

An exotic souvenir on a German traveler returning from South Africa

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Abstract

The study reports the finding of a nymph attached to a German tourist during a holiday in South Africa. The nymph specimen was morphologically identified and confirmed by sequence analysis of the 16S rRNA and 12S rRNA genes. The nymph was identified as *Rhipicephalus maculatus*, a rare tick reported in humans. Screening using the pan-Rickettsia-PCR for infection gave a negative result.

Keywords: Human; Tick; South Africa; Germany

Introduction

Ixodid ticks are important arthropod vectors of pathogens, and human parasitism by these ticks are a common event in the world (Sonenshine et al. 2002). The hard tick family Ixodidae currently comprises 729 species worldwide (Guglielmone and Robbins 2018). In the genus *Rhipicephalus*, 85 species are listed, with the majority (63 species) having an Afrotropical distribution. The remaining *Rhipicephalus* species are either distributed exclusively in the Palearctic and Oriental regions or, probably due to anthropogenic activities, are prevalent also in the Australasian, Nearctic, and Neotropical regions (Guglielmone et al. 2014). So far, 48 species have been reported as human parasites. Most of them are sporadic or rarely parasitize humans; few species, however, more frequently may be found on humans globally (among them, *Rhipicephalus sanguineus* and *Rhipicephalus bursa*). According to Horak et al. (2018), approximately 88 hard tick species are known to occur in the southern African region, with 34 belonging to the genus *Rhipicephalus* (Horak et al. 2018).

According to literature, the majority of imported African tick-bite fever cases in travelers originate from South Africa (Mazhetese et al. 2021). This is likely due to the fact that South Africa is the main tourist destination in the southern Africa region, reporting more than 8 million international tourist arrivals per year (Fernández Ruiz 2015). In this regard, “wildlife tourism” increases the probability of contact between animals and humans and, therefore, also human contact with disease vectors (Hall 2019). Some studies also report the importation of South African ticks on human hosts. As such, a recent study described the importation of a nymph from *Amblyomma marmoreum* species complex into Argentina on an infested human

(Tarragona et al. 2021) and *Rhipicephalus capensis* into the USA (Stafford et al. 2022). Here, we report a human tick infestation on a German tourist in South Africa, which was detected shortly before returning to Germany with a tick, which so far was only rarely found on humans.

Material and methods

From 1 to 7 November 2021, a resident of Munich, Germany, made a trip to South Africa. According to her trip itinerary, she started 1 November in Munich and arrived on 2 November in Durban. There, she stayed overnight until 3 November in Mtubatuba, South African Province of KwaZulu-Natal (28°24'21.36"S 32°12'51.6"E). From 3 to 4 November, she visited the Falaza Game Park, Hluhluwe (27°59'39.0"S 32°20'43.4"E; South African Province of KwaZulu-Natal), and during this stay, she reported a tick crawling on her shoe. From 4 November, she stayed in St Lucia, (28°7'23.59"S 32°33'22.9"E; South African Province of KwaZulu-Natal) and flew back on 6 November to Cape Town to take the plane back to Munich. She stayed overnight in Cape Town (Muizenberg), (34°5'23.59"S 18°29'45.09"E) South Africa. On the morning of 7 November, she detected a tick on her hip and removed it manually before the flight back to Munich.

She deposited the live tick in a plastic box for contact lenses and brought it to a medical practice specializing in travel medicine in Munich asking for advice and treatment, respectively. The head of the practice contacted the author (LCD) and sent the tick for investigation. The tick specimen was first identified using morphological keys (Walker et al. 2000), under a KEYENCE VHX-900F microscope (Itasca, IL, USA). For confirmation of the morphological identification and potential infection with *Rickettsia* spp., molecular testing was performed.

DNA was extracted using the QIAamp mini Viral RNA extraction kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. For molecular identification of the tick specimen, two genes were amplified by PCR and Sanger sequenced, namely the 16S rRNA (Halos et al. 2004) and 12S rRNA (Beati and Keirans 2001) genes as described. Sequences were deposited in Genbank with accession numbers OM574775 and OM538420 for the 12S rRNA and 16S rRNA, respectively. The sequences obtained were manually checked and compared with sequences deposited in GenBank by BLASTN and phylogenetic analysis. Sequences for each gene were aligned using MAFFT (Kato and Standley 2013) with default parameters and phylogenetic analyses performed with IQ-Tree2 v1.6.12 (Minh et al. 2020). An optimal evolutionary model was calculated for each gene partition: 16S (TIM2 + F + I + G4) and 12S (HKY + F + I + G4), and absent genes were treated as missing data. An edge-proportional partition model with proportional branch lengths (-spp) was used to allow each partition its own specific rate to accommodate different evolutionary rates between partitions. Nodal support was estimated using ultrafast bootstrap ($n = 10,000$), and the 50% consensus tree was reported. Additionally, the *Rickettsia* spp. screening was performed using a previously published real-time PCR assay targeting a part of the *gltA* gene (Wölfel et al. 2008).

Results and discussion

The tick was identified as a nymph of the genus *Rhipicephalus* with morphological characters specific for *Rhipicephalus maculatus* Neumann, 1901. It is one of the three ornate *Rhipicephalus* species. The specific name *maculatus*, from the Latin meaning "spotted," is

due to a characteristic diffuse light-colored pattern that is seen only on the scutum of adults (Walker et al. 2000).

The analysis of the morphology of the nymph (Fig. 1a, b, c, d) revealed the following characteristics: scutum wider than long (0.675 mm width, 0.527 mm long); posterior margin a broad deep curve; cervical pits deep, convergent, continuous with the shallow; eyes at the widest point, prominent; basis capituli over three times broad as long (0.395 mm width, 0.128 mm long), the widest anteriorly where the exceptionally long sharp lateral angles project sideways over the scapulae, ventrally small bulges represent the ventral spurs and long setae. Hypostome spatulate, dental formula 2/2. Coxae I each with a long sharp external spur and a shorter broader internal spurs; coxae II to IV each with an external spur, decreasing gradually in size. Spiracular plate round.



Fig. 1. A nymph of *Rhipicephalus maculatus* parasitizing a German tourist with a prior trip to South Africa: **a** dorsal view, **b** ventral view, **c** scutum and capitulum dorsal, and **d** capitulum ventral

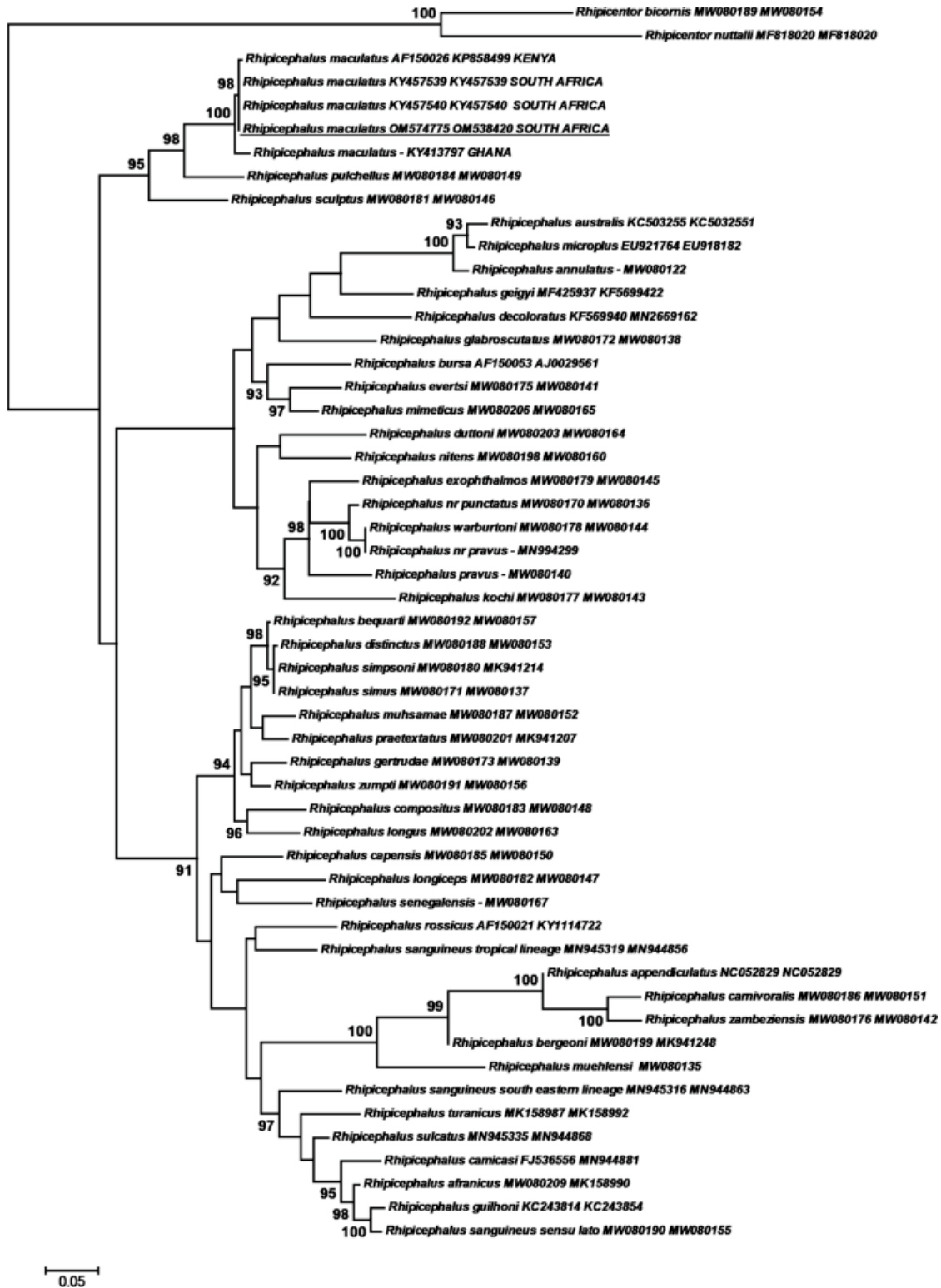


Fig. 2. Maximum likelihood analysis of the 12S and 16S rRNA genes for the genus *Rhipicephalus*. Bootstrap support above 90% is indicated, and the tree was rooted with *Rhipicentor* species. The accession numbers used for the 12S and 16S rRNA genes are indicated behind the species names, respectively, and the tick sequenced in the current study is underlined

Rhipicephalus maculatus rarely infests humans. Horak et al. (2018) reported 7 humans infested with 13 males, 5 females, and 2 nymphs with the current study are the first report of a *R. maculatus* nymph on a German tourist. *Rhipicephalus maculatus* is prevalent in the eastern and southern regions of Africa, at altitudes from sea level up to 1500 m (Walker et al. 2000). In South Africa, it was specifically described in the northeastern part of the South African province KwaZulu-Natal (Walker et al. 2000; Horak et al. 2018). Three of the locations where the German tourist spent most of her time, Mtubatuba, St. Lucia, and Falaza Game Park are located in the KwaZulu-Natal Province in the region that exactly correlate with the limited distribution of this tick species (Horak et al. 2018). In Falaza Game Park, the tourist saw a tick on her shoe during the observation of fenced wild animals, mainly impalas, zebras, warthogs, and southern green monkeys. Among other wild animals, impalas and warthogs are well-known hosts for *R. maculatus* (Walker et al. 2000; Horak et al. 2018).

The 16S rRNA (243 bp) and 12S rRNA (246 bp) sequences obtained showed 100% sequence identity, respectively, to *R. maculatus* sequences from South Africa and Kenya as confirmed by phylogenetic analysis (Fig. 2). *Rickettsia* spp. DNA was not amplified in the sample obtained from the *R. maculatus* nymph.

In our observation, a nymph of *R. maculatus* parasitizing a German tourist during a trip in South Africa is reported. No tick-borne pathogens could be detected in the tick. Besides the regular introduction of ticks by birds from other countries or continents (Petney et al. 2012; Chitimia-Dobler et al. 2019) or domestic animals traveling with tourists (Petney et al. 2012), this is another example that humans may acquire exotic ticks in foreign countries and bring them to their home country. Other similar examples, e.g., *Amblyomma mixtum*, infected with *Rickettsia amblyommatis*, imported from Cuba (Chitimia-Dobler et al. 2020), or *Dermacentor auratus* imported from a human from Cambodia (Chitimia-Dobler et al. 2021) to Germany have been described. Hoyle et al. (2001) summarized the tick's species found on quarantined dogs and cats in the UK and the potential pathogens for which the respective tick species can act as a vector. It is important that imported ticks are correctly identified, as in the recent study describing the importation of an *Amblyomma* nymph into Argentina on an infested human, which could not be morphologically and genetically identified, and it was attributed to *Amblyomma marmoreum* species complex (Tarragona et al. 2021). Another interesting report is the importation of *Rhipicephalus capensis* into the USA (Stafford et al. 2022). The analysis of the presented images in the manuscript shows that the morphological features (folded alloscutum, scutum, trochanter spur seen on the top of the scapulae and genital aperture) do not match *R. capensis* (Walker et al. 2000), while the analysis of the 16S rRNA gene only indicates 95% sequence identity. The possibility, therefore, exists that this might be a new species closely related to *R. capensis*. The newly described case of human parasitism by *R. maculatus*, together with the examples given from the literature underscores the potential risk for tourists when they visit areas where ticks with zoonotic potential occur. It also again shows that the correct identification of ectoparasitic ticks is important first to estimate the potential risk of introduction (e.g., example of *Haemaphysalis longicornis*) and to specifically test for pathogens, which are known to be transmitted by the respective tick species.

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Conflict of interest

The authors declare no competing interests.

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