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Detection of a novel bacterium of the genus *Midichloria* (family *Midichloriaceae*) in avian-borne *Hyalomma marginatum* ticks and their trans-Saharan migratory hosts.

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Ticks are haematophagous ectoparasites of vertebrates habitually parasitizing avian species, which may contribute to tick dispersal across continents during migrations (Hasle 2013; Altizer et al., 2011). *Midichloria* bacteria can be transmitted to the vertebrate host during the tick bite (Bazzocchi et al., 2013; Serra et al., 2018). Although many avian species are common hosts of ticks harbouring *Midichloria* (e.g. *Ixodes, Hyalomma*), the circulation of this bacterium in birds has never been investigated. The aims of this study are: 1) evaluate the presence of *Midichloria* DNA in *H. marginatum* ticks and blood collected from trans-Saharan migratory birds; 2) quantify *Midichloria* bacteria in ticks through a novel quantitative PCR (qPCR).

A total of 256 *H. marginatum* ticks and 97 blood samples were collected from three different migratory species (*Phoenicurus phoenicurus*, *Saxicola rubetra* and *Sylvia communis*) on Ventotene Island (Central Italy) and DNAs were extracted. A nested-PCR targeting the 16S rRNA gene of *Midichloria* was used to detect bacterial presence. Subsequently, primers targeting the gyrB gene of *Midichloria* and the cal gene of *H. marginatum* were designed and used in a qPCR for *Midichloria* quantification. Results were expressed as gyrB/cal copy numbers ratio.

94% of *Hyalomma* ticks harbored DNA of *Midichloria* belonging to the monophylum associated with ticks, while the bacterial DNA was detected in 44.3% of blood samples. Furthermore, engorged ticks showed significantly higher bacteria load than unengorged ticks (Table 1; Wilcoxon sum-rank test: z=3.14; p=0.0017), similarly to what has been observed for *M. mitochondrii* in *I. ricinus* ticks.

This work provides evidence for the presence of circulating Midichloria DNA in long-distance migratory birds, suggesting an enhanced worldwide spread of these bacteria across haematophagous ectoparasite populations. Future studies are necessary to increase the knowledge of *Midichloria* role in the biology of this tick species.

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Table 1: Range values of gyrB and cal copy numbers and of gyrB/cal ratios obtained in H. marginatum ticks through qPCR analysis.

	***gyrB* copy number range**	***cal* copy number range**	***gyrB*/*cal* range**
Unengorged ticks	$6.8 \times 10^2 – 1.5 \times 10^5$	$7.2 \times 10 – 5.8 \times 10^3$	$7.7 – 5.2 \times 10^2$
Engorged ticks	$2.2 \times 10^2 – 1.8 \times 10^5$	$6.6 \times 10 – 6.2 \times 10^2$	$1.5 \times 10 – 8.2 \times 10^3$

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