely related to *Hepatozoon* sp. found in lizards (*Quedenfeldtia moerens,* HQ734789) from Morocco, and then to *Hepatozoon* sp. found in snakes (*Lycognathophis seychellensis,* HQ292773, HQ292774) from Seychelles (Fig. 2). In addition, the *Hepatozoon* sp. found in snakes (*Elaphe carinata,* KF939622, KF939626) also from China was determined to be most closely related to *Hepatozoon* sp. found in snakes (*Python regius,* EF157822) from Ghana. In the phylogenetic tree, as an external population, *Adelina bambarooniae* is most distantly related to any other genus. From the above, there might be no significant difference in the geographical distribution of *Hepatozoon* parasites and limited host specificity. For example, most similar *Hepatozoon* sp. isolated appears to infect the hosts which belong to the same family, but these hosts are from different countries, such as the host caimans, snakes, murids, felines and canines. However, the lizards occupy two branches and shows a relatively distant genetic relationship.

DISCUSSION

Snakes are valuable source of traditional Chinese medicinal materials and used for food. Snake farming in China and Southeast Asia has greatly increased over the last twenty years, and the total quantity of snakes traded in China is estimated to be 7000-9000 tons every year (Xiao *et al*., 2019; Zhihua, 2004). Therefore, the protection of snakes is closely related to the development of the snake industry and traditional Chinese medicine. Currently, there are many reports on the genus *Hepatozoonosis*

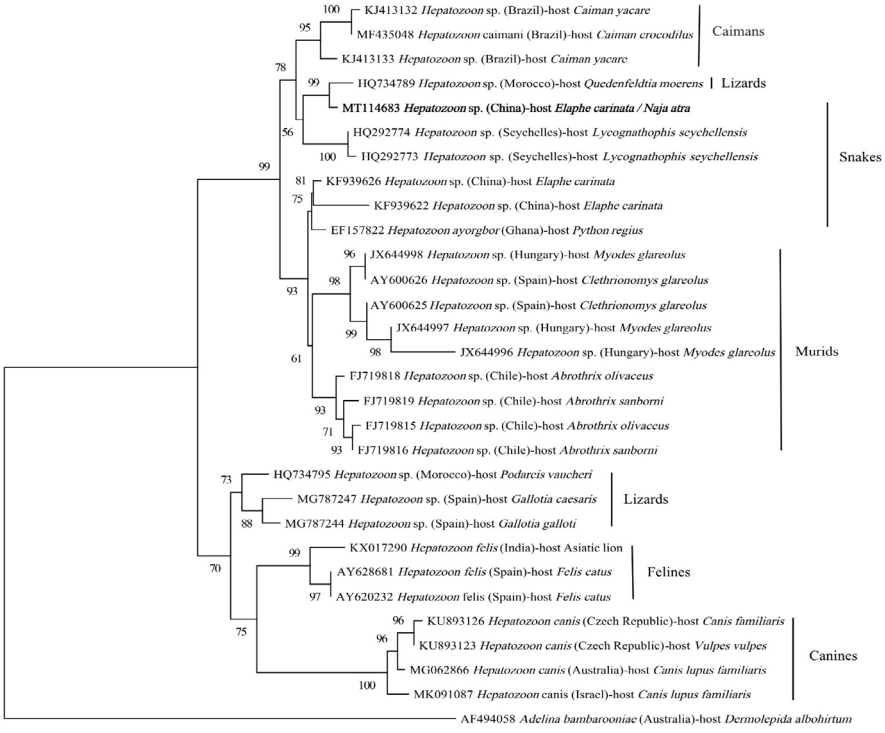

Fig. 2. Phylogenetic tree based on 18SrRNA sequences of *Hepatozoon* species (NJ).

all over the world. However, *Hepatozoon* has not been widely recognized in China. In 1987, Li described a new species *Hepatozoon guangdongense* that infected snakes, which was the first report of snake *Hepatozoon* in China (Li, 1987). Few studies on snake *Hepatozoon* have been reported since another new species of *Hepatozoon chinensis* was reported in the blood of king snakes (*Elaphe carinata*) in 2015 (Han *et al*., 2015). The latest report is that *Hepatozoon* was detected in blood samples of snakes *Lycodon rufozonatus* and *Gloydius brevicaudus* by nested PCR and sequencing (Xiao *et al*., 2019). The present study provides a report on the infection rate and intensity of *Hepatozoon* infection in *Naja atra, Elaphe carinata* and *Ptyas mucosus* from Wuhu. The infection rates were 25% and 40% in *Elaphe carinata* and *Naja atra*, respectivly. No *Hepatozoon* has been found in *Ptyas mucosus* in the present study. This could be due to the small sample size even though there has been no report that *Hepatozoon* infected in the snake *Ptyas mucosus* in China, while there is a report that *Hepatozoon* infected the Indochinese rat snake *Ptyas korros* from Khon Kaen (Sumrandee *et al*., 2015).

The cobra snake *Naja atra* is one of the endemic species in China. They secrete a mixture of neurotoxic blood-circulatory venom, which can be life-threatening if not treated in time after being bitten. Meanwhile, the venom secreted by cobra can be made into freeze-dried products and venom enzymes to treat serious snake bites. This cobra used to be a least concerned species. In the past two decades, its population in the wild has declined sharply, and it is sliding from vulnerable species to endangered species. In order to protect the ecological environment, it is necessary to protect this species. Yet, the *Hepatozoon* infected *Naja atra* has not been reported so far; this study is the first report that *Hepatozoon* infected *Naja atra* in China.

The early classification and identification of *Hepatozoon* were mainly based on the morphological characteristics of the polypide, such as the mature gamonts and schizonts, as well as the length and width of the polypide. However, it is obviously quite difficult to study each stage throughout its life cycle. Therefore, it is not reliable to classify *Hepatozoon* only according to morphology. With the development of modern science and technology, molecular markers were all used to identify species. The variety of morphological and morphometric forms of gamonts also emphasizes the need for molecular confirmation of the involved *Hepatozoon* species. 18S rRNA was an important molecular marker for the currently known *Hepatozoon*. According to the data, *Hepatozoon* sp. infections in several snake species worldwide have been reported based on microscopy and molecular techniques (Bouer *et al*., 2017; Cook *et al*., 2018; Han *et al*., 2015; Harris *et al*., 2011). Above all, the identification of parasitic species can be based on morphological characteristics, combined with the 18S gene sequences. Two-way verification from morphology and molecular biology can not only quickly identify species but can also increase the accuracy and reliability of species identification.

In this study, the sequences of 1,416 bp of the 18S rRNA gene derived from PCR amplification using HepF300/900 and HEMO1/HEMO2 that target different but over-lapping parts of the 18S rRNA gene (Han *et al*., 2015). The *Hepatozoon* sp. recovered in this study does not form a clade with the other *Hepatozoon* sp. species found in *Elaphe carinata* from China, but are instead related to the *Hepatozoon* sp. from Morocco and Seychelles. And in this study the *Hepatozoon* sp. found in snakes was determined to be most closely related to the *Hepatozoon* sp. found in lizards (*Quedenfeldtia moerens,* HQ734789). The phylogenetic results showed that there is limited host specificity and no significant difference to the geographical distribution of *Hepatozoon* parasites. The results are the same as the research about *Hepatozoon* species in lizards from North Africa and the study on *Hepatozoon caimani* in *Caiman crocodilus yacare* from North Pantanal, Brazil (Bouer *et al*., 2017; Maia *et al*., 2011). With regard to some species of *Hepatozoon* seem to have limited host-specificity, parasitologists presumed that the *Hepatozoon* sp. host spectrum is limited to the host ecology rather than to the host phylogenetic relationships (Bouer *et al*., 2017; Maia *et al*., 2011). In the current study, it is not very clear how parasites from different geographical locations are grouped together, particularly how some *Hepatozoon* sp. found in snakes from China are more related to *Hepatozoon* sp. found in lizards from Morocco than to other snakes from China. Moreover, although the molecular characterization used in the present study (18S rRNA) is highly conservative and widely used in molecular characterization of *Hepatozoon* sp. (Bouer *et al*., 2017; Han *et al*., 2015; Xiao *et al*., 2019). To better illuminate the diversity of *Hepatozoon* species and extend a better phylogenetic analysis of this group of parasites, it is necessary to identify more *Hepatozoon* species from different hosts and different geographical locations.

ACKNOWLEDGMENTS

The authors were grateful to Dr. Marisa Tellez from the University of California Santa Barbara for advice on the manuscript and encouragement in the process of experiment. We greatly appreciate the snake raisers of Yongchang biotechnology in Wuhu for providing guidance and technical assistance during our sample collection. This research was supported by Anhui Provincial Natural Science Foundation of China (No: 1608085MC77), Key

Program in the Youth Elite Support Plan in Universities of Anhui Province (No: gxyqZD2016171) and Academic Aid Program for top-notch talents in disciplines and specialties in the Universities of Anhui Province.

Statement of conflict of interest

The authors have declared no conflict of interest.

REFERENCES

Baneth, G., Mathew, J.S., Shkap, V., Macintire, D.K., Barta, J.R. and Ewing, S.A., 2003. Canine hepatozoonosis: two disease syndromes caused by separate Hepatozoon spp. *Trends Parasitol.,* **19**: 27-31. https://doi.org/10.1016/S1471-4922(02)00016-8

Bouer, A., Andre, M.R., Goncalves, L.R., Luzzi, M.C., Oliveira, J.P., Rodrigues, A.C., Varani, A.M., Miranda, V.F.O., Perles, L., Werther, K. and Machado, R.Z., 2017. Hepatozoon caimani in Caiman crocodilus yacare (Crocodylia, Alligatoridae) from North Pantanal, Brazil. *Rev. Bras Parasitol. Vet.,* **26**: 352-358. https://doi.org/10.1590/s1984-29612017041

Cook, C.A., Netherlands, E.C., As, J.V. and Jacobus Smit, N., 2018. Two new species of Hepatozoon (Apicomplexa: Hepatozoidae) parasitising species of Philothamnus (Ophidia: Colubridae) from South Africa. *Folia Parasitol. (Praha),* **65**: 2018.004. https://doi.org/10.14411/fp.2018.004

Han, H., Wu, Y., Dong, H., Zhu, S., Li, L., Zhao, Q., Wu, D., Pei, E., Wang, Y. and Huang, B., 2015. First report of Hepatozoon (Apicomplexa: Adeleorina) from king ratsnakes (Elaphe carinata) in Shanghai, with description of a new species. *Acta Parasitol.,* **60**: 266-274. https://doi.org/10.1515/ap-2015-0038

Harris, D.J., Maia, J.P. and Perera, A., 2011. Molecular characterization of Hepatozoon species in reptiles from the Seychelles. *J. Parasitol.,* **97**: 106-110. https://doi.org/10.1645/GE-2470.1

Li, D., 1987. Description of Hepatozoon guangdongense sp. nov. (Eucoccodia: Haemogregarinidae). *Acta Zoot. Sin.,* **12**: 113-118.

Madsen, T., Ujvari, B. and Olsson, M., 2005. Old pythons stay fit; effects of haematozoan infections on life history traits of a large tropical predator. *Oecologia,* **142**: 407-412. https://doi.org/10.1007/s00442-004-1742-9

Maia, J.P., Harris, D.J. and Perera, A., 2011. Molecular survey of Hepatozoon species in lizards from North Africa. *J. Parasitol.,* **97**: 513-517. https://doi.org/10.1645/GE-2666.1

Mitchell, M.A., 2011. Zoonotic diseases associated with reptiles and amphibians: An update. *Vet. Clin. North Am. Exot. Anim. Pract.,* **14**: 439-456. https://doi.org/10.1016/j.cvex.2011.05.005

Pedersen, A.B., Jones, K.E., Nunn, C.L. and Altizer, S., 2007. Infectious diseases and extinction risk in wild mammals. *Conserv. Biol.,* **21**: 1269-1279. https://doi.org/10.1111/j.1523-1739.2007.00776.x

Soares, P., Borghesan, T.C., Tavares, L.E.R., Ferreira, V.L., Teixeira, M.M.G. and Paiva, F., 2017. Hepatozoon caimani Carini, 1909 (Adeleina: Hepatozoidae) in wild population of Caiman yacare Daudin, 1801 (Crocodylia: Alligatoridae), Pantanal, Brazil. *Parasitol. Res.,* **116**: 1907-1916. https://doi.org/10.1007/s00436-017-5467-1

Sumrandee, C., Baimai, V., Trinachartvanit, W. and Ahantarig, A., 2015. Hepatozoon and Theileria species detected in ticks collected from mammals and snakes in Thailand. *Ticks Tick Borne Dis.,* **6**: 309-315. https://doi.org/10.1016/j.ttbdis.2015.02.003

Ungari, L.P., Santos, A.L.Q., O'Dwyer, L.H., da Silva, M.R.L., Rodrigues Santos, T.C., da Cunha, M.J.R., de Melo Costa Pinto, R. and Cury, M.C., 2018. Molecular characterization and identification of Hepatozoon species Miller, 1908 (Apicomplexa: Adeleina: Hepatozoidae) in captive snakes from Brazil. *Parasitol. Res.,* **117**: 3857-3865. https://doi.org/10.1007/s00436-018-6092-3

Wozniak, E.J., Kazacos, K.R., Telford, S.R., Jr. and McLaughlin, G.L., 1996. Characterization of the clinical and anatomical pathological changes associated with Hepatozoon mocassini infections in unnatural reptilian hosts. *Int. J. Parasitol.,* **26**: 141-146. https://doi.org/10.1016/0020-7519(95)00110-7

Xiao, X., Qi, R., Han, H.J., Liu, J.W., Qin, X.R., Fang, L.Z., Zhou, C.M., Gong, X.Q., Lei, S.C. and Yu, X.J., 2019. Molecular identification and phylogenetic analysis of Cryptosporidium, Hepatozoon and Spirometra in snakes from central China. *Int. J. Parasitol. Parasites Wildl.,* **10**: 274-280. https://doi.org/10.1016/j.ijppaw.2019.10.001

Zhihua, Z.Z.J., 2004. International trade status and crisis for snake species in China. *Conserv. Biol.,* **18**: 1386-1394. https://doi.org/10.1111/j.1523-1739.2004.00251.x