



Short communication

## First detection of *Hyalomma rufipes* in Germany

Lidia Chitimia-Dobler<sup>a,\*</sup>, Santiago Nava<sup>b</sup>, Malena Bestehorn<sup>a</sup>, Gerhard Dobler<sup>a</sup>,  
Silke Wölfel<sup>a</sup>



<sup>a</sup> Bundeswehr Institute of Microbiology, Neuherbergstrasse 11, D80937 Munich, German Center of Infection Research (DZIF) partner Munich, Germany

<sup>b</sup> Instituto Nacional de Tecnología Agropecuaria Estación Experimental Agropecuaria Rafaela, Argentina

### ARTICLE INFO

#### Article history:

Received 27 May 2016

Received in revised form 16 August 2016

Accepted 16 August 2016

Available online 17 August 2016

#### Keywords:

*Hyalomma rufipes*

Horse

Germany

### ABSTRACT

*Hyalomma rufipes*, a two-host tick, is the most widespread *Hyalomma* species in Africa. In December 2015, an ixodid tick male with an unusual morphology was detected on a horse in a stable near Mainz in the Federal State Rhineland-Palatine. For identification purposes, the tick was preserved in alcohol and sent to our laboratory. The morphology of the tick showed specific characteristics of *H. rufipes*. The 16S rDNA sequence of *H. rufipes* from Germany was identical to the corresponding 16S rDNA sequence of *H. rufipes* from Tanzania, and they both were closely related to *Hyalomma marginatum*. The tick was tested with a real-time PCR for rickettsiae and Crimean-Congo hemorrhagic fever (CCHF) virus with negative results.

© 2016 Elsevier GmbH. All rights reserved.

### 1. Introduction

*Hyalomma rufipes* Koch, 1844 is known as "the hairy *Hyalomma*" or "the coarse bont-legged *Hyalomma*" (Walker et al., 2003; Latif, 2013a). *Hyalomma rufipes* was considered a subspecies of *Hyalomma marginatum* (Camicas et al., 1998; Horak et al., 2002), but currently it is accepted as a valid species (Apanaskevich and Horak, 2008). *Hyalomma rufipes* is the most widespread *Hyalomma* species in Africa, but is also present in Greece, Northern China, Russia, Turkey, Iraq, Syria, Pakistan, Egypt (Nile Valley), Yemen, and Oman (Walker et al., 2003; Kolonin, 2009; Chen et al., 2010; Bakirci et al., 2011; Latif, 2013b; Chaligiannis et al., 2014). Both larvae and nymphs use small mammals and birds as hosts, while adults are mainly found on cattle, sheep, goats, wild ungulates and horses (Walker et al., 2003; Latif, 2013b). As with certain other *Hyalomma* species, especially of the *H. marginatum* complex, *H. rufipes* is known to be a vector of Crimean-Congo hemorrhagic fever (CCHF) virus (Walker et al., 2003; Latif, 2013a; Kayedi et al., 2015) as well as of *Rickettsia conorii* (Walker et al., 2003; Latif, 2013a), *Rickettsia aeschlimannii* (Mediannikov et al., 2010; Djerbouh et al., 2012; Bitam, 2012), *Anaplasma marginale* and *Babesia occultans* (Walker et al., 2003; Latif, 2013a). Some authors implicated *Hyalomma* species in tick facial paralysis in humans (Gürbüz et al., 2010; Dogan et al., 2012). Larvae and nymphs of *H. rufipes* have been occasionally found on migratory birds in some European countries (e.g. The

Netherlands and Norway) (Nijhof et al., 2007). However, probably due to climatic conditions, no permanent populations have been recognized in these northern countries or in Central Europe, so far. The aim of the present study was the morphological and genetic identification of a single specimen of an exotic tick species for Germany and the testing of this individual tick for rickettsiae and CCH virus.

### 2. Material and methods

The *H. rufipes* male was removed from a horse by the owner in Ober-Olm, near the city of Mainz, Rhineland-Palatinate, on 13th December 2015, below the tail during a routine care. After removal, the tick was preserved immediately in 70% alcohol and sent to our laboratory. The tick was identified using morphological characters according to Walker et al. (2003) and Apanaskevich and Horak (2008).

Total nucleic acid was extracted using a Viral RNA mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The extracted total nucleic acid was stored at  $-80^{\circ}\text{C}$  until use.

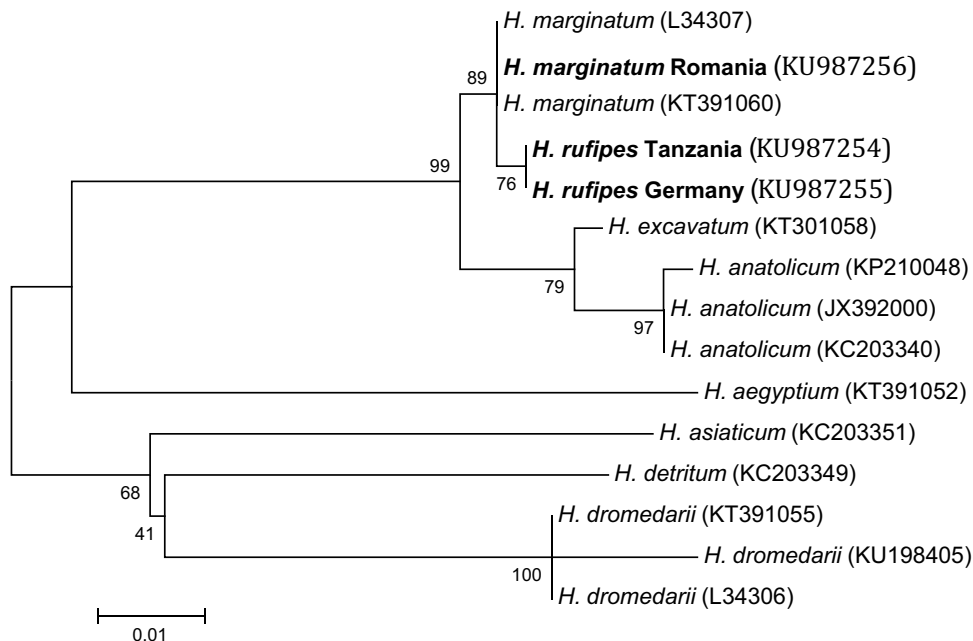
The 16S rDNA gene was amplified using a previously described polymerase chain reaction (PCR) protocol (Mangold et al., 1998). The obtained 420 bp fragment was analyzed by gel electrophoresis and Sanger sequencing (GATC Biotech, Konstanz, Germany). The phylogenetic analysis of a 395 bp, partial 16S rDNA sequence, was performed with Maximum-likelihood (ML) method using Mega 5.0 (Tamura et al., 2011). Best fitting substitution models were determined with the Akaike Information Criterion using the ML

\* Corresponding author.

E-mail address: [lidychitimia@yahoo.com](mailto:lidychitimia@yahoo.com) (L. Chitimia-Dobler).



**Fig. 1.** a: *Hyalomma rufipes* male, dorsal view: conscutum of male shiny black coloured and body evenly rounded posteriorly. Punctations dense and small covering evenly the dorsum (1); lateral grooves are short and obscured by dense punctations (2). Fig. 1b: *Hyalomma rufipes* male, ventral view: subanal plates distinct and directly posterior (in-line) to adanal plates (1); adanal plates shape with square ends (2); spiracle tail narrow and long extending to the margin of the body (3).



**Fig. 2.** Phylogenetic tree based on 16S rDNA sequences obtained from specimens of *Hyalomma* (*H.*) *rufipes* collected in Germany and in Tanzania and *H. marginatum* (KU987256) collected in Romania. The sequences obtained for this paper are marked in bold. GenBank accession numbers of the new sequences *H. rufipes* Germany (KU987255) and *H. rufipes* Tanzania (KU987254). Other sequences include data as available in GenBank, from other reported *Hyalomma* species from different countries.

model test implemented in MEGA 5.0. Support for the topologies was tested by bootstrapping over 1000 replicates and gaps were excluded from the comparisons. The substitution model was GTR +I. 16S rDNA sequences of *Hyalomma* spp. available in the GenBank were included in the sequences comparison.

Finally, the tick was tested for *Rickettsia* DNA using a panRickettsia real-time PCR (Wölfel et al., 2008) and for CCHF virus using a real-time RT-PCR according to the protocol of Atkinson et al. (2012).

### 3. Results

At the tick feeding location on the horse a localized inflammatory process compatible with a local reaction after a *Hyalomma* bite was detected. The horse did not show any other signs of dis-

ease. The tests performed to detect nucleic acids of possible human pathogens like rickettsiae or CCHF virus revealed negative results. The tick's rostrum had been broken during removal. The tick was morphologically identified as a male of *H. rufipes* (Fig. 1a and b) according to the identification keys of Walker et al. (2003) and Apanaskevich and Horak (2008). On the dorsal view, the *H. rufipes* male's conscutum was shiny black coloured and the body evenly rounded posteriorly. The punctations were dense and small covering the whole dorsum evenly. The lateral grooves were short and obscured by dense punctations. The legs were distinctly brightly banded. Differential characteristics of males of other *Hyalomma* species are: a) a posteromedian groove and a paramedian groove are absent; b) posterior ridges are absent; c) a caudal depression is absent. On the ventral view, the subanal plates are distinct and

directly posterior (in-line) to the adanal plates. The adanal plates shape with square ends, the spiracle tail is narrow and long extending to the margin of the body.

The obtained 16S rDNA sequence (KU987255) was compared with a sequence from one *H. rufipes* adult male from Tanzania (KU987254; pers. data) and showed 100% identity with this sequence (Fig. 2). These two sequences were closely related to *H. marginatum*. In fact, the pairwise difference between the 16S rDNA sequences of *H. rufipes* and *H. marginatum* was 0.5% (Fig. 2).

#### 4. Discussion

*Hyalomma rufipes* is a member of the *Hyalomma marginatum* complex, formed by *Hyalomma isaaci*, *Hyalomma marginatum sensu stricto*, *Hyalomma rufipes*, *Hyalomma turanicum* and *Hyalomma glabrum* (Capek et al., 2014). In the phylogenetic analysis, for the first time we proved that *H. rufipes* is genetically closely related to *H. marginatum*. The morphological and genetic proximity of these two species is exemplified by the fact that different authors (Hoogstraal and Kaiser, 1960; Camicas et al., 1998; Horak et al., 2002) considered *H. rufipes* a subspecies of *H. marginatum*, called *H. marginatum rufipes*. Currently, the former *H. marginatum rufipes* is considered a species called *H. rufipes* (Apanaskevich and Horak, 2008), and we follow this view, but future studies with additional lines of evidence could be helpful to validate or reject the current status of these taxa.

Ticks belonging to the *H. marginatum* complex including *H. rufipes* are known to transmit viral and bacterial agents with the potential to cause diseases with variable severity in humans, among them CCHF virus, *R. conorii* and *R. aeschlimannii* (Bitam, 2012; Parola et al., 2013). *R. aeschlimannii* was detected in ticks collected from birds in Pakendorf and Zerbst, Saxony-Anhalt, Germany, in May 2007. However, these ticks were not clearly identified as a species of the *H. marginatum* complex because the phylogenetic analysis of the three analyzed genes revealed different conflicting results (Rumer et al., 2011).

The immature stages of *H. rufipes* have been found on birds in some European countries (Hasle et al., 2009; Capek et al., 2014). *Hyalomma rufipes* larvae and nymphs feed on the same host individual, often a bird, and remain there up to 28 days (Knight et al., 1978). It is well known that ticks may be transported by migratory passerine birds over long distances (Hoogstraal et al., 1961; Olsen et al., 1995; Ishiguro et al., 2000; Alekseev et al., 2001). However, adult *H. rufipes* have only rarely been found outside of their known distribution area, so far. So far, one adult *H. rufipes* was reported on a horse in the Netherlands (Nijhof et al., 2007), two males were found on cows in Hungary (Hornok and Horvath, 2012), and a female parasitized a wild boar in south-central Spain (Ruiz-Fons et al., 2006).

In the United Kingdom, 21% of the birds, migrating from Africa in spring, were infested with *H. marginatum* nymphs (Jameson et al., 2012). *Hyalomma* species can also be transported from one continent to the other by humans, as shown e.g. a *H. truncatum* male imported to the United States of America attached to a photographer returning from an expedition in Ethiopia (Mathison et al., 2015). To exclude the potential transmission of relevant human pathogens mentioned above, the tick was tested for rickettsial DNA using a panRickettsia real-time PCR (Wölfel et al., 2008) and for CCHF virus (Atkinson et al., 2012) with negative results, respectively.

To the best of our knowledge this is the first record of *H. rufipes* in Germany and one of so far only three reports on adults of this species found in Central Europe (Nijhof et al., 2007; Hornok and Horvath, 2012). Neither the infested horse nor its stable mates had ever travelled to Africa or to southern Europe. As *Hyalomma* larvae and nymphs are regularly found on migratory birds, there is good reason to assume that this tick was probably imported as a feed-

ing nymph by a migratory bird during the spring migration from an endemic area to Central Europe. Due to the comparatively mild weather conditions in the year 2015 until December it is likely that the engorged tick had enough time to develop to the adult stage and to infest the horse. This is another example that tropical or subtropical tick species can molt to the next life stage under favorable weather conditions north of their common distribution area.

As *Hyalomma* ticks may carry important human pathogens—despite the fact that the tick described in this case did not harbor any rickettsiae or CCHF virus—a surveillance system for ticks invading Central Europe should be established as already existing for mosquitoes in Germany ([www.mueckenatlas.de](http://www.mueckenatlas.de)).

#### Conflict of interest

The authors declare no competing personal or financial interests.

#### Acknowledgements

We thank Prof. Dr. Abdalla Latif (Onderstepoort Veterinary Institute, Onderstepoort, South Africa) and Prof. Dr. Dmitry Apanaskevich (Georgia Southern University, Statesboro, Georgia, USA) for confirming our morphological identification.

We thank Dr. Olaf Kahl for his valuable comments and for the help with English language.

Part of this work was funded by the German Center of Infection Research (DZIF).

#### References

- Alekseev, A., Dubinina, H.V., Semenov, A.V., Bolshakov, C.V., 2001. Evidence of Ehrlichiosis agents found in ticks (Acari: Ixodidae) collected from migratory birds. *J. Med. Entomol.* 38, 471–474.
- Apanaskevich, D.A., Horak, I.G., 2008. The genus *Hyalomma* Koch, 1844: V. Re-evaluation of the taxonomic rank of taxa comprising the *H. (Euhyalomma) marginatum* Koch complex of species (Acari: Ixodidae) with redescription of all parasitic stages and notes on biology. *Int. J. Acarol.* 34, 13–42.
- Atkinson, B., Chamberlain, J., Logue, C.H., Cook, N., Bruce, C., Dowall, S.D., Hewson, R., 2012. Development of a real-time RT-PCR assay for the detection of Crimean-Congo hemorrhagic fever virus. *Vector Borne Zoonotic Dis.* 12, 786–793.
- Bakirci, S., Sarali, H., Aydin, L., Latif, A., Eren, H., Karagenc, T., 2011. *Hyalomma rufipes* (Koch, 1844) infesting cattle in the West Aegean region of Turkey. *Turk. J. Anim. Sci.* 35 (5), 359–363.
- Bitam, I., 2012. Vectors of rickettsiae in Africa. *Ticks Tick Borne Dis.* 3, 381–385.
- Camicas, J.L., Hery, J.P., Adam, F., Morel, P.C., 1998. *The Ticks of the World Nomenclature, Described Stages, Hosts, Distribution* (Acarida, Ixodida). Orston Editions, France, 233 pp.
- Capek, M., Literak, I., Kocianova, E., Sychra, O., Najer, T., Trnka, A., Kverek, P., 2014. Ticks of the *Hyalomma marginatum* complex transported by migratory birds into Central Europe. *Ticks Tick Borne Dis.* 5, 489–493.
- Chaligiannis, I., Papa, A., Sotiraki, S., 2014. Ticks feeding on ruminants and humans in Greece. *Parasite Vectors* 7 (Suppl. 1), O1.
- Chen, Z., Yang, X., Bu, F., Yang, X., Yang, X., Liu, J., 2010. Ticks (Acari Ixodoidea: Argasidae, Ixodidae) of China. *Exp. Appl. Acarol.* 51, 393–404.
- Djerbouh, A., Kernif, T., Beneldjouzi, A., Socolovschi, C., Kechemir, N., Parola, R., Raoult, D., Bitam, I., 2012. The first molecular detection of *Rickettsia aeschlimannii* in the ticks of camels from southern Algeria. *Ticks Tick Borne Dis.* 3, 374–376.
- Dogan, M., Devge, C., Tanriöver Pata, Y.S., Sönmezoglu, M., 2012. Case report: facial nerve paralysis due to intra-aural *Hyalomma* tick infestation. *Turiye Parazitol. Derg.* 36, 254–257.
- Gürbüz, M.K., Erdogan, M., Dogan, N., Birdane, L., Cingi, E., 2010. Case report: isolated facial paralysis with a tick. *Turiye Parazitol. Derg.* 34, 61–64.
- Hasle, G., Bjuene, G., Edvardsen, E., Jakobsen, C., Linnehol, B., Roer, J.E., Mehl, R., Røed, K.H., Pedersen, J., Leinaas, H.L., 2009. Transport of ticks by migratory passerine birds to Norway. *J. Parasitol.* 95, 1342–1351.
- Hoogstraal, H., Kaiser, M.N., 1960. Observations on ticks (Ixodoidea) of Lybia. *Ann. Entomol. Soc. Am.* 53, 445–457.
- Hoogstraal, H., Kaiser, M.N., Traylor, M.A., Gaber, S., Guindy, E., 1961. Ticks (Ixodidae) on birds migrating from Africa to Europe and Asia. *Bull. World Health Organ.* 24, 197–212.
- Horak, I.G., Camicas, J.L., Keirans, J.E., 2002. The Argasidae, Ixodidae and Nuttalliellidae (Acari: Ixodida): a world list of valid tick names. *Exp. Appl. Acarol.* 28, 27–54.

- Hornok, S., Horvath, G., 2012. First report of adult *Hyalomma marginatum rufipes* (vector of Crimean-Congo haemorrhagic fever virus on cattle under a continental climate in Hungary. *Parasite Vectors* 5, 170.
- Ishiguro, F., Takada, N., Masuzawa, T., Fukui, T., 2000. Prevalence of Lyme disease *Borrelia* spp. in ticks from migratory birds on the Japanese mainland. *Appl. Environ. Microbiol.* 66, 982–986.
- Jameson, L.J., Morgan, P.J., Medlock, J.M., Watola, G., Vaux, A.G.C., 2012. Importation of *Hyalomma marginatum*, vector of Crimean-Congo haemorrhagic fever virus, into United Kingdom by migratory birds. *Ticks Tick Borne Dis.* 3, 95–99.
- Kayedi, M.H., Chinikar, S., Mostafavi, E., Khakifirouz, S., Jalali, T., Hosseini-Chegeni, A., Naghizadeh, A., Niedrig, M., Fooks, A.R., Shahhosseini, N., 2015. Crimean-Congo hemorrhagic fever virus clade IV (Asia1) in ticks of western Iran. *J. Med. Entomol.* 52, 1144–1149.
- Knight, M.M., Norval, R.A.L., Rechav, Y., 1978. The life cycle of the ticks *Hyalomma marginatum rufipes* Koch (Acarina: Ixodidae) under laboratory conditions. *J. Parasitol.* 64, 143–146.
- Kolonin, G., 2009. Fauna of Ixodid Ticks of the World (Acari: Ixodidae), <http://www.kolonin.org>. (accessed 15.02.16).
- Latif, A.A., 2013a. Ixodid Ticks of Major Economic Importance and Their Distribution in South Africa. *Ticks and Tick-borne Diseases Monograph 1. AGRI Connect (PTY) LTD.*
- Latif, A.A., 2013b. Illustrated Guide to Identification of African Tick Species. *Ticks and Tick-borne Diseases Monograph 2. AGRI Connect (PTY) LTD.*
- Mangold, A.J., Barges, M.D., Mas-Coma, S., 1998. Mitochondrial 16S rRNA sequences and phylogenetic relationships of *Rhipicephalus* and other tick genera among Metastriata (Acari: Ixodidae). *Parasitol. Res.* 84, 478–484.
- Mathison, B.A., Gerth, W.J., Pritt, B.S., Baugh, S., 2015. Introduction of the exotic *Hyalomma truncatum* on a human with travel to Ethiopia: a case report. *Ticks Tick Borne Dis.* 6, 152–154.
- Mediannikov, O., Diatta, G., Fenollar, F., Sokhna, C., Trape, J.F., Raoult, D., 2010. Tick-borne rickettsioses, neglected emerging diseases in rural Senegal. *PLoS Negl. Trop. Dis.* 4, 1–13.
- Nijhof, A.M., Bodaan, C., Postigo, M., Nieuwenhuijs, H., Opsteegh, M., Fransses, L., Jebbink, F., Jongejan, F., 2007. Ticks and associated pathogens collected from domestic animals in the Netherlands. *Vector Borne Zoonotic Dis.* 7, 585–595.
- Olsen, B., Jaenson, T.G.T., Bergström, S., 1995. Prevalence of *Borrelia burgdorferi* sensu lato-infected ticks on migrating birds. *Appl. Environ. Microbiol.* 61, 3082–3087.
- Parola, P., Paddock, C.D., Socolovschi, C., Labruna, M.B., Mediannikov, O., Kernif, T., Abdad, M.Y., Stenos, J., Bitam, I., Fournier, P.E., Raoult, D., 2013. Update on tick-borne rickettsioses around the world: a geographic approach. *Clin. Microbiol. Rev.* 26, 657–700.
- Ruiz-Fons, F., Fernández-de-Mera, I.G., Acevedo, P., Höfle, U., Vicente, J., de la Fuente, J., Gortazár, C., 2006. Ixodid ticks parasitizing Iberian red deer (*Cervus elaphus hispanicus*) and European wild boar (*Sus scrofa*) from Spain: geographical and temporal distribution. *Vet. Parasitol.* 140, 133–142.
- Rumer, L., Graser, E., Hillebrand, T., Talaska, T., Dautel, H., Mediannikov, O., Roy-Chowdhury, P., Sheshukova, O., Mantke, O.S., Niedrig, M., 2011. *Rickettsia aeschlimannii* in *Hyalomma marginatum* ticks, Germany. *Emerg. Infect. Dis.* 17, 325–326.
- Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., Kumar, S., 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol. Biol. Evol.* 28, 2731–2739.
- Wölfel, R., Essbauer, S., Dobler, G., 2008. Diagnostics of tick-borne rickettsioses in Germany: a modern concept for a neglected disease. *Int. J. Med. Microbiol.* 298 (S1), 368–374.
- Walker, A.R., Bouattour, A., Camicas, J.L., Estrada-Peña, A., Horak, I.G., Latif, A.A., Pegram, R.G., Preston, P.M., 2003. Ticks of domestic animals in Africa: guide to identification of species. ICTTD.