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RESEARCH ARTICLE

An investigation on the presence of apicomplexan parasites of the genus *Babesia* in ticks collected from wild mammals in Türkiye

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ABSTRACT: Members of the genus *Babesia*, belonging to the group Apicomplexa, are protozoan parasites transmitted by ticks to humans and animals, infecting the red blood cells of mammals. In the present study, a total of 112 ticks collected from 80 different hosts belonging to 23 mammalian species from 16 provinces in Türkiye were screened for the presence of *Babesia* using PCR with primers targeting the 411-452 base pair region of the 18S rRNA gene. As a result of molecular analyses, *Babesia* positivity was detected in 7 ticks (6.25%). Phylogenetic analysis revealed the presence of *Babesia microti* in *Ixodes acuminatus* ticks collected from Edirne, Balıkesir, and Tokat provinces and *Babesia caballi* in a *Hyalomma* species tick collected from Kars province. These findings highlight the presence of *Babesia* pathogens in ticks collected from wild mammals in Türkiye, underscoring the ecological and epidemiological significance of these species. The study provides valuable data for understanding the spread of tick-borne diseases and for developing control strategies.

Keywords: Acari, *Babesia*, diseases, ectoparasites, Mammalia, ticks, zoonoses
Zoobank: http://zoobank.org/59B43D83-6DE6-4C88-88EF-8F89084E6FF2

INTRODUCTION

Ticks (Acari: Ixodida) are significant ectoparasites that feed on the blood of terrestrial vertebrates during all active life stages (larvae, nymphs, and adults). Ticks, distributed almost globally, play an important role in the natural cycles of various disease agents and are responsible for transferring several medically and veterinary significant pathogens to their hosts. During blood-feeding, ticks transmit disease-causing pathogens to humans and animals through secretions, leading to serious health problems (Jongejan and Uilenberg, 2004). Today, ticks are represented by about 1,000 species across three living families: Ixodidae, Nuttalliellidae, and Argasidae. Additionally, in 2017, the Deinocrotonidae family was described based on fossil materials from Myanmar, with the extinct species *Deinocroton draculi* representing this family (Dantas-Torres, 2018). A study conducted in 2022 identified the Khimairidae family as a fifth family, with the species *Khimairia fossus* described (Chitimia-Dobler et al., 2022). In 2024, a study on fossil tick species led to the identification of new tick species within the genus *Deinocroton*. However, all identified *Deinocroton* species (*bicornis*, *copia*, *draculi*, and *lacrimus*) were transferred to the Nuttalliellidae family, and the Deinocrotonidae family was dissolved (Chitimia-Dobler et al., 2024).

Ticks range in size from approximately 2 to 20 mm and are relatively large mites that are visible to the naked eye. Ticks belonging to the family Ixodidae are typically found in open, pasture-like environments, whereas those in the family Argasidae are usually located in sheltered, enclosed spaces such as dwellings and shelters. Due to its geographic location and rich habitat diversity, Türkiye is situated in an area where ticks are commonly found. Studies have identified the presence of a total of 56 tick species in Türkiye, including 8 species from the Argasidae family and 47 from the Ixodidae family (Bursalı et al., 2012, 2020; Keskin et al., 2014; Orkun and Karaer, 2018; Orkun and Vatansever, 2021; Keskin and Doi, 2025). Research conducted throughout Türkiye has predominantly focused on ticks parasitising humans and livestock, as well as the identification of tick-borne pathogens (Gargili et al., 2011, 2012; Bakirci et al., 2012; Bursalı et al., 2013; Keskin et al., 2016; Orkun et al., 2014; Karasartova et al., 2018). However, there has been limited research on ticks collected from wild animals and the pathogens carried by these ticks in the country (Keskin et al., 2014; Orkun et al., 2014; Orkun and Karaer, 2017; Orkun and Emir, 2020).

One of the most significant pathogens transmitted by ticks is the protozoan parasite *Babesia*, which causes a disease known as babesiosis in both humans and animals. The first known case was reported by Victor Babes in Romania in 1888, who identified the causative agent of a severe hemolytic disease in cattle and sheep and named it *Haematococcus bovis*, initially classifying it as a bacterium. A few years later, Theobald Smith and Fred Kilborne (1893) discovered that the agent responsible for Texas cattle fever in the United States was the same organism previously described by Babes. They named it as *Pyrosoma bigeminum*, later reclassified as *Babesia bigemina*. Crucially, Smith and Kilborne also revealed that the organism was not a bacterium but a protozoan blood parasite and that ticks were its biological vectors.

The genus *Babesia* currently includes over 100 species, several of which are known to cause disease in humans and animals (Dantas-Torres et al., 2017). In Europe, *Babesia di-*

vergens was historically the most reported species infecting humans, with only 22 documented cases before 1998. However, more than 50 human cases have been recorded since then, including infections caused by *B. divergens* (35 cases), *B. microti* (11 cases), and *B. venatorum* (5 cases) (Hildebrandt et al., 2021). Globally, approximately 95% of human babesiosis cases occur in the United States and Canada, with the vast majority attributed to *B. microti*. In addition to its relevance in human health, babesiosis also affects animals. *Babesia caballi*, for instance, is a primary causative agent of equine piroplasmosis (Ochi et al., 2023). This tick-borne disease poses a significant threat to the equine industry, particularly in tropical and subtropical regions (Mahmoud et al., 2020), as it affects not only horses but other equids as well (Wise et al., 2014). Due to its impact on international trade and equestrian activities, equine piroplasmosis is classified as a notifiable disease by the World Organization for Animal Health (WOAH) (Venter et al., 2024).

While the presence of *Babesia* species in ticks collected from humans and livestock in Türkiye has been investigated in various studies, there is limited research on the presence of *Babesia* in ticks collected from wild animals. This study aimed to investigate the presence of *Babesia* in ticks collected from wild animals captured in different regions of Türkiye.

MATERIALS AND METHODS

Morphological identification of ticks and host animals

Within the scope of the project, the ticks constituting the study material were collected from 80 host animals belonging to 23 different species, across 33 locations in 16 provinces of Türkiye (Adana, Artvin, Balıkesir, Bolu, Bursa, Edirne, Eskişehir, Hakkari, Iğdır, Kahramanmaraş, Kars, Kilis, Niğde, Samsun, Tekirdağ, and Tokat), under the appropriate permit numbers (B.30.2.ODM.0.20.09.00-050.04-97, B.30.2.ODM.0.20.09.00-050.04-09, and 21264211-288.04-E.72185). All tick specimens were preserved in the tick collection housed at the Parasitology Laboratory, Department of Biology, Faculty of Art and Science, Tokat Gaziosmanpaşa University. Species identification of the tick specimens was conducted based on morphological characteristics using stereomicroscopes (Leica MZ16 and Olympus SZ61), following the identification keys provided by Filippova (1997, 1977) and Apanaskevich and Horak (2008). Identification of mammalian host species was performed using the diagnostic keys described by Kryštufek and Vohralík (2001, 2007, 2009). The taxonomic classification of mammals in the Palearctic region remains incompletely resolved and is undergoing continuous revisions, particularly in light of recent advances in morphometric and molecular studies. In our study, the nomenclature of certain small mammal species was therefore adopted based on the most up-to-date and widely accepted taxonomic assessments currently available (Mahmoudi et al., 2022; Kryštufek and Shenbrot, 2022; İbiş et al., 2023).

Molecular Studies (DNA isolation, PCR, Sanger Sequencing and bioinformatics analysis)

Total DNA was individually extracted from ticks used in the study, following the protocol provided by the manufacturer (Biobasic, Canada). The quantity of DNA in the samples was measured using a spectrophotometer (Thermo Scientific™ Multiskan™ GO, Finland), and samples containing DNA of sufficient quality were stored at -80°C.

To investigate the presence of *Babesia* in the tick samples, a primer set targeting the ~ 411–452 base pair region of the 18S rRNA gene was used: BJ1 (GTCTTGTAATTGGAATGATGG) and BN2 (TAGTTTATGGTTAGGACTACG) (Casati et al., 2006). PCR reactions were prepared with a total volume of 25 µl, consisting of Dream Taq™ PCR Master Mix 2x (Thermo Fisher Scientific, Vilnius, Lithuania) containing DNA Taq polymerase, dNTPs, and buffer), 2 µl of forward primer, 2 µl of reverse primer, 5 µl of template DNA, and 16 µl of DNA-grade water. The PCR thermal cycling conditions were as follows: initial denaturation at 95°C for 5 minutes; 35 cycles of denaturation at 94°C for 60 seconds, annealing at 55°C for 60 seconds, and extension at 72°C for 120 seconds; followed by a final extension at 72°C for 5 minutes.

The PCR products obtained were loaded onto a 1% agarose gel and electrophoresed for approximately 30 minutes. The presence of positive bands was visualized using a UV transilluminator (UVP Transilluminator, Thermo Fisher Scientific). The PCR products were then sent to a commercial company (Macrogen Inc., Amsterdam, Netherlands) for sequencing and bidirectional reading. Each sequence result was imported into the BioEdit software as forward and reverse reads, and consensus sequences were generated. These consensus sequences were submitted to the NCBI GenBank Database and compared to previously deposited *Babesia* sequences using the BLAST tool. Phylogenetic relationships between the detected specimens and other *Babesia* species were determined by constructing a phylogenetic tree using the Maximum Likelihood method in the MEGA 12 software (Kumar et al., 2024).

RESULTS

Ticks identified on wild mammals

A total of 112 ticks were collected from 80 individual mammalian hosts representing 23 species across 16 provinces in Türkiye. Morphological identification revealed the presence of eight tick species: *Haemaphysalis erinacei* (2 males, 2 nymphs, 1 larva), *Haemaphysalis parva* (2 nymphs), *Hyalomma aegyptium* (1 female, 1 male, 1 nymph), *Hyalomma* sp. (7 nymphs), *Ixodes acuminatus* (7 females, 64 nymphs, 1 larva), *Ixodes ricinus* (8 larvae, 1 nymph), *Ixodes trianguliceps* (2 nymphs, 1 larva), and *Rhipicephalus turanicus* (5 females, 2 males, 4 nymphs).

The host mammals from which ticks were collected included: *Apodemus flavicollis* (n=9), *Apodemus uralensis* (n=1), *Apodemus witherbyi* (n=3), *Apodemus* sp. (n=4), *Clethrionomys glareolus* (n=1), *Crocidura gueldenstaedtii* (n=29), *Dryomys laniger* (n=1), *Erinaceus concolor* (n=3),

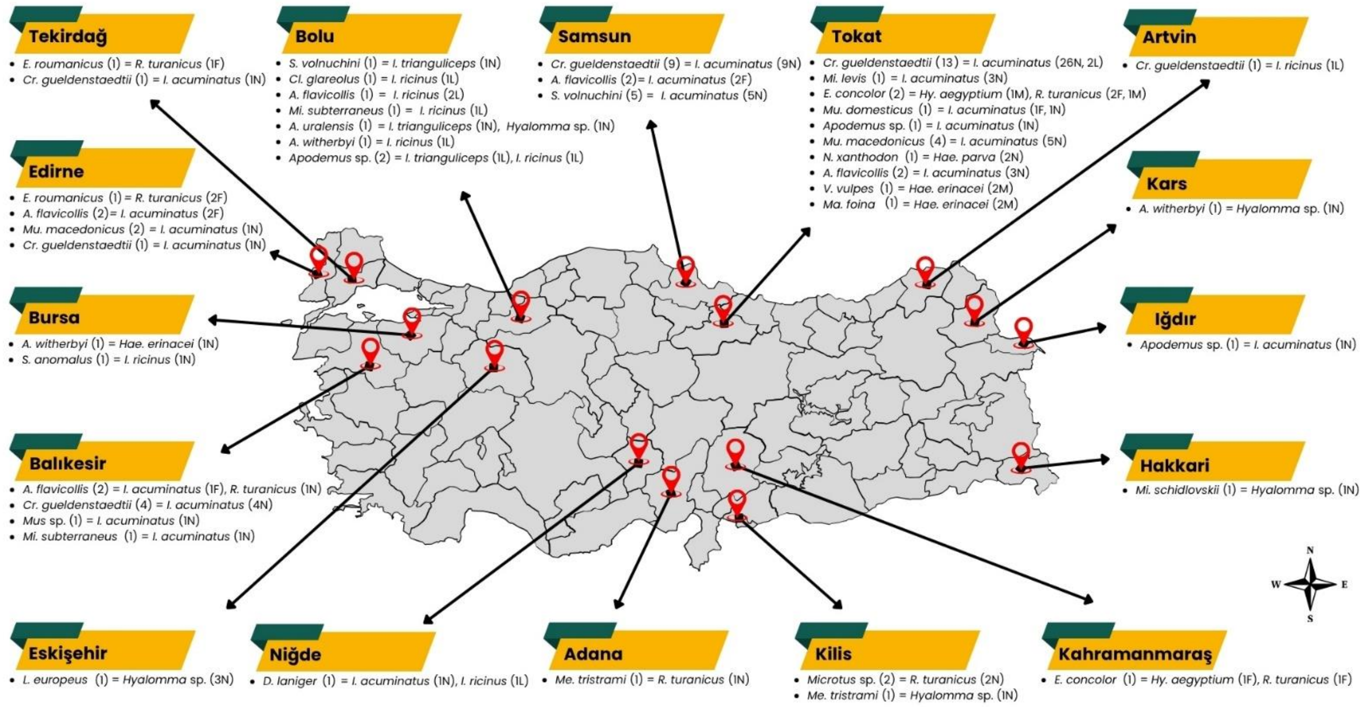

Figure 1. The host-associations and localities ticks collected in the present study.

Erinaceus roumanicus (n=2), *Lepus europaeus* (n=1), *Martes foina* (n=1), *Meriones tristrami* (n=2), *Microtus levis* (n=1), *Microtus schidlovskii* (n=1), *Microtus subterraneus* (n=2), *Microtus* sp. (n=2), *Mus domesticus* (n=1), *Mus macedonicus* (n=6), *Mus* sp. (n=1), *Nannospalax xanthodon* (n=1), *Sciurus anomalus* (n=1), *Sorex volnuchini* (n=6), and *Vulpes vulpes* (n=1).

Host associations and collection localities of the ticks identified in this study are presented in Figure 1.

Detection of Babesia species in ticks

As a result of molecular analyses, 7 out of the 112 specimens (6.25%) tested positive for *Babesia* spp. Upon comparison with the NCBI GenBank database, the sequence (PQ818402) obtained from a *Hyalomma* species collected from an *Apodemus witherbyi* in the Kars/Sarıkamış region showed 99.36% similarity with *Babesia caballi* (FJ209026-Croatia) and 99.35% similarity with *Babesia caballi* (PQ044789-France).

The sequences obtained from an *Ixodes acuminatus* specimen collected from *Apodemus flavicollis* (PQ818403) in Edirne and from *Crocidura gueldenstaedtii* (PQ818404–PQ818408) in Balıkesir (Kapıdağ Peninsula) and Tokat (Kumocağı and Taşlıçiftlik Village) exhibited 99.60–100% similarity to zoonotic *Babesia microti* Jena isolates (EF413181–Germany, KJ508857–Slovakia, JX627356–Türkiye, AB085191–Germany, MN355503–Türkiye). Additionally, our *Babesia microti* sequences (PQ818403–PQ818408) were compared with previously reported *B. microti* sequences from Erzurum (MN355502–MN355504), showing high similarity levels ranging from 99.60% to 100%. In contrast, BLASTn comparisons with sequences from Giresun (MH523087–MH523097) revealed slightly lower similarity values, ranging from 99.32% to 99.79%. Comparisons with *B. microti* sequences reported from Bartın showed similarity values ranging from 98.74% to 100%. Moreover, sequence comparisons with *Babesia microti* Munich (LC770217), a strain considered non-pathogenic in humans, revealed lower similarity rates, ranging between 96.74% and 97.19%. The similarity rates of our *Babesia microti* sequences with the *Babesia microti* Jena and *Babesia microti* Munich strains are presented in Table 1.

The phylogenetic relationship of the *Babesia* species detected in the ticks collected from wild mammals in our project, prepared using the Maximum Likelihood method, is shown in Figure 2.

Table 1. The similarity rates of our *Babesia microti* sequences with the *Babesia microti* Jena and *Babesia microti* Munich strains.

GenBank Accesion no.	Ticks	Host	Localities	*Babesia microti* Jena	*Babesia microti* Munich
PQ818403	*I. acuminatus*	*A. flavicollis*	Edirne	99.60%	96.86%
PQ818404	*I. acuminatus*	*C. gueldenstaedtii*	Balıkesir	99.80%	96.97%
PQ818405	*I. acuminatus*	*C. gueldenstaedtii*	Tokat	100%	97.18%
PQ818406	*I. acuminatus*	*C. gueldenstaedtii*	Tokat	99.80%	97.04%
PQ818407	*I. acuminatus*	*C. gueldenstaedtii*	Tokat	100%	97.19%
PQ818408	*I. acuminatus*	*C. gueldenstaedtii*	Tokat	99.42%	96.74%

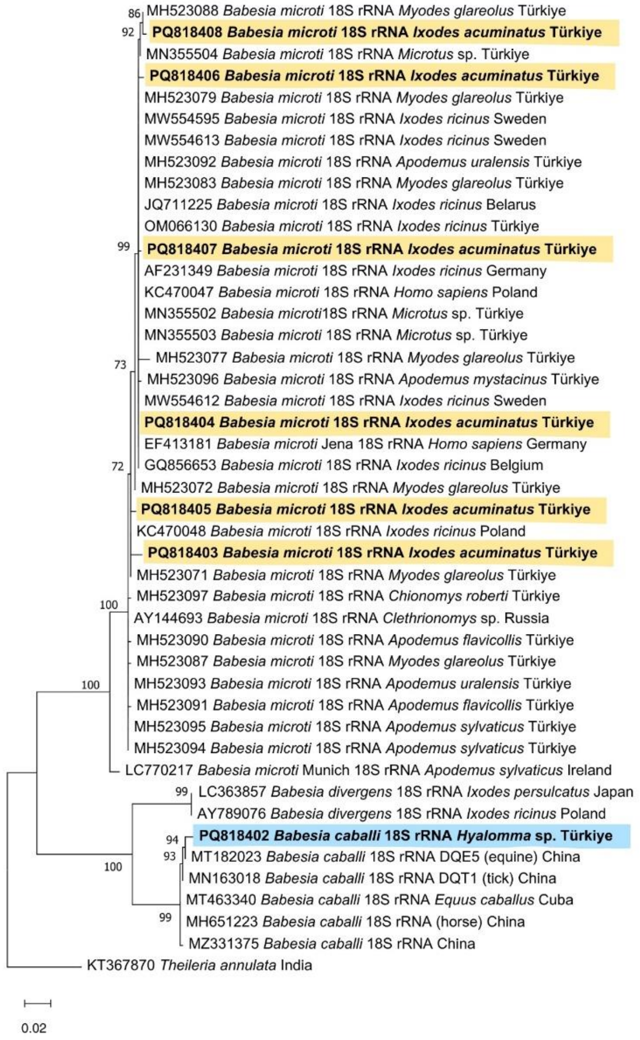

Figure 2. Phylogenetic tree based on the 18S rRNA gene region, showing the relationship between *Babesia* species identified in this study (indicated in bold and colored) and sequences retrieved from NCBI GenBank.

DISCUSSION

Ticks play a critical role in the life cycle and transmission of *Babesia* species. Following sexual reproduction in the midgut of adult ticks, the parasites invade ovarian tissues, allowing for transovarial transmission to offspring. Larvae subsequently migrate to the salivary glands and transmit the pathogen during blood feeding. Numerous hard tick species have been identified as vectors for *Babesia*, with 22 tick species reported to transmit 18 different *Babesia* species affecting livestock, pets, and humans (Ozubek et al., 2020).

In Türkiye, babesiosis is also recognized as a significant tick-borne disease, primarily reported in domestic animals such as cattle, horses, sheep, goats, and dogs. Surveillance studies on domestic and limited wild animals in Türkiye have identified various *Babesia* species, including *B. bigemina*, *B. bovis*, *B. caballi*, *B. canis*, *B. crassa*, *B. divergens*, *B. gibsoni*, *B. major*, *B. microti*, *B. motasi*, *B. ovis*, *B. occultans*, and *B. vogeli* (Orkun and Karaer, 2017, Aslantaş et al., 2020). Furthermore, studies involving human-biting ticks have detected *B. crassa*, *B. major*, *B. occultans*, *B. ovis*, and *B. rossi* (Aktas et al., 2012; Orkun et al., 2014; Karasartova et al., 2018).

Several studies in Türkiye have investigated *Babesia* infections in wild animal populations. For example, *Babesia* species have been detected in ticks collected from wild boars, rabbits, and foxes. In a study conducted by Orkun and Karaer (2017), *Babesia* DNA was screened by PCR in *Hyalomma marginatum* and *Haemaphysalis parva* collected from wild boars, as well as in *Hyalomma* spp. collected from rabbits. Sequence analyses revealed the presence of *Babesia occultans* in *Hy. marginatum* from wild boars and in *Hyalomma* spp. (nymphs) from rabbits. *Babesia rossi* was identified in *Hae. parva* collected from wild boars, and *Babesia crassa* was detected in *Hae. parva* from wild boars. Additionally, blood samples from foxes tested positive for *Babesia vulpes*, while rabbits harbored *Babesia* sp. tavsan 1 and *Babesia* sp. tavsan 2.

In a study performed by Aktas et al. (2007), the prevalence of *Babesia ovis* was investigated in sheep and goats. Among 400 samples (300 sheep and 100 goats), PCR analysis identified 33 positive cases, whereas microscopic examination detected only 6 positive samples. This finding highlights the higher sensitivity of molecular methods compared to traditional microscopy.

Studies on humans also underline the presence of *Babesia* species in ticks collected from individuals. In a study conducted in Çorum, 322 ticks collected humans were analyzed, detecting *B. microti*, *B. occultans*, and *B. ovis* in these ticks at varying rates (Karasartova et al., 2018). Similarly, research involving 498 wild rodents in Erzurum demonstrated that wild rodents act as reservoirs for *Babesia* agents, although no *Babesia* DNA was detected in ticks collected from these animals (Guven et al., 2022). In another study conducted on wild rodents in Türkiye, the study area included the provinces of Burdur, Yozgat, Giresun, and Bartın. Within the scope of this study, a total of 536 wild rodent specimens were collected from these four provinces, comprising the following species: *Myodes glareolus* (n=54), *Apodemus flavicollis* (n=135), *Apodemus sylvaticus* (n=24), *Apodemus witherbyi* (n=93), *Apodemus uralensis* (n=56), *Apodemus mystacinus* (n=74), *Chionomys roberti* (n=13), *Microtus subterraneus* (n=4), *Microtus guentheri* (n=26), and *Mus macedonicus* (n=57). Blood samples from these rodents were analyzed for the presence of *B. microti*. As a result, *B. microti* was detected in 31 out of 536 wild rodent samples. The positive samples originated from the provinces of Bartın and Giresun (Usluca et al., 2019).

In the present study, the presence of *Babesia* species in ticks collected from wild mammals across various regions of Türkiye was investigated using molecular techniques. Two species were identified: *B. microti* and *Babesia caballi*, with *B. microti* exhibiting a wider geographic distribution. Out of 112 tick samples analyzed, 7 tested positive for *Babesia*, corresponding to a positivity rate of 6.25%. Sequence analysis revealed that our *B. microti* isolates shared 99.60–100% similarity with the zoonotic *Babesia microti* Jena isolates, which have been implicated in human infections in Europe. This high degree of genetic similarity indicates that the *B. microti* strains circulating in ticks parasitizing wild mammals in Türkiye may possess zoonotic potential and should not be overlooked in public health assessments.

Consequently, these findings reinforce the notion that wild mammals and their associated ticks in Türkiye constitute an important reservoir for zoonotic *Babesia* species. Continuous surveillance using sensitive molecular tools is critical for understanding the epidemiology of babesiosis and for assessing the potential zoonotic risks posed by wildlife reservoirs.

Authors' contributions

Muhsin Kişi: Data curation, formal analysis, funding acquisition, visualization, software, writing - original draft (supporting). **Ayşe Sarı:** Data curation, formal analysis, investigation. **Ahmet Yesari Selçuk:** Resources, data curation, investigation. **Adem Keskin:** Conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, software, writing - original draft, project administration, supervision, writing - original draft (lead), writing - review & editing. All authors discussed the results and contributed to the final manuscript.

Statement of ethics approval

Not applicable.

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Conflict of interest

The authors declare that there is no conflict of interest.

Data availability

All sequences detected in this study have been deposited in the National Center for Biotechnology Information (NCBI, USA) GenBank database under the accession numbers from PQ818402-PQ818408.

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