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Molecular identification of *Colpodella* sp. of South China tiger *Panthera tigris amoyensis* (Hilzheimer) in the Meihua Mountains, Fujian, China

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Abstract: A three-year-old male South China tiger died in the tiger enclosure of the China Tiger Park in the Meihua Mountains on December 2018 after being bitten by a tick. This tiger presented clinical symptoms like whole-body severe jaundice, hepatosplenomegaly, kidney, and lymph node hemorrhages. The *Colpodella* sp.-specific 18S rRNA gene was detected using nested PCR. Interestingly, the DNA isolated from the blood of the tiger was found to be 100% similar to that of the tick by NCBI BLAST analysis. However, the DNA fragments isolated from the tiger's blood were 90.1% similar to the *Colpodella* sp. strain human erythrocyte parasite (HEP, MH208621) and 90.4% similar to the *Colpodella* sp. strain Heilongjiang (HLJ, KT364261). To investigate the species of ticks and ticks-carried *Colpodella* parasites in this region, the species of ticks obtained from the grasses outside the tiger enclosure and the species of *Colpodella* carried by ticks were identified. The DNA from ticks as well as that from the tick-borne *Colpodella* sp. were amplified from each tick using PCR followed by amplicon sequencing. In total 402 adult ticks samples were collected, among which 22 were positive for *Colpodella* sp. (5.5%), and the species were further determined by morphology, DNA sequencing and phylogenetic analyses. Interestingly, one *Colpodella* sp. was found to have 94.2% sequence similarities to the *Colpodella* sp. strain HEP (MH208621). This strain was previously reported to infect a woman in Yunnan, China. In addition, three *Colpodella* sp. showed 87–91% sequence similarities to the *Colpodella* sp. strain HLJ (KT364261), which was previously reported to infect human in Heilongjiang, China. This study disclosed the possibility of zoonotic transmission of *Colpodella* sp. by ticks in China. Finally, it provides a basis for urgently determining and monitoring the repertoire of ticks-borne piroplasmid pathogens, with the ultimate aim of strategic control.

Keywords: ticks, Yunnan, 18S rRNA, 16S rRNA, ITS2 rRNA

The South China tiger *Panthera tigris amoyensis* (Hilzheimer) is a unique subspecies of tiger in China and one of the most endangered animals in the world (Liu et al. 2013). It has been classified as the first-class national protected animal in China and included in the Washington Convention (the Convention on International Trade in Endangered Species of Wild Fauna and Flora) of the endangered species in 1988.

Parasites of the order Piroplasmida, includes species of *Babesia* Starcovici, 1893 and *Theileria* Bettencourt, França et Borges, 1907. They reportedly infect both wild and domestic animals (Brown 1997). In addition, a new species called *Colpodella* sp., a close relative of apicomplexans was identified (Kuvardina et al. 2002). To date, only two cases of human infections by *Colpodella* sp. are reported, which suggests that opportunistic infections of humans with free-living protists might occur (Yuan et al. 2012, Jiang et al. 2018).

Species of *Colpodella* are free-living protists, freshwater, or marine predators that feed on protists and algae (Patterson and Simpson 1996). The life cycles of *Colpodella* spp. include the trophozoite and cyst stages. These protists have three-membraned pellicle and apical complex organelles containing rhoptries, micronemes, pseudo-conoid, polar rings, and subpellicular microtubules, which promote predation (Simpson and Patterson 1996). The trophozoites

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Fig. 1. Sampling sites in the Meihua Mountains. The red triangle indicates the location of the Meihua Mountains, Fujian, China.

have the pseudo-conoid in the rostrum, which is used for feeding. They also possess two hetero-dynamic flagella originating from separate flagellar pockets with transversal plates in the transitional zone (Brugerolle 2002). Some species of *Colpodella* possess trichocysts, which are organelles that are ejected in response to stimuli (Brugerolle 2002). To date the life cycles of *Colpodella vorax* (Kent, 1880), *C. unguis* Patterson et Simpson, 1996; *C. turpis* Simpson et Patterson, 1996; and *C. pugnax* Cienkowski, 1865 have been described (Simpson and Patterson 1996, Brugerolle 2002).

The genus *Colpodella*, includes a scantily-studied species which are almost indistinguishable from those of *Perkinsus* Levine, 1978 except for their free-living habits. These organisms penetrate the cell membrane of their protistan prey and using a reinforced rostrum either consume the cytoplasmic contents or ingest the entire cells (Brugerolle and Mignot 1979). The rostrum of *Colpodella* species is structurally and functionally identical to the so-called ‘‘conoid’’ of zoospores of species of the *Perkinsus* and *Parvilucifera* Norén, 1999. However, Siddall et al. (1997) observed all the alveolate taxa to inhibit micropores and showed that the anterior structures in species of *Perkinsus* resemble the feeding peduncles in some dinoflagellates rather than the apical complex (Siddall et al. 1997).

The 18S rRNA analysis indicated *Colpodella* sp. to be phylogenetically related to apicomplexans such as species of *Plasmodium* Marchiafava et Celli, 1885 and the plastid-containing *Chromera velia* Moore, 2008 (Janouškovec et al. 2015). Phylogenetic analysis indicated *Colpodella* sp. (ATCC 50594) to be related to *C. pontica* Mylnikov, 2000 (renamed *Voromonas pontica*), *C. tetrahymenae* Cavalier-Smith et Chao, 2004, *Cryptosporidium serpenti* Levine, 1980 and *Toxoplasma gondii* (Nicolle et Manceaux, 1908) (Kuvardina et al. 2002).

Ticks are not only obligate blood-feeders but also the most important vectors of zoonotic diseases since they can act as vectors for several pathogens, like viruses, protists, fungi, bacteria and helminths, and can cause diseases in wild as well as domestic animals (Jongejan and Uilenberg 2004). They are divided into four families, the Nuttalliellidae and Laelaptidae, Ixodidae or hard tick, and Argasidae or soft tick (Anderson and Magnarelli 2008, Vesco et al. 2011), which are distributed across the world from the tropics to subarctic regions. However, they demonstrate the greatest species diversity in the tropics and subtropics (Anderson and Magnarelli 2008).

The tick *Haemaphysalis flava* Neumann is an important ectoparasite, which not only damages its hosts directly but also serves as a vector for various infectious pathogens in China (Cheng et al. 2013, Lu et al. 2013, Li et al. 2017). Recent studies have also reported *Haemaphysalis longicornis* Neumann and *Haemaphysalis hystricis* Supino in China (Lu et al. 2017, Wang et al. 2018, Zhang et al. 2018, Li et al. 2019b, Liu et al. 2019, Yang et al. 2019, Zheng et al. 2019). *Haemaphysalis bispinosa* Neumann has been believed to occur in China, especially in the southern part. However, the widely-distributed *H. longicornis* has been often mistaken for *H. bispinosa* in many Chinese studies (Chen et al. 2015).

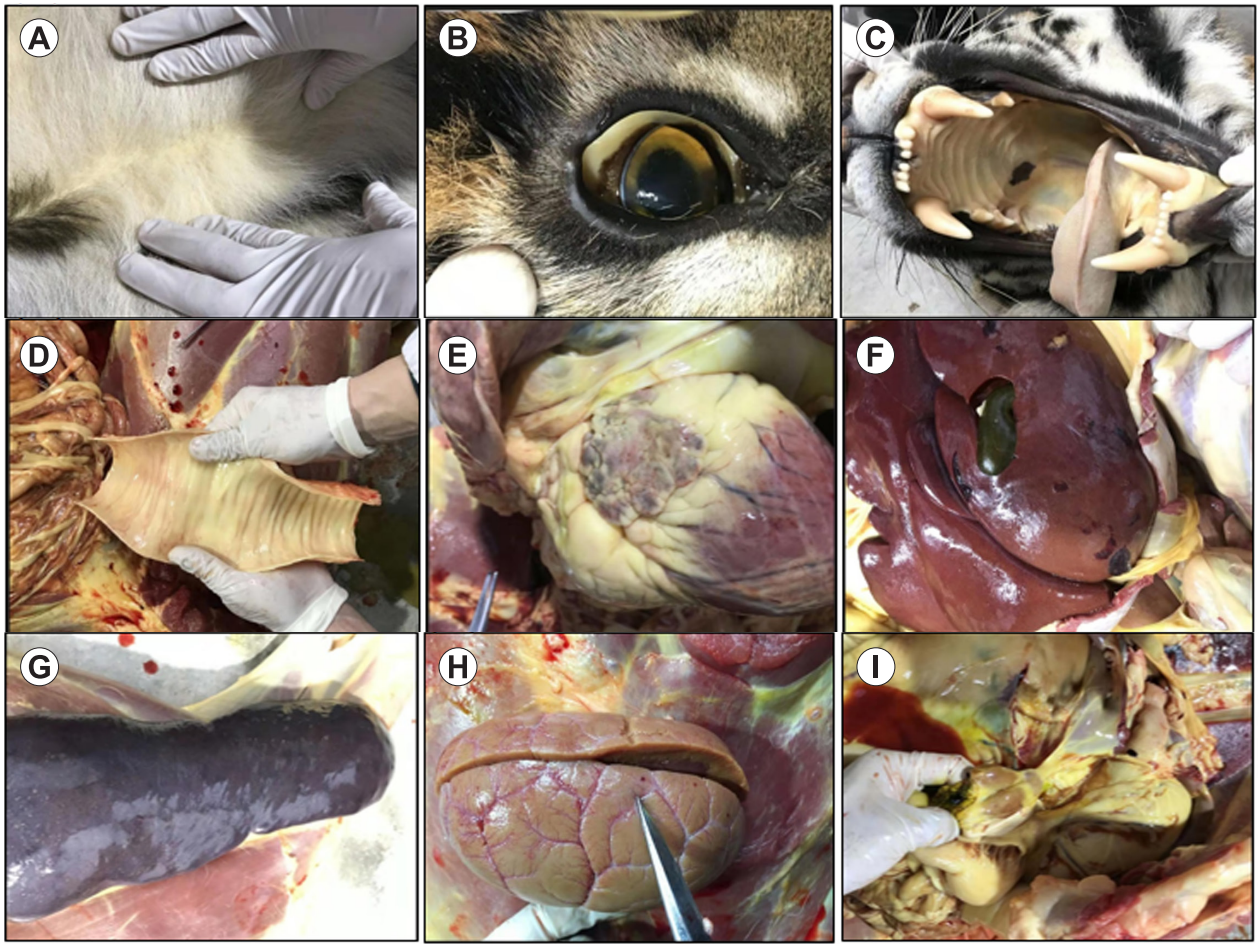

Fig. 2. The appearance of anatomical pathology and the lesions in the main organs. **A** – skin; **B** – eye conjunctiva; **C** – oral mucosa; **D** – trachea; **E** – coronary fats around the heart were identified with pathological characteristics like severe jaundice; **F** – hepatomegaly; **G** – splenomegaly; **H** – kidney with hemorrhages; **I** –mesenteric lymph nodes.

The Fujian Meihua Mountains are a well-preserved original forest area abounding in vegetation which includes coniferous and broad-leaved mixed forest, coniferous broad-leaved bamboo mixed forest, and shrub forest. Therefore, there are abundant biological resources in this area. These forests are homes to several nationally protected animals such as the South China tigers, leopards, black deers, sika deers, and wild boars (Liu et al. 2013).

Although the identification of ticks based on morphological features is convenient, it needs prior experience (Qin et al. 2011). Molecular techniques, harnessing the mitochondrial (mt) and ribosomal DNA (rDNA) fragments, to serve as useful tools for the accurate identification and characterisation of pathogens (Blouin 2002, Padgett et al. 2005, Zhao et al. 2012). The ITS2 rDNA can distinguish morphologically similar tick species of *Ixodes* and *Amblyomma* (Marrelli et al. 2007, Chao et al. 2011).

To date, there are very limited studies on the tick-borne piroplasms as well as the ticks in these areas (Wang et al. 2020). This study aims to elucidate the associations between the ticks and ticks-borne piroplasms in the Meihua Mountains. Moreover, it also attempts to better understand the extent of zoonotic transmission of pathogens in this region since humans in this region are in frequent contact with wild animals. Therefore, the possibility of zoonotic transmission of tick-borne pathogens from the tigers to humans in this region cannot be ruled out. Hence, epidemiological investigation of ticks-borne piroplasms is very important in this area.

MATERIALS AND METHODS

Study sites

The Meihua Mountains lie between the latitudes 25.2539N to 25.5956N and longitudes 116.7569E to 116.9592E. This area has a warm climate with abundant rainfall. The average temperature ranges between 13 to 18ºC with maximum temperature of 24ºC and minimum temperature of 8ºC. Owing to the relatively distinctive geographical location, forest ecology and natural environment, the Meihua Mountains and large area of forest resources serve as the protective umbrella and refuge for various organisms. The abundant and unique biological resources in the area, along with a variety of rare animals and plants, make this mountain a natural gene pool and the ideal base for biological science research (Liu et al. 2013).

Table 1. Tick samples and ticks-carried piroplasmids compared in this study.

Tick species	GenBank accession number		Tick-carried piroplasmids	GenBank accession number	Percentage identify by BLAST	Genetic distances (expressed in percentages)
	ITS-2	16S rRNA		18S rRNA		
Haemaphysalis bispinosa	MT297637	KY825195	*Colpodella* sp. (1)	FN598219	90.5%	90.4
Dermacentor andersoni	EU520395	EF636463	*Colpodella* sp. (2)	MH012047	87.6%	82.4
H. longicornis	JQ625700	MT555304	*Colpodella* sp. (3)	KC486095	82.7%	79.2
H. hystricis	-	KC170733	*Colpodella* sp. (4)	GU067926	83.3%	82.6
D. andersoni	S83084	EF636463	*Colpodella* sp. (5)	KC487929	91.2%	90.6
H. hystricis	-	KC170733	*Colpodella* sp. (6)	MH208617	90.4%	91.4
H. bispinosa	MK621331	KY825195	*Colpodella* sp. (7)	MN640807	98.2%	97.7
H. flava	JQ737122	MT294425	*Colpodella* sp. (8)	FN598253	92.2%	90.4
D. atrosignatus	MT297636	KC170745	*Colpodella* sp. (9)	FN598253	87.7%	85.4
H. flava	AB861941	MT294425	*Colpodella* sp. strain HEP (10)	**MH208622**	99.7%	99.7
H. longicornis	JQ346684	MK439888	*Colpodella* sp. (11)	KT600661	98.1%	98.7
D. taiwanensis	-	MT294309	*Colpodella* sp. (12)	MH012045	90.5%	91.7
R. duttoni	-	MF425976	*Colpodella* sp. (13)	KC488120	81.4%	95
H. hystricis	-	MT294298	*Colpodella* sp. (14)	MH208619	84.4%	81.5
D. atrosignatus	MT297636	KC170745	*Colpodella* sp. (15)	KY914473	92.9%	93.9
H. longicornis	JQ346684	KX083342	*Colpodella* sp. (16)	MH012047	95.2%	95.3
H. hystricis	-	MT294298	*Colpodella* sp. strain HLJ (17)	**KT364261**	89.8%	89.8
H. bispinosa	MT297637	KY825195	*Colpodella* sp. strain HLJ (18)	**KT364261**	87.2%	87.1
H. longicornis	KX450329	MN956527	*Colpodella* sp. strain HLJ (19)	**KT364261**	90.8%	90.9
H. flava	JQ737122	MT294425	*Colpodella* sp. strain HEP (20)	**MH208621**	94.2%	92.1
H. flava	AB861941	MT294425	*Colpodella* sp. strain HEP (21)	**MH208620**	95%	93.8
H. flava	MT297641	MT294425	*Colpodella* sp. (22)	MH208619	89.3%	86.7

The accession numbers of *Colpodella* sp. strain HEP and HLJ are in bolded characters.

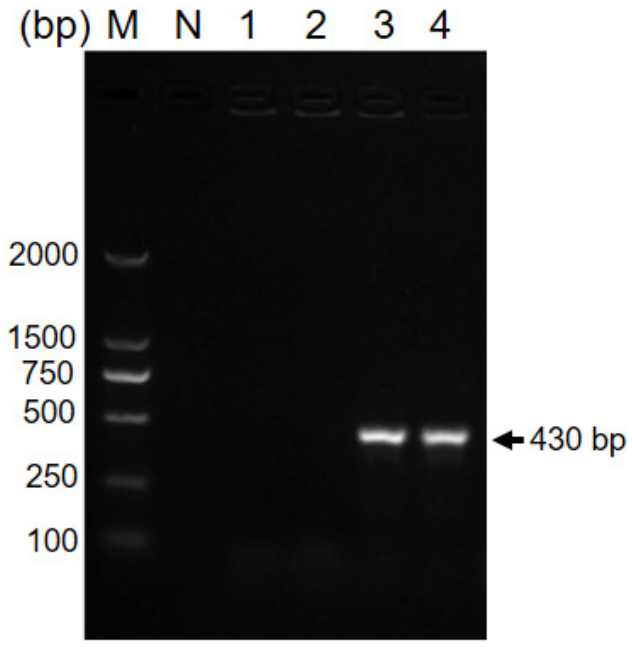

Fig. 3. Detecting *Colpodella* sp. from the blood samples of the South China tiger *Panthera tigris amoyensis* (Hilzheimer) bitten by a tick and DNA from this bitten tick by PCR. M – marker; N – negative control (ddH_2O was taken as the template and *Colpodella* sp. 18S rRNA universal primers were used for PCR). The DNA isolated from the blood of the South China tiger served as the template and the primers specific for *Mycoplasma suis* (lane 1), *Toxoplasma gondii* (Nicolle et Manceaux, 1908) (lane 2), and *Colpodella* sp. (lane 3) were used for PCR. The DNA from ticks was amplified using the *Colpodella* sp. universal primers for PCR (lane 4).

Collection of the soil water samples and ticks

In this study, five soil samples from the grasses and five water samples from the small ditches nearby the tiger enclosure were collected and stored at 4°C for further PCR examination. In total 402 adult ticks were collected in 2019 from the China Tiger Park of the Fujian Meihua Mountains, China (Fig. 1). The adult ticks were collected from the grasses using forceps and were fixed in 70% ethanol with storage at -20°C until further use. The species of the collected ticks were identified based on the morphology according to standard morphological keys (Anderson and Magnarelli 2008), and the identity of each tick sample was confirmed based on the mitochondrial 16S and ITS2 rRNA sequences.

DNA extraction from the blood of the tiger and ticks

Blood samples were extracted using a blood DNA extraction kit and EZNA tissue DNA extraction kit (Omega Bio-tek Inc., Norcross, USA). The tick samples were rinsed twice in sterile phosphate-buffered saline (PBS) solution, cut into small pieces with sterile scissors and then placed in sterile 1.5 ml microtubes. The DNA was extracted from all the tick samples using the Tissue DNA Extract Kit (Omega Bio-tek Inc.) according to the manufacturer's instructions. The extracted DNA was eluted in 100 µl elution buffer and frozen at −80°C for further use.

DNA from the soil and water samples

In brief, 5 g of soil samples were collected from the grasses nearby the tiger enclosure and suspended in 10 ml ddH_2O. 5 µl of ddH_2O were taken as the template for the following *Colpodella* sp. 18S rRNA nested PCR programs. 5 µl of water samples of the small ditches near the tiger enclosure were collected and directly served as the template for the same nested PCR programs of *Colpodella* sp. 18S rRNA.

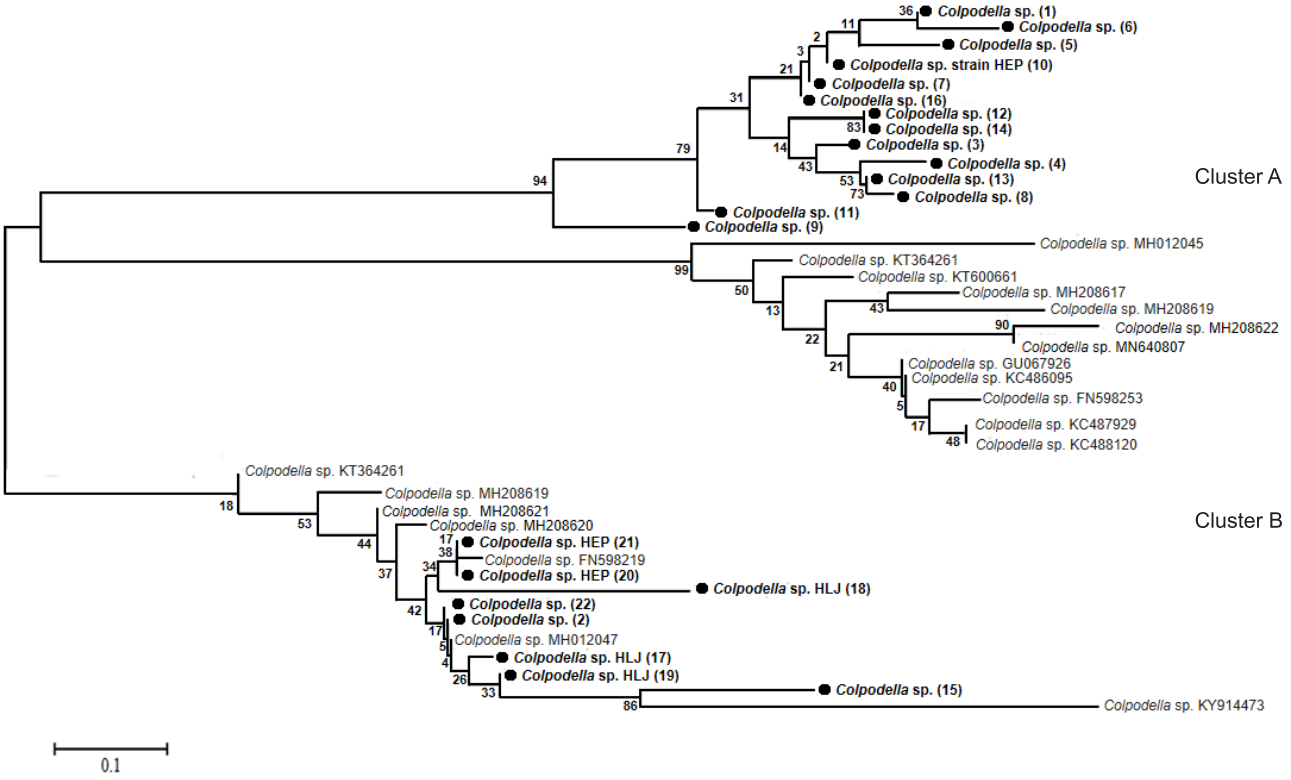

Fig. 4. Phylogenetic analysis based on the18S rRNA gene of strains of *Colpodella* spp. isolated from the Meihua Mountains, Fujian, China. A phylogenetic tree was generated using the NJ method. Scale bar indicates the degree of divergence represented by a given length of the branch. Black dots and boldface letters indicate 18S rRNA gene sequences acquired in this study.

PCR amplification

A nested PCR was performed to detect the expression of the *Colpodella* sp. 18S rRNA gene. Primer sequences specific to the 18S rRNA gene included Piro-1-S: 5′-CTTGACGGTAGGGTATTGGC-3′ and Piro-3-AS: 5′-CCTTCCTTTAAGTGATAAGGTTCAC-3′ for the first round of PCR, PIRO-A: 5′-AAATACCCAATCCTGACACAGGG-3′ and PIRO-B: 5′-TTAAATACGAATGCCCCCAAC-3′ for the second round of PCR (430-bp product) (Matsimbe et al. 2017). The PCR was performed with 50 ng of template DNA, 0.5 µl of rTaq DNA polymerase (Takara Bio, San Jose, USA), 5 µl of 10 × Taq buffer, 2 µl of 2.5 mM dNTP (Takara Bio, USA), 10 µM of primers and adjusted to a final volume of 50 µl with ddH_2O. The nested PCR conditions were as follows: first round PCR condition: 95°C for 5 min, followed by 35 cycles of 95°C for 45s, 55°C for 1 min, and 72°C for the 90s, followed by a final 10 min extension at 72°C. For the second-round PCR, 5 µl of PCR products served as the template, the PCR condition: 95°C for 5 min, followed by 35 cycles of 95°C for 45s, 55°C for 1 min, and 72°C for 90s, followed by a final 10 min extension at 72°C. The primer sequences for detecting the tick-specific 16S rRNA gene expression, included the 16S rRNA-1: 5′-CTGCTCAATGATTTTTTAAATTGCTGTGG-3′ and 16S-rRNA-2: 5′- CCGGTCTGAACTCAGATCAAGT-3′ for the PCR (500-bp product) (Li et al. 2018); PCR condition followed the previous report. To detect ticks ITS2 rRNA gene expression, the primer sequences included ITS2 rRNA-F: 5′-CGAGACTTGGTGTGAATTGCA-3′ and ITS2-rRNA-R: 5′- TCCCATACACCACATTTCCG-3′ for the PCR (1500-bp product) (Barker and Walker 2014), PCR condition was according to the previous report. In addition, primers (F: 5′-CAGCGGTGAGAAAGCAAG-3′; R: 5′-CTGGGTGTATGAAGAGTGGTGT-3′) and PCR conditions for detecting *Mycoplasma suis* were according to the GeneBank accession number AJ504999 ORF6 (Ba et al. 2009). Primers (F: 5′-TGCATAGGTTGCAGTCACTG-3’; R: 5′-TCTTTAAAGCGTTCGTGGTC-3′) and PCR condition for detecting of *T. gondii* were designed according to the previous study (Costa et al. 2016). PCR products were electrophoresed on a 1% agarose gel and visualised under ultraviolet light. DNA bands of the correct size were gel extracted, purified and sequenced by BGI Tech Solutions Co., LTD (Liuhe, Beijing, China).

Sequence analyses

The PCR products and genetic distances were analysed using the Lasergene version 7.1 software (DNASTAR Inc., Madison, WI, USA) and compared with the sequences published in the GenBank database.

Phylogenetic analysis

To estimate the neighbor-joining (NJ) trees for nucleotide alignment, the clustalW and MEGA v6.06 software was used with the empirical base frequencies. The bootstrap values were determined for 1,000 replicates.

RESULTS

The clinical features of the South China tiger

On December, 2018, a three-year-old male South China tiger died at the South China tiger breeding institute in the

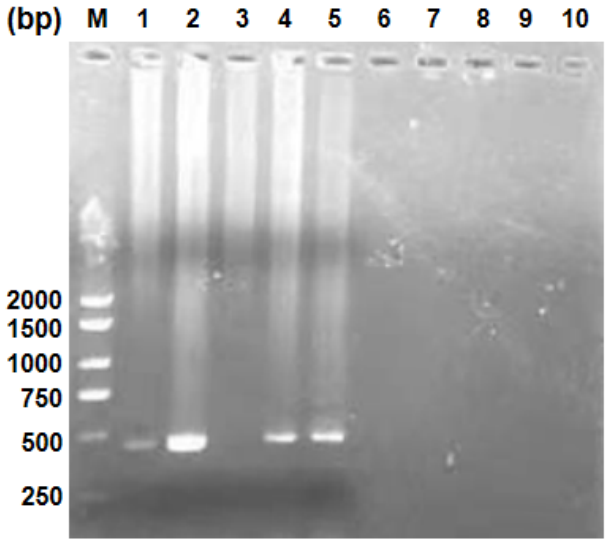

Fig. 5. Detecting *Colpodella* sp. from the soil and water samples near the tiger enclosure using PCR. M – marker. Five water samples (lane 1–5) from the small ditches and five soil samples from the grasses (lane 6–10) near the tiger enclosure. Five grams of soil samples were suspended in 10 ml ddH_2O and 5 µl were taken as the template for PCR and 5 µl of water samples directly served as the template for PCR.

Meihua Mountains after being bitten by a tick. Few days before dying, this tiger not only exhibited clinical symptoms of anorexia along with a runny nose and drool, but its excrements were bluish-green. After treating with sulfonamide, cephalosporin and ampicillin, the urine turned yellow. Anatomical and pathological examinations suggested the tiger has whole-body severe jaundice, including skin (Fig. 2A), eye conjunctiva (Fig. 2B), oral mucosa (Fig. 2C), trachea (Fig. 2D), and the coronary fats around the heart (Fig. 2E). Moreover, the tiger also presented pathological features such as hepatomegaly (Fig. 2F), splenomegaly (Fig. 2G), with hemorrhage in the kidney (Fig. 2H) and mesenteric lymph nodes (Fig. 2I). Although the blood samples of the South China tiger were also collected, the blood smear and routine blood test were not possible since the blood samples were too thin.

Detection of *Colpodella* sp. from the blood sample of the South China tiger and tick by PCR

The template DNA was isolated from the South China tiger's blood and tick and different primers were used for PCR. The PCR results indicated the tiger's blood DNA to be negative using the *Mycoplasma suis* and *Toxoplasma gondii* primers (Fig. 3, lanes 1, 2) but positive using the *Colpodella* sp. universal primers (Fig. 3, lane 3). Furthermore, DNA from the tick was also found to be positive for *Colpodella sp.* (Fig. 3, lane 4). After DNA sequencing and NCBI BLAST analysis, the DNA sequences from the blood samples showed 100% similarity to that of the tick. Both of them were found to be 90.1% similar to the *Colpodella* sp. strain human erythrocyte parasite (HEP, MH208621) and 90.4% similar to the *Colpodella* sp. strain Heilongjiang (HLJ, KT364261). According to the analysis of the DNA sequences, this tick was proposed to transmit *Colpodella* sp. to the South China tiger through biting. Additionally, based on the morphology, the analysis of the 16S and ITS2 rRNA sequences revealed the species of the tick to be *Haemaphysalis flava*.

Molecular characterisation of 22 *Colpodella*-positive ticks in the Meihua Mountains

In total of 402 ticks were collected and screened for identifying the presence of *Colpodella* sp. Out of the 402 ticks, only 22 of them were detected to be *Colpodella*-positive (5.5%). These ticks housed 8 species belonging to 3 genera and all the specimens belonged to the family Ixodidae. The three genera of ticks were *Haemaphysalis* Koch, *Dermacentor* Koch, and *Rhipicephalus* Koch (Table 1). The species collected from 22 ticks were most frequently *H. flava* (n = 5; 23%), *Haemaphysalis longicornis* (n = 4; 18%), *H. hystricis* (n = 4; 18%), *H. bispinosa* (n = 3; 14%), *Dermacentor andersoni* Stiles (n = 2; 9%), *D. atrosignatus* Neumann (n = 2; 9%), *D. taiwanensis* Sugimoto (n = 1; 5%), *Rhipicephalus duttoni* Neumann (n = 1; 5%), in that order. Three ticks were found to harbour *Colpodella* sp. that had 94 to 100% similarity with the sequence of the *Colpodella* sp. strain HEP (MH208620-22). The other three ticks were identified to carry *Colpodella* sp., with 87 to 91% similarity with the sequence of the *Colpodella* sp. strain HLJ (KT364261). Both of the *Colpodella* sp. HEP (Yuan et al. 2012) and HLJ (Jiang et al. 2018) strains have been previously reported to cause tick-borne diseases in humans.

Analysis of the genetic distance based on the 18S rRNA sequences of *Colpodella* sp.

The results revealed that the genetic distances between various *Colpodella* sp. strains and alignment sequences from the GenBank™ ranged from 79.2% to 99.7% (Table 1). The genetic distances of the *Colpodella* sp. strain HEP (10) was found to be 99.7%, that with *Colpodella* sp. strain HEP (20) and (21) were 92.1% and 93.8%, respectively. In addition, the other three ticks were found to carry the *Colpodella* sp. strain HLJ (17–19) with genetic distances of 87.1 to 90.9% (Table 1).

Construction of the phylogenetic tree based on the 18S rRNA sequence of *Colpodella* sp.

The NJ phylogenetic tree based on the 18S rRNA gene nucleotide sequence is shown in Fig. 4. There are two distinct clusters (cluster A and B). Cluster A included had only *Colpodella* sp. strain HEP (10) and was distant from the other two *Colpodella* sp. HEP strains (20) and (21) in cluster B. Cluster B, contained *Colpodella* sp. (15) and reference strain *Colpodella* sp. (KY914473). In addition, the *Colpodella* sp. strains HEP (20) and (21) were found to be within a closer clade, which was the same as the *Colpodella* sp. strain HLJ (17) and (19).

PCR detection of *Colpodella* sp. from soils and water samples

Five soil samples were collected from the grasses and five water samples were collected from the small ditches near the tiger enclosure. The results of PCR indicated that *Colpodella* sp. was not present in soils but was almost detected in the four water samples of the small ditch near the tiger enclosure (Fig. 5, lane 1, 2, 4, 5).

DISCUSSION

Excessive hunting and loss of habitats in China has led to extinction of wild animals such as the South China tigers. Therefore, there is an urgent need to protect and restore their populations. In recent years, there has been an increase in the number of inbred captive South China tigers in the China Tiger Park of the Meihua Mountains, but these animals in captivity take time before they are left into the wild.

In the present study, it has been found that *Colpodella* sp. may occur in South China tigers. Therefore, the prevention and control of infectious diseases caused by piroplasms should be considered. When a three-year-old male South China tiger was bitten by a tick in December 2018, it died in the tiger enclosure of the China Tiger Park, in China, after developing several characteristics similar to those evident in babesiosis, such as severe jaundice in the whole body and swollen major organs (Beugnet and Moreau 2015).This tiger may have been be infected with a piroplasma transmitted by ticks. To date, there has not been reported any death of the South China tiger due to this protist. However, screening ticks that may harbour *Colpodella* spp. should be performed to detect possible occurrence of piroplasms.

The collected ticks were identified based on their morphology, and 16S and ITS2 rRNA sequences, but none of the sequences obtained were identical, possibly because ITS2 rDNA shows little intraspecific variation but considerable interspecific difference (Zhu et al. 2007). Additionally, the ITS2 rDNA of species of *Haemaphysalis* deposited in the GenBank database was reported to vary in length from 1,159 bp to 1,707 bp (Cheng et al. 2013). The previous report has demonstrated that the ITS2 rDNA of hard ticks evolve mainly by increasing and decreasing the lengths of the nucleotide sequences to altering the stems length of the secondary structure, and the increase in the size of the ITS2 rDNA might have been caused by replication slippage generating large repeats (Hlinka et al. 2002). Alternatively, in this study, the length of the 16S rRNA sequence deposited in the GenBank database was found to vary in length from 316 bp to 473 bp, which is consistent with the report described by Zhao et al. (2012).

Ticks are among the most significant blood-sucking arthropods in the world because they transmit various pathogens causing disease and death in humans, domesticated animals and wildlife (Anderson and Magnarelli 2008). Vectors of *Babesia* belong to at least four Ixodidae genera: *Rhipicephalus*, *Ixodes* Latreille, *Haemaphysalis*, and *Hyalomma* Koch (Antunes et al. 2017). In this study, ticks of the genera *Haemaphysalis*, *Dermacentor* and *Rhipicephalus* were identified as the major host vectors for *Colpodella* sp. in the Meihua Mountains (Table 1). These data are consistent with a previous report suggesting that a *R. microplus* (Canestrini) was positive for *Colpodella* sp. (Matsimbe et al. 2017).

Haemaphysalis spp. and *Dermacentor* spp. were found both to be *Colpodella* vectors, which have not been reported previously. In our study, *Haemaphysalis* spp. represented approximately 73% (16/22) of the *Colpodella* sp.-positive ticks, with *Haemaphysalis flava* and *H. longicornis* being the dominant tick species, suggesting that it is widely distributed in this region (Table 1).

Haemaphysalis longicornis is the most frequently collected species in the grassland from Jiangxi, China (Zheng et al. 2019) and has become invasive in multiple regions of the world, mainly because of its parthenogenetic reproduction, broad habitat use and high diversity of avian and mammalian hosts (Heath 2016). *Haemaphysalis flava* was the most collected species in the grasses; it is a vector of various infectious diseases in China (Li et al. 2017). This study suggested that *H. flava* might be the dominant species carrying the *Colpodella* sp. strain HEP, which infected the South China tiger. In our recent study, *Babesia* sp., a close relative of *Colpodella* sp., was also detected in an *H. flava* tick (Wang et al. 2020). Besides the South China tiger, *H. flava* was also found in the giant pandas in Shaanxi province and wild hedgehogs in Henan and Hunan provinces of China (Cheng et al. 2013, Li et al. 2017).

To reveal the origin of *Colpodella* sp. in the Meihua Mountains, five soil samples from the grasses and five water samples were collected from the small ditch nearby the tiger enclosure. *Colpodella* sp. was not detected in the soils but was present in 4 water samples. Therefore, it could be suggested that the origin of *Colpodella* sp. was from the small ditch near the tiger enclosure. The ticks might have carried *Colpodella* sp. while sucking water from the small ditch. However, the presence of *Colpodella* sp. in the soils cannot be ruled out. *Colpodella* sp. was not detected in the soils possibly due to too low titre and therefore could not be detected by PCR.

To date, *Colpodella* sp. infection in humans has been reported only in Yunnan and Heilongjiang Provinces of China. The 18S rRNA sequences of the *Colpodella* sp. strain HEP were detected in blood samples from a 57-year-old woman in Yunnan Province (Yuan et al. 2012). In addition beside, a patient with a recent history of a tick bite, in the Heilongjiang Province, was also found to express nucleotide sequences of *Colpodella* sp. in a cerebrospinal fluid sample using a PCR assay. This isolate was named as *Colpodella* sp. HLJ strain (Jiang et al. 2018). Our data revealed that three ampicons from *H. flava* were 94 to 100% similar to the sequence of the *Colpodella* sp. strain HEP. Besides, the other three ticks were found to harbour *Colpodella* sp., which was 87 to 91% similar to the sequence of the *Colpodella* sp. strain HLJ (Table 1). Based on phylogenetic analysis, the *Colpodella* sp. strain HEP (20) and (21) seemed to be not closely related to the *Colpodella* sp. strain HEP (10), which is placed within cluster A. In addition, *Colpodella* sp. (15) and its reference strain *Colpodella* sp. (KY914473) belong to the same clade. Molec-

ular analysis suggests that the *Colpodella* sp. strain HEP (20) and (21) are closes to reference strains *Colpodella* sp. (MH208621) and (MH208620) than to the *Colpodella* sp. strain HLJ (17) and (19).

The present study confirmed the presence of *Colpodella* strains in the Meihua Mountains, and the possibility of transmission from the tick-carried *Colpodella* to the South China tigers. Zoonotic transmission to humans in this region cannot be ruled out.

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