



Short communication

Hyalomma rufipes of Asian origin transported to Europe by a migrant birdAttila D. Sándor^{a,b,c,*}, Áron Péter^b, Joanna B. Wong^d, Reto Burri^d, Sándor Hornok^{a,b}^a HUN-REN-UVMB Climate Change: New Blood-Sucking Parasites and Vector-Borne Pathogens Research Group, Hungary^b Department of Parasitology and Zoology, University of Veterinary Medicine, Budapest, Hungary^c STAR-UBB Institute, Babes-Bolyai University, Cluj-Napoca, Romania^d Swiss Ornithological Institute, CH-6204 Sempach, Switzerland

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ABSTRACT

Hyalomma rufipes is one of the most epidemiologically important ticks in Africa and the Middle East. It is regularly transported by migratory birds and there are chances that these ticks may become sources for the emergence of resident populations in the temperate region due to recent changes in climatic conditions. In May 2023, a *Hyalomma* sp. nymph was collected in SE Romania from a long-distance migrant host, the Pied Wheatear (*Oenanthe pleschanka*) with known migratory route. The tick was identified morphologically and genetically as *H. rufipes* and based on the timing of the migratory track it attached to its avian host in Saudi Arabia, the Middle East. This is the first ever *H. rufipes* recorded in Europe with known Middle Eastern/Asian origin.

1. Introduction

Ticks are currently the most important vectors of zoonotic diseases in the Northern Hemisphere, with several tick-borne diseases showing emerging patterns (Madison-Antenucci et al., 2020). Bacterial diseases like Lyme borreliosis, human granulocytic anaplasmosis, or relapsing fevers are on the rise both in Europe, as well as in North America, while the impact of viral tick-borne encephalitis or Crimean-Congo Haemorrhagic fever (CCHF) seems to be vastly underestimated both in Africa and Asia (Heyman et al., 2010; Mackenzie and Williams, 2009; Temur et al., 2021). All these emerging health risks have a common background: the changes in geographic occurrences of their main vectors, the ticks. Several epidemiologically important tick species were documented to expand ranges, with northward or elevational shifts, or new colonisations (Cunze et al., 2022; Medlock et al., 2013). Among these tick species, one bears particular importance, *Hyalomma rufipes* Koch, 1844, locally also known as "the hairy *Hyalomma*" or "the coarse bont-legged *Hyalomma*" (Latif, 2013; Walker, 2003).

Hyalomma rufipes is a two-host tick species, which was considered a subspecies of *Hyalomma marginatum* for a long period (Horak et al., 2002), but it is currently classified as a valid species (Apanaskevich and Horak, 2008). It is primarily an African species, occurring in more arid habitats, with a distribution split in two by the tropical areas. It does occur outside Africa, with resident populations in some countries in the

Middle East (Yemen, Oman and Saudi Arabia), but its sporadic occurrence was also reported from most Mediterranean countries, and Central Europe east to Caucasus, Russia, Iraq and China (Vatansver, 2017). *Hyalomma rufipes* is the most common vector of Crimean-Congo Haemorrhagic Fever (CCHF) virus in Africa, while blamed for the transmission of several *Rickettsia* species, *Theileria annulata*, but also *Babesia occultans* (Bonnet et al., 2023), thus making it a very important tick species both from a human epidemiologic, as well as a livestock health perspective. Adult ticks rely on large herbivores (mainly cattle, horses, sheep and camels, but also wild ungulates and ostriches) for feeding, while both larvae and nymphs use small mammals, reptiles and birds as hosts (Vatansver, 2017). As such, the immatures of the species regularly occur on Palearctic-African long-distance migrant birds, which may transfer these ticks from Africa, all along their northward spring migration (Hoogstraal and Kaiser, 1958). Although larvae and mostly nymphs were regularly recorded on such migrants in several countries in Europe (Keve et al., 2022), the occurrence of adult *H. rufipes* in Europe is rare and mostly confined to single individuals found mainly on horses (Chitimia-Dobler et al., 2016). An establishment of permanent populations in Europe was considered unlikely due to climatic conditions, however, there are signs of changes in this situation with a small, reproductive population recorded in Hungary (Keve et al., 2023). The origin of these ticks was hypothesised to be of sub-Saharan Africa, with all molecularly tested, European *H. rufipes* records showing high

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similarity with African sequences (Chitimia-Dobler et al., 2016; Hansford et al., 2019; Hornok et al., 2022; Keve et al., 2023; Lesiczka et al., 2022; Uiterwijk et al., 2021). The aim of the present study was to present a record of a *H. rufipes* with Asian origin collected in Romania from a bird with known migratory route.

2. Materials and methods

The tick was removed from an adult male Pied Wheatear (*Oenanthe pleschanka*) (Fig. 1.) on 05 May 2023 near Mihail Kogalniceanu, SE Romania (44.4182 N; 28.5072E). It was fully engorged and self-detached while the host was processed. For the estimated developmental period of *H. rufipes* subadults, previously published data based on laboratory rearing of ticks were used (Knight et al., 1978; Magano et al., 2000).

After removal, the tick was preserved in 97 % ethanol. The capture of the host bird was a targeted action, as this individual was marked with color rings before and was bearing a miniature data logger. It was initially captured and deployed with the datalogger in the same place in May 2022, to follow its post-breeding migration and wintering. The geolocator used (a multi-sensor 1.2 g GDL3-PAM geolocator, Swiss Ornithological Institute) was mounted using a leg-loop back-harness and collected data on ambient light intensity (Lisovski et al., 2020). Using a preset threshold method (threshold = 1; log-scale), the sunrise and sunset times of each day of the journey was determined from the recorded light data using the R package TwGeos (R Core Team, 2023; Wotherspoon et al., 2016). The tag was calibrated at the breeding site to derive the median reference solar zenith angle, and subsequent location estimation, construction and refinement of the most probable median route (from 2000 final Markov chain Monte Carlo iterations) was modelled using R package SGAT (Wotherspoon et al., 2013).

The collected tick was identified using standard morphological characters (Estrada-Peña et al., 2018). The DNA was extracted from one leg using the QIAamp DNA Mini Kit (QIAGEN, Hilden, Germany), including an overnight digestion in tissue lysis buffer and Proteinase K at 56 °C. PCR amplification of an approx. 710-bp-long fragment of the cytochrome c oxidase subunit I (*cox1*) barcoding gene was attempted with the primer pairs LCO1490 (5'-GGT CAA ATC ATA AAG ATA TTG G-3') and HCO2198 (5'-TAA ACT TCA GGG TGA CCA AAA AAT CA-3') (Folmer et al., 1994). In addition, a second genetic marker, an approx. 460-bp-long fragment of the 16S rDNA gene of Ixodidae was also amplified with the primers 16S+1 (5'-CTG CTC AAT GAT TTT TTA AAT TGC TGT GG-3') and 16S-1 (5'-CCG GTC TGA ACT CAG ATC AAG T-3') (Black and Piesman, 1994). PCR reaction components included 5 µl of

extracted DNA, added to 20 µl of reaction mixture containing 1 U Hot-Star Taq Plus DNA Polymerase (5U/µl) (QIAGEN, Hilden, Germany), 0.5 µl dNTP Mix (10 mM), 0.5 µl of each primer (50 µM), 2.5 µl of 10x Coral Load PCR buffer (15 mM MgCl₂ included) and 15.8 µl ddH₂O. For amplification, an initial denaturation step at 95 °C for 5 min was followed by 40 cycles of denaturation at 94 °C for 40 s, annealing for 1 min at 48 °C or 51 °C in case of the *cox1* and 16S rRNA genes, respectively, and extension at 72 °C for 1 min. Final extension was performed at 72 °C for 10 min. PCR products were visualized in 1.5 % agarose gel.

Purification and sequencing of the PCR products were done by Eurofins Biomi Ltd. (Gödöllő, Hungary). Quality control and trimming of sequences were performed with the BioEdit program. Obtained sequences were compared to GenBank data by the nucleotide BLASTN program (<https://blast.ncbi.nlm.nih.gov>).

3. Results

The collected tick was identified as a fully engorged *Hyalomma* sp. nymph (based on the presence of eyes on the scutum, form and length of hypostome and palps, as well coxa I, see Fig. 2). The *cox I* gene sequence (accession no PV810458) showed a 100 % identity with sequences of *H. rufipes* from Nigeria (GenBank accession no MN601293), Kenya (OQ540949), and Cameroon (MK648422). The 16S rRNA sequence (accession no PV765685) showed 100 % identity to sequences of *H. rufipes* collected in Namibia (KU130461), South Africa (KU130465), and Senegal (KU130457). Considering the 23–28 days as the developmental period of *H. rufipes* subadults, we localised the geographical area of probable presence of the host 23–28 days prior to its recapture. This time period (ca. on dates between 7–11 April 2023) partly overlaps with a minor migratory staging east of Riyadh, in the region of Haradh, SE Saudi Arabia (a region of ca. 50–70 km radius circle with central coordinates at 23.46 N, 48.78 E). Here the bird interrupted its northward migration for eight days in the mentioned period (see Fig. 3.), this being the likely geographical region where *H. rufipes* attached as larvae, which developed into the nymph removed from the adult Pied Wheatear in Romania.

4. Discussion

Long-distance Palearctic-African migrants are known to regularly



Fig. 1. Adult male Pied Wheatear (*Oenanthe pleschanka*) at the moment of initial capture on 27 May 2022.



Fig. 2. Fully engorged nymph of *Hyalomma rufipes*, collected from a Pied Wheatear (*Oenanthe pleschanka*) on 08 May 2023 near Mihail Kogalniceanu, SE Romania.

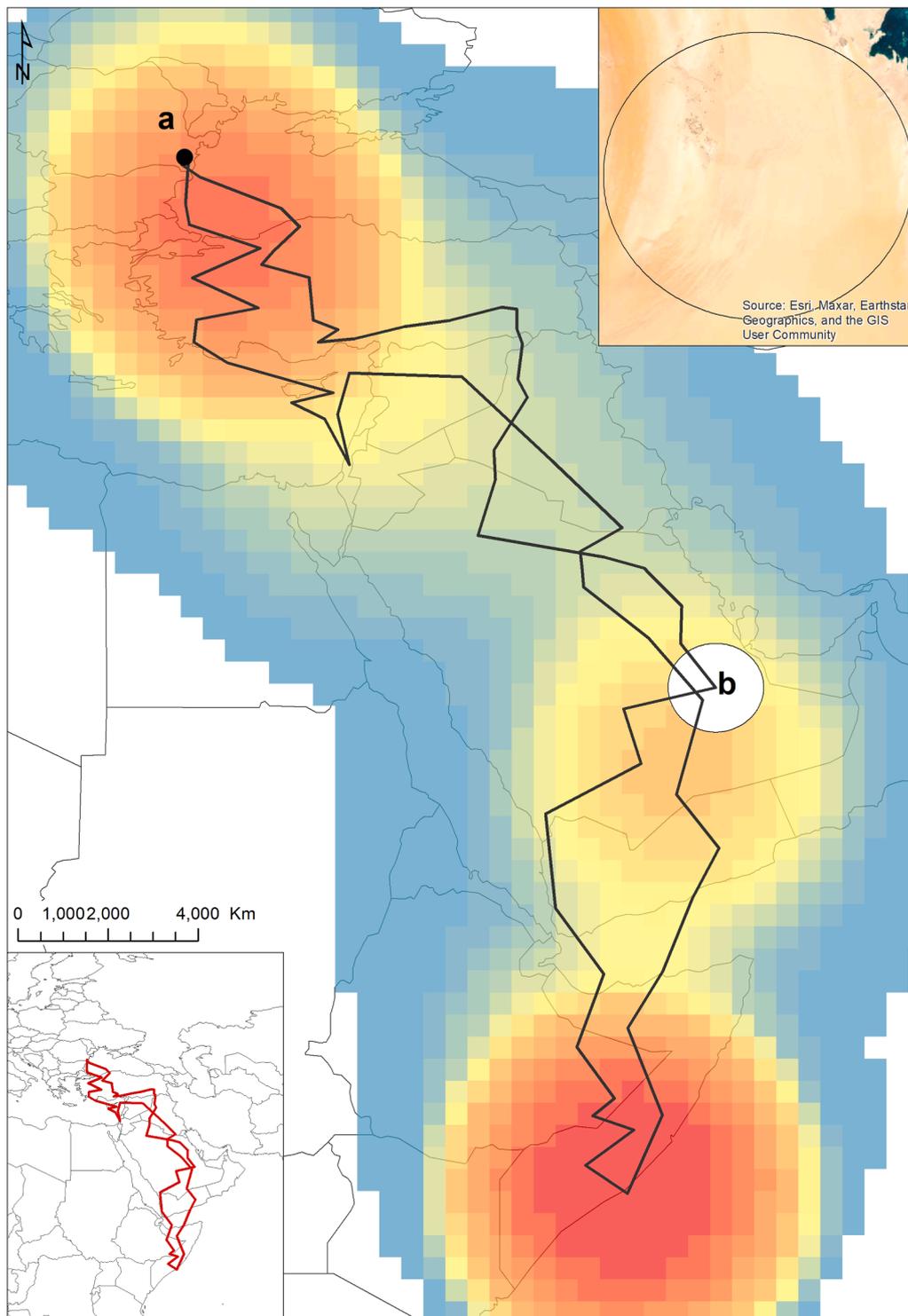


Fig. 3. Reconstructed migratory track of the adult Pied Wheatear (*Oenanthe pleschanka*), ring no COR R032468, heatmap generated using coordinates based on sunrise/sunset records collected by a geolocator, showing ringing/recapture site (point *a*) as well probable region of tick acquisition (ca. 200 km radius circle *b*, enlarged in the right upper corner showing landscape composition using satellite image).

transport ticks of tropical or subtropical origin to temperate regions of Europe or Asia (Hornok et al., 2022; Keve et al., 2022). Among these migrants, open landscape specialists, like *Oenanthe* spp. wheatears are considered important hosts for *Hyalomma* spp. ticks (Hoogstraal et al., 1961; Hoogstraal and Kaiser, 1958; Kaiser et al., 1974; Keve et al., 2022), with regular records of these species hosting *H. rufipes* in North Africa (Hoogstraal and Kaiser, 1958), Europe (Mancuso et al., 2022; Saikku et al., 1971) or the Middle East (Kaiser et al., 1974). The Pied

Wheatear is a typical representative of this group, with a primarily Asian distribution, and having the most western breeding population along the shores of the Black Sea (Schweizer et al., 2019). These birds winter in East Africa (especially in the Horn of Africa, see Cramp and Perrins, 1993), and spring migrants were already recorded to host nymphs of *H. rufipes* in Cyprus (Kaiser et al., 1974) and in coastal Saudi Arabia (Fain et al., 1995). Based on the migratory route of this tracked individual Pied Wheatear, the most likely point of origin of the nymphal tick

lays in the SE part of Saudi Arabia, east to Riyadh, in the region of Haradh. Unfortunately, with the considerable uncertainty of geolocator provided data (Lisovski and Hahn, 2012), we are not able to pinpoint the exact location the bird used. However, this whole region shows fairly similar habitat pattern of extensive areas of sandy-gravel desert, with rocks and salt lakes (sabkha-s) to the south with low vegetation cover and ideal habitats for larks and wheatears (Boland et al., 2020). The region is a hotspot of Pied Wheatear records in Saudi Arabia. The largest integrated dairy farm in the world is also located here, with extensive arable and grazing fields, providing suitable conditions for ticks, like *H. rufipes*, which is a common parasite of livestock in the region (Abdally et al., 2020; Alghamdi, 2024).

To the best of our knowledge, this is the first geolocated record of any tick of Middle Eastern/Asian origin in Europe and it is also the first observation of *H. rufipes* in Romania. Immatures of *H. rufipes* are commonly found on many bird species (52 species listed by Keve et al., 2022), and it is known that such tick individuals may harbour pathogens, too. Molecular analyses of bird-derived ticks registered DNA of several rickettsial species in *H. rufipes* nymphs, with *Rickettsia aeschlimannii* being the most common (Battisti et al., 2020; Mancuso et al., 2022; Olivieri et al., 2021). However, this tick is the most important vector for CCHF virus in Africa and it is involved in the circulation of two apicomplexans with huge impact on livestock health, *Babesia occultans* (Decaro et al., 2013) and *Theileria annulata* (Bonnet et al., 2023). While neither of these pathogens were recorded in Romania up to now, serological screening of livestock showed 27–37 % seroprevalence for CCHF virus in SE Romania (Bratuleanu et al., 2022; Ceianu et al., 2012), suggesting at least occasional presence of this pathogen. As current changes in land use and climatic conditions may favour the establishment of new vector species in non-endemic areas, the screening of migratory birds may be used as an early warning system to highlight such colonisations and to trigger timely response in case of any new pathogen introduction by these vectors.

Author statement

Hereby the authors declare that they have not used any type of generative artificial intelligence for the writing of this manuscript, nor for the creation of images, graphics, tables, or their corresponding captions.

Ethics approval and consent to participate

Birds were captured and tagged during routine bird ringing operations required for the study of population dynamics of migratory birds according the national legislation (Law 49/2011, Annex 8, art. C), number of ringing permit COR828265/2001 (issued to ADS).

Consent for publication

Not applicable.

CRediT authorship contribution statement

Attila D. Sándor: Writing – review & editing, Writing – original draft, Investigation, Data curation, Conceptualization. **Aron Péter:** Writing – review & editing, Visualization, Resources, Investigation, Data curation. **Joanna B. Wong:** Writing – review & editing, Resources, Investigation, Formal analysis, Data curation. **Reto Burri:** Writing – review & editing, Resources, Project administration, Methodology, Funding acquisition, Formal analysis, Data curation. **Sándor Hornok:** Writing – review & editing, Resources, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no competing interests.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ttbdis.2025.102518.

Data availability

All data related to this study is presented in the manuscript.

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