



# *Rhipicephalus sanguineus* s.l. detection in the Slovak Republic

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## Abstract

Herein we report the first occurrence of *Rhipicephalus sanguineus* s.l. in the Slovak Republic. Sixty fed and unfed *Rh. sanguineus* s.l. ticks were collected from an apartment in the capital city of the Slovak Republic (Bratislava) and a family house in the town of Sered' in southwestern Slovakia. Based on molecular analyses of the cytochrome C oxidase subunit 1 (*cox1*) and 16S rRNA genes, all the ticks clustered in the *Rhipicephalus* sp. Ila mitochondrial “temperate” lineage, which contains geographically similar ticks from the mid- and western Mediterranean regions. *Rhipicephalus sanguineus* s.l. is not a part of the Slovak hard tick fauna.

**Keywords** *Rhipicephalus sanguineus* s.l. · Tick · Tick-borne diseases · The Slovak Republic

## Introduction

The brown dog tick or kennel tick *Rhipicephalus sanguineus* (Latreille, 1806) is one of the most widely distributed tick species (Walker 2000). It belongs to the *Rhipicephalus sanguineus* group of the African genus *Rhipicephalus* (Krantz and Walter 2009). Currently, *Rh. sanguineus* is taxonomically classified as a complex comprising at least 10 closely related species (Dantas-Torres 2008). These ticks are latitudinally distributed from 50° N to 35° S, which includes mostly warm, humid, coastal or continental climate zones (Filippova 1997). In Europe, it mainly occurs in countries in the Mediterranean region, including Portugal, Spain, France, Italy, Croatia, Greece, Cyprus and Turkey (ECDC 2018). *Rhipicephalus sanguineus* is a three-host ectoparasite that mainly feeds on dogs and lives in the cracks of walls or dog substrate areas (Filippova 1997; Dantas-Torres 2008). Adult

*Rh. sanguineus* have also been detected on other mammals such as horses, buffaloes, cattle, cats and many rodents (Filippova 1997; Uspensky 2009). There are some single records from wild animals, including leopard, jackal, porcupine and hedgehog (Filippova 1997; Pomerancev 1950).

Due to its medical and veterinary significance *Rh. sanguineus* is one of the most studied tick species. It is a vector of many different bacteria (*Rickettsia rickettsii*, *R. conorii*, *R. conorii* subsp. *israelensis*, *R. conorii* subsp. *caspia*, *R. conorii* subsp. *indica*, *R. massiliae*, *Ehrlichia canis*, *Coxiella burnetti*), protozoa (*Babesia canis*, *B. gibsoni*, *Hepatozoon canis*) and viruses (the Crimean-Congo hemorrhagic fever orthonavirus, the Wad Medani) that are dangerous to animals and humans (Taylor et al. 1966; Filippova 1997; Walker 2000; Dantas-Torres 2008; Parola et al. 2009; Tahmasebi et al. 2010).

*Rhipicephalus sanguineus* ticks are not a part of the Slovak tick fauna. Up to now, two cases of *Rh. sanguineus* detection in companion animals in former Czechoslovakia have been reported (Černý 1985, 1989). This article aims to present the first case of *Rh. sanguineus* introduction to the capital of the Slovak Republic and a city in southwestern Slovakia.

## Material and methods

Ticks were collected with forceps from the walls in the apartment and from two mixed-breed dogs in Bratislava

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(48.157149, 17.135584) during 2017 and 2018 years, as well as, from the walls and furniture in a house in the city of Sered' (48.293202; 17.729897), about 60 km from Bratislava, in 2020. Ticks were identified to species and stage according to taxonomic keys (Emchuk 1960; Filippova 1997; Walker 2000). Genomic DNA was isolated individually from each questing tick by the method of alkaline hydrolysis with modifications (Guy and Stanek 1991) and with DNeasy blood & tissue kit (Qiagen, Hilden, Germany) following the manufacturer's instructions for engorged individuals. DNA samples were stored at  $-20\text{ }^{\circ}\text{C}$  until further analyses.

Portions of the 16S rRNA and the *cox1* genes were chosen as targets for molecular analyses of tick species identification (Hornok et al. 2017). PCR amplified approximately 710 bp of the *cox1* gene using the primers LCO1490 and HCO2198 (Hornok et al. 2017) and approximately 460 bp of 16S rRNA of Ixodidae, using the primers 16S + 1 and 16S-1 (Black and Piesman 1994).

PCR amplification of the *gltA* gene of rickettsiae belonging to the spotted fever and typhus groups was performed by using the oligonucleotide pairs RpCS.877p and RpCS.1258n for the primary PCR amplification and RpCS.896p and RpCS.1233n for the secondary amplification as described by Choi et al. (2005). The presence of the *ompA* gene of *Rickettsia* spp. belonging to the spotted fever group was tested by a conventional PCR assay using the primers 190.70p and 190.701n as already described (Fournier et al. 1998).

PCR detection of 18S rRNA of *Babesia* spp. was performed using the genus specific primers BJ1 and BN2, as published previously by Casati et al. (2006). Amplicons were purified using a QIAquick Spin PCR Purification Kit (Qiagen, Hilden, Germany) as described by the manufacturer. The sequencings were performed by Eurofins Genomics Europe. DNA sequences were compared with available databases in GenBank®. The sequences were submitted to the GenBank® database under accession numbers MW152142, MW152143, MW152144 and MW152145 (*cox1*) and MW145165, MW145166 and MW145167 (16S). The MEGA model selection method was applied to choose the appropriate model for phylogenetic analyses. Phylogenetic analyses were conducted using the Maximum Likelihood method based on the Tamura-Nei model using MEGA version 7.0.

## Results

In October 2017, we found hundreds of ixodid ticks feeding on two mixed-breed dogs and crawling on the walls of a private apartment in Bratislava (the capital of the Slovak Republic). All the ticks were small, with elongated bodies, short palps, eyes, festoons and hexagonal basis capituli (Figs. 1, 2, 3a, b). Males had a deeply cleft coxa I and comma-shaped spiracular plates (Figs. 4a, b).



Fig. 1 Capitulum of moulted nymph

The owners had no ticks, and the family had not traveled abroad in recent years. The apartment and dogs were disinfected after examination. New ticks were observed crawling on the wall by the owners in March and then again in June 2018.

In January–February 2020, adult ticks were found by the inhabitants in a house in Sered', and 10 males and 10 females were sent to our laboratory.

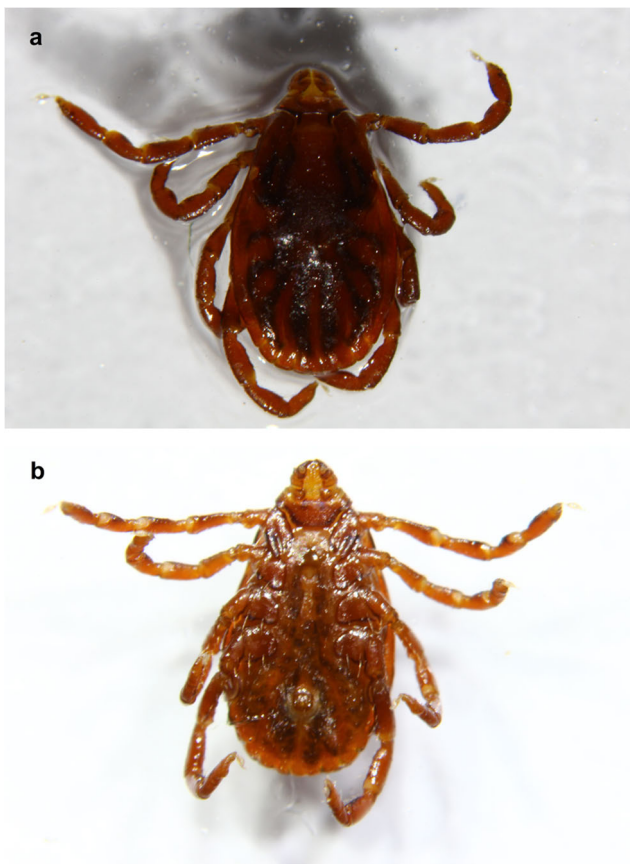
In total, 60 ticks (6 larvae, 30 nymphs, 12 males and 12 females) were morphologically and molecularly identified as *Rh. sanguineus*. Samples sent for identification contained questing and engorged ticks of all stages. Molecular analyses of amplified parts of the *cox1* and 16S rRNA genes of the ticks showed that they belonged to the subgroup *Rhipicephalus* sp. IIa “temperate lineage” (Figs. 5, 6).

Our samples (MW152142, MW152143, MW152144 and MW152145) showed 99.86% identity by the *cox1* gene sequences with isolates of *Rh. sanguineus* s.l. from Portugal (KU556745), Malta (KX519712) and 99.71% with samples from the USA: Georgia and Texas (MN593344, MN585197).

The portion of the 16S rRNA gene showed 99.53% identity of our samples (MW145165, MW145166 and MW145167) with *Rh. sanguineus* s.l. sequences from GenBank®,



Fig. 2 Nymph *Rh. sanguineus*



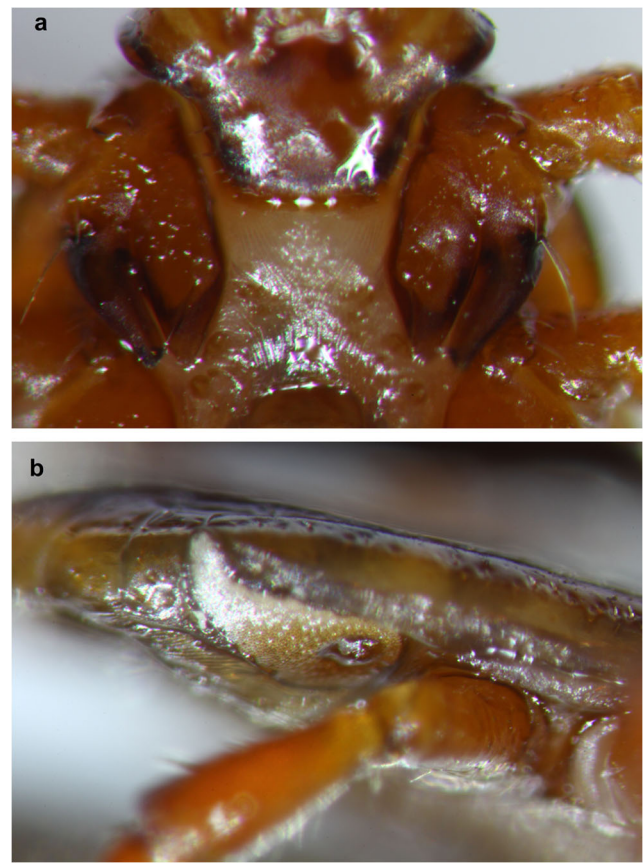
**Fig. 3** Dorsal (a) and ventral (b) view of female *Rh. sanguineus*

originating from Spain (JX997393), France (MH630342), Serbia (KX793738), China (MG651945) and the USA (KT382469).

All the samples were negative for *Rickettsia* spp. and *Babesia* spp. pathogens.

## Discussion

*Rhipicephalus sanguineus* s.l. is a cosmopolitan species. Dogs are likely the most suitable host for *Rh. sanguineus* ticks which can also infest other domestic or wild animals and humans. Their wide distribution is probably due to global transportation of domestic dogs by humans (Filippova 1997; Walker 2000; Uspensky 2004; Dantas-Torres and Otranto 2017). In recent years, *Rh. sanguineus* feeding on humans have been described in Europe (Parola et al. 2009), Israel (Uspensky 2009) and some South American countries (Dantas-Torres 2010). In Central and Eastern Europe, ticks have been reported in the natural environment in Hungary (Hornok et al. 2020), Croatia (Chitimia-Dobler et al. 2019), Serbia (Potkonjak et al. 2016), Albania (Xhaxhiu et al. 2009), Greece (Latrofa et al. 2017), Bulgaria (Ivanov et al. 2011), Romania (Sándor et al. 2014), Ukraine (Rogovskyy et al. 2017), Turkey (Ozubek et al. 2018), Russia (near the Black

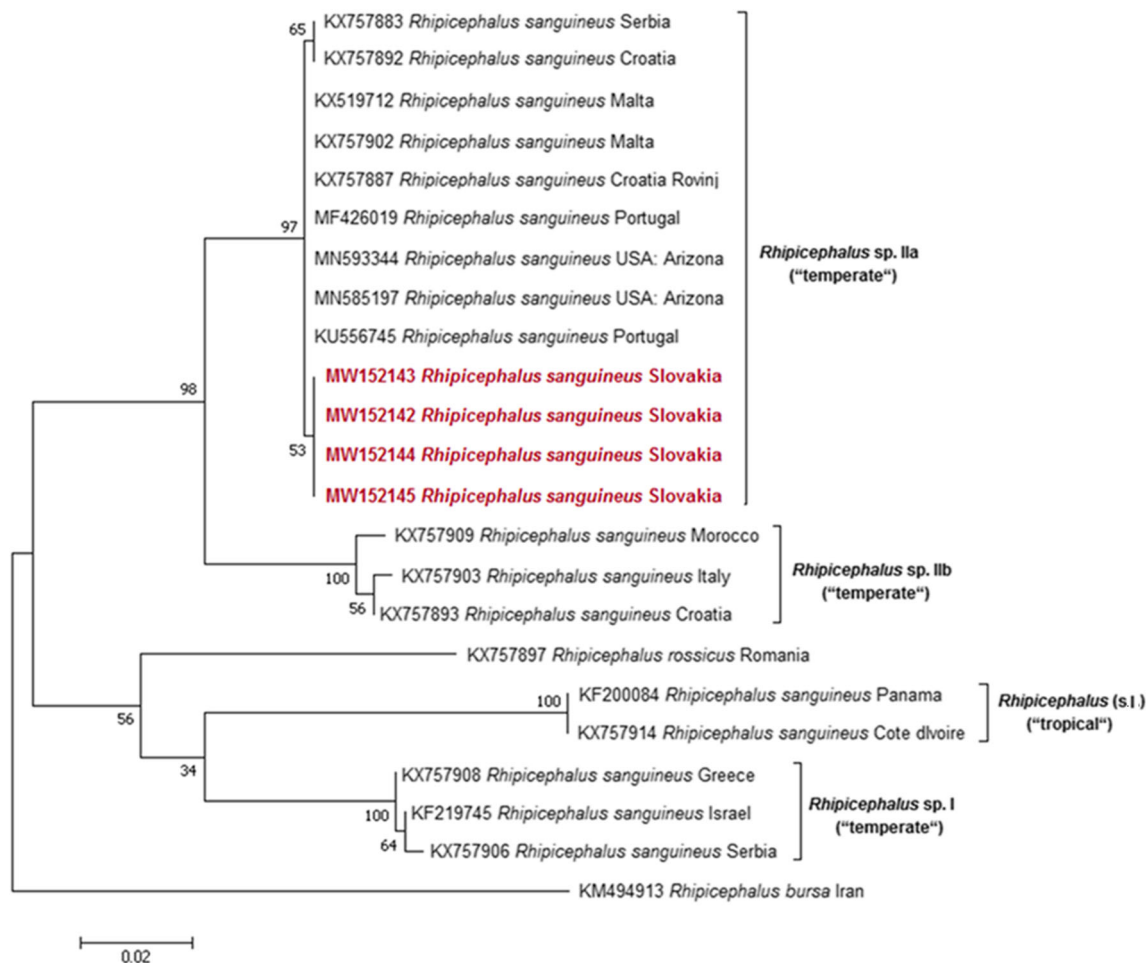


**Fig. 4** Left coxa I (a) and comma-shaped spiracular plates (b) of male *Rh. sanguineus*

and Caspian seas) (Filippova 1997) and Georgia (Sukhiashvili et al. 2020).

Molecular-phylogenetic analyses refer to two clades of *Rh. sanguineus*, “tropical species” and “temperate species”, both of which occur in the New World and in the Old World (Filippova 1997; Dantas-Torres 2008). Phylogenetic analyses of the *cox1* gene of ticks from private flat in Bratislava city and the house in Sereď confirmed that they belonged to the subgroup *Rhipicephalus* sp. Ila “temperate lineage”, with the closest relationship to samples of *Rh. sanguineus* ticks from Portugal, France, Malta, Croatia, Serbia and the USA (Fig. 5). Phylogenetic analyses of the 16S rRNA gene sequences also confirmed this and showed the closest relationship to samples from Serbia, Croatia, Malta and China (Fig. 6). According to data from the latest phylogenetic analyses of *Rh. sanguineus* (Hornok et al. 2017), our samples belonged to a heterogeneous group that occurs both in the New and Old Worlds.

*Rhipicephalus sanguineus* s.l. do not occur in the natural environment in the Slovak Republic. Two brief notes about the introduction of *Rh. sanguineus* ticks in former Czechoslovakia were reported by Černý (1985, 1989). In 1985, nymphs and larvae of *Rh. sanguineus* were introduced into an unknown city in Czechoslovakia by a dog from former Yugoslavia (from the territory of Croatia). In 1989, Černý



**Fig. 5** Phylogeny of *Rhipicephalus* spp. following the Maximum Likelihood analysis of the *cox1* gene. Partial sequences that were obtained in this work are indicated with color. Bootstrapped 1000 bp. Branch lengths represent the number of substitutions per site inferred according to the scale shown

presented another case report of tick introduction by a dog traveling from Cuba to Prague (now the Czech Republic) in 1976 (the material was collected and stored for identification in a museum collection).

*Rhipicephalus sanguineus* s.l. has multiple survival strategies due to its high adaptability. It can be a typical exophilic three-host tick (under natural conditions), endophilic (under indoor conditions) or monotropic (all stages feed on the same host). In recent years, *Rh. sanguineus* ticks were detected on humans and animals in northern European countries far from their natural distribution area. Mainly, this happens after people return from a vacation in Southern Europe or other warm climate countries. There are many case reports from England (Bates et al. 2002; Jameson et al. 2010; Hansford et al. 2017) due to the work of the Veterinary Laboratories Agency. From 2002 to 2009 in the UK, 53 tick collections were registered from animals in quarantine kennels, while 40 collections were registered from 2012 to 2016. All ticks were imported from countries where they were common. There are some published case reports of tick collections from Norway (Hamnes

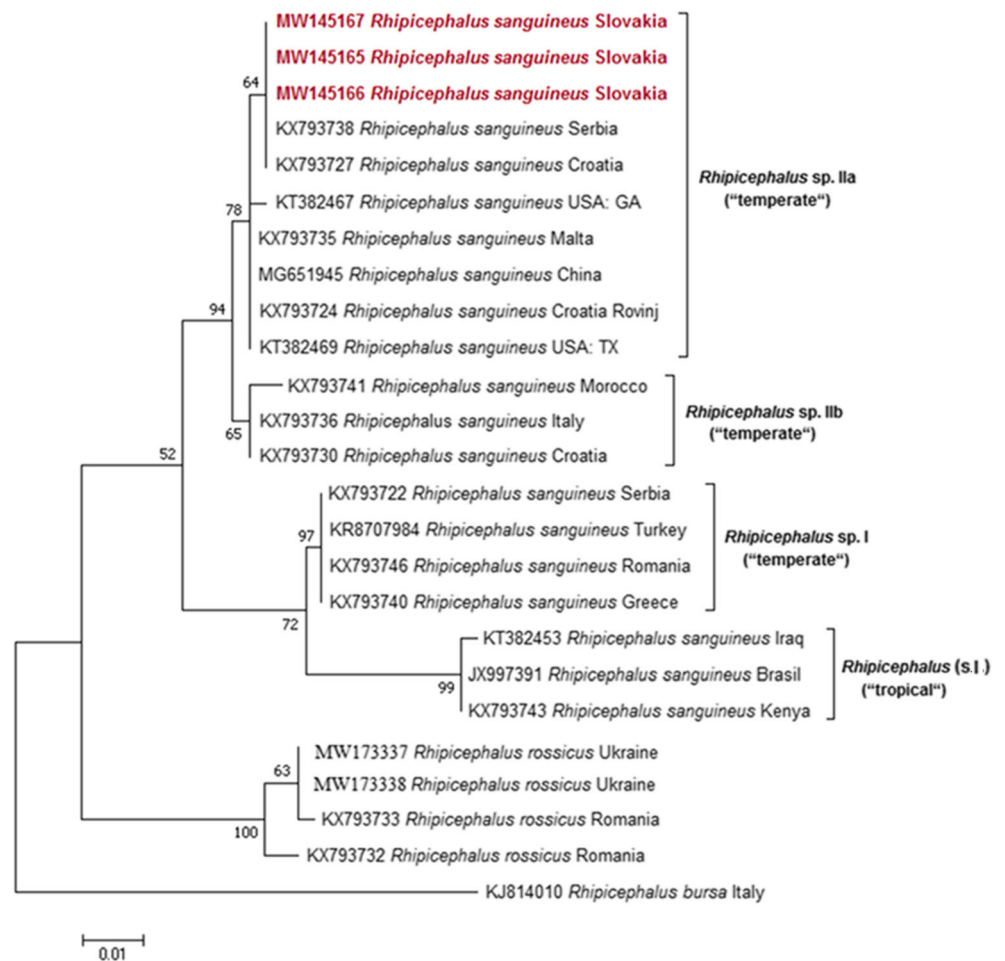
et al. 2012), the Netherlands (Garben and Bosman 1980), Denmark (Haarlof 1969; Willeberg 1970), and Poland (Szymański 1980). According to Dantas-Torres and Otranto (2017), *Rh. sanguineus* populations cannot be established in such cold areas.

Sometimes, introduced ticks are infected with different pathogens. In Germany, imported ticks were infected with *Ehrlichia canis* (Dongus et al. 1996). *Rhipicephalus sanguineus* s.l. ticks introduced to Austria in the 1970s–1980s were infected with *Babesia canis* (Prosl and Kutzer 1986). In 1980–1981 in Switzerland, near Geneva, ticks carried by the family dog in 1976 infected four persons with boutonneuse fever (*R. conori*) (Péter et al. 1984).

## Conclusions

This is the first documented case of *Rh. sanguineus* s.l. introduction to the Slovak Republic. It is possible that *Rh. sanguineus* s.l. ticks are introduced into the Slovak Republic

**Fig. 6** Phylogeny of *Rhipicephalus* spp. following the Maximum Likelihood analysis of the 16S rRNA gene. Partial sequences that were obtained in this work are indicated with color. Bootstrap 1000 bp. Branch lengths represent the number of substitutions per site inferred according to the scale shown



more often than reported. Under suitable conditions, brown dog ticks can live in new indoor habitats for several months or years. There are many possible *Rh. sanguineus* s.l. transmission routes from its natural habitats to the Slovak Republic or any European country. Additionally, with the increasing number of people and dogs traveling, it is likely that importation and infestation cases in the Slovak Republic will continue. When tourists are accompanied by their pets on vacation, they should be aware of possible infestation with *Rh. sanguineus* s.l. ticks and their pathogens.

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**Authors' contributions** All authors contributed to the study conception and design. Material collection [Markéta Derdáková, Jasna Kraljik and Eva pitalská]. Morphological identification performed by [Michal Stanko].

Material preparation, data collection and analysis were performed by [Yuliya M. Didyk] and [Barbara Mangová]. The first draft of the manuscript was written by [Yuliya M. Didyk] and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript. Supervision performed by [Markéta Derdáková].

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**Availability of data and material (data transparency)** The DNA sequences analysed during the current study are available in the Genbank®.

**Code availability (software application or custom code)** Not applicable.

**Declarations**

**Ethics approval (include appropriate approvals or waivers)** Not applicable.

**Consent to participate (include appropriate statements)** Not applicable.

**Consent for publication (include appropriate statements)** Not applicable.

**Conflicts of interest/competing interests (include appropriate disclosures)** The authors declare that they have no conflict of interest.

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