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MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION OF *AMBLYOMMA SCUTATUM* (ACARI: IXODIDAE) ACCIDENTALLY INTRODUCED IN ITALY

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KEY WORDS

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ABSTRACT

Eight ticks were found in Comacchio (FE), Italy parasitizing a young black iguana (*Ctenosaura similis*) that had been accidentally transported in a commercial plant container from Costa Rica. Specimens were identified morphologically as *Amblyomma scutatum* and then confirmed by the barcoding of the mitochondrial cytochrome c oxidase subunit 1 gene. *Amblyomma scutatum* is a common tick known to infest reptiles in Central America, Mexico, and Venezuela, but not in Europe. In Italy, the possibility for this tick to become endemic is unlikely because of the absence of its principal hosts. Nevertheless, this finding confirms the high risk of introducing exotic species that is linked with global commerce and therefore the need for veterinary control of shipments.

The increasing levels of international travel and trade correspond to unprecedented rates of exotic species introductions in the European landscape (Costello and McAusland, 2003). Ticks are particularly suited for stowing away and entering new habitats on their endemic hosts (Barrè and Uilenberg, 2010). Although ticks are generally visible to the naked eye during quarantine inspections, their larval stages can be rather small. Some ticks may attach to the host in hidden anatomical sites (e.g., within nostrils or ear canals) where they cannot be easily detected or removed.

Various species of exotic ticks from Asia have been recorded on imported exotic reptiles in many countries, especially in Europe and the U.S.A. (Pietzsch et al., 2006; Nowak, 2010). For example, an Asian water monitor *Varanus salvator* brought from Indonesia to Poland was found to be parasitized by *Amblyomma varanense* (Nowak, 2010).

Amblyomma is a genus of hard ticks of the Ixodidae family, widespread in tropical and subtropical zones, where it parasitizes terrestrial and aquatic vertebrates such as amphibians, reptiles, birds, and mammals (Guzmán-Cornejo et al., 2011). On the American continent several species, including *Amblyomma americanum*, *Amblyomma cajennense*, and *Amblyomma maculatum*, are of economic significance as the adults prefer to feed on cattle (Jongejan and Uilenberg, 2004).

Moreover, since *Amblyomma* ticks can transmit pathogens such as *Rickettsia* (Sumrandee et al., 2014) and *Ehrlichia* (Fargnoli et al., 2020), they have both medical and veterinary importance. In Latin America in particular, many species of *Amblyomma* are vectors of human rickettsiosis (Enríquez et al., 2020).

Neumann (1899) described *Amblyomma scutatum* found in Guatemala for the first time. It is a Neotropical species that lives in tropical and subtropical dry broadleaf forest (Guglielmone et al., 2014). Its introduction in Nearctic and Palearctic regions was reported by Keirans and Durden in 2001, although there was no evidence of its actual establishment in those regions (Guglielmone et al., 2014). *Amblyomma scutatum* has not yet been found in Europe, but other similar species, such as *Amblyomma dissimile*, have been reported (Mihalca, 2015).

Amblyomma scutatum is considered a parasite of squamata species, but it was collected on a broad range of hosts such as *Cervidae*, *Noctilionidae*, *Didelphidae*, *Dasyproctidae*, and *Cathartidae* (Robinson, 1926), although these now appear to be unusual hosts for this tick (Guglielmone et al., 2014).

On 23 March 2018, a young black iguana (*Ctenosaura similis*) was found in a floral shop in Comacchio (Italy) (44°41′28″N, 12°11′05″E) inside a commercial container of yucca plants that was imported from Costa Rica by a Dutch import plant company. The iguana, accidentally imported in the shipment, carried 8 ticks that were collected manually and placed alive in vials. In the laboratory, ticks were frozen, separated according to sex, and then identified to species using morphological keys (Voltzit, 2007; Guzmán-Cornejo et al., 2011). The ticks were morphologically identified as 4 males and 4 females of *A. scutatum*.

After morphological identification, 5 specimens (3 males and 2 females) were individually processed for molecular analyses, whereas the remaining were preserved in ethanol 70%. Deoxyribonucleic acid was extracted from ticks using a commercial kit Qiagen© Biosprint one-for-all vet-kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's instructions. Successively, we amplified and sequenced the target 658-base-pair fragment of mitochondrial

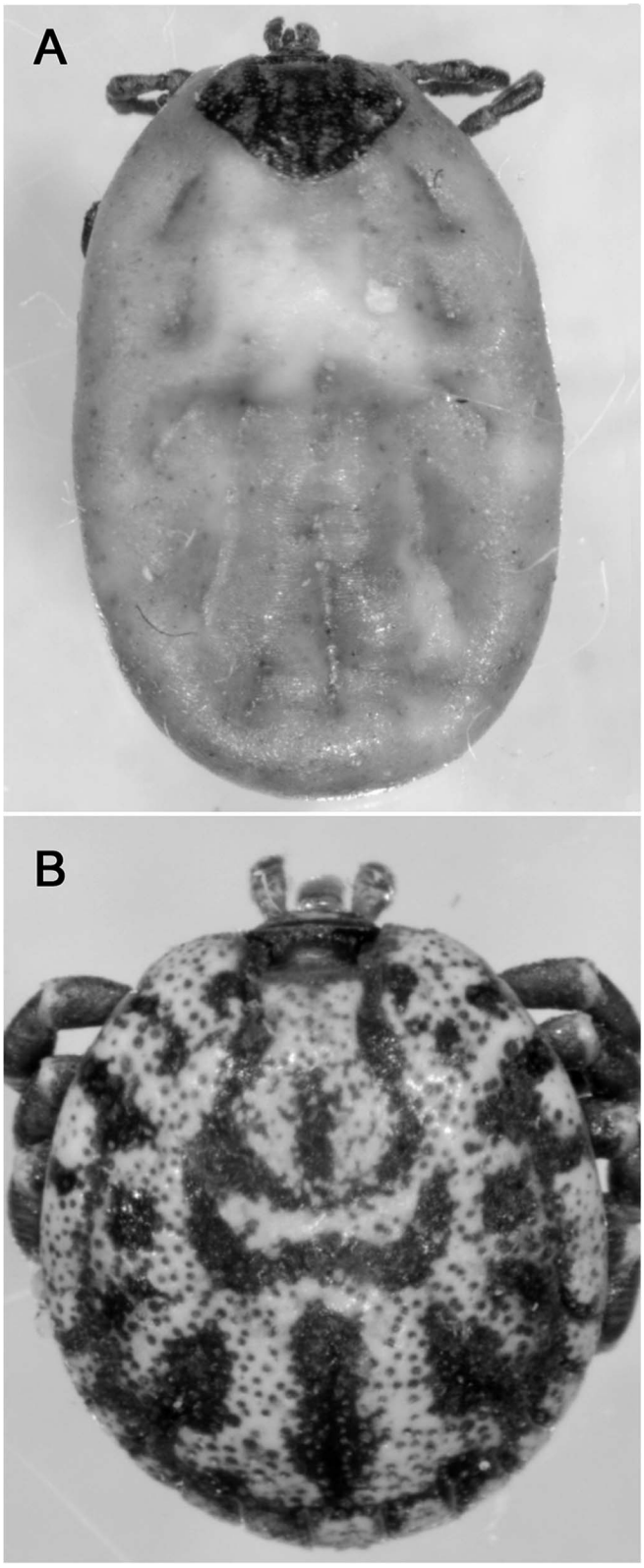

Figure 1. Dorsal view of female (**A**) and male (**B**) of two of the *Amblyomma scutatum* specimens collected from the iguana (*Ctenosaura similis*) in Comacchio, Italy.

Figure 2. Capitulum of the *Amblyomma scutatum* male.

cytochrome c oxidase (*COI*) gene (Park et al., 2011). Sequences are deposited (accession number: LR745649, LR745650, LR745651, LR745652, LR745653) in the European Bioinformatics Institute database (https://www.ebi.ac.uk/).

The obtained sequences were used to retrieve homologous sequences from the barcode of life database (BOLD) using the BOLD identification engine tool, as well as from the GenBank database using the basic local alignment search tool (Ratnasingham and Hebert, 2007; https://blast.ncbi.nlm.nih.gov/Blast.cgi). Then, sequences were aligned and used to construct a maximum likelihood (ML) tree with the software MEGA X by selecting the substitution model with the lowest Bayesian information criterion (namely the Tamura 3-parameter + G) (Kumar et al., 2018).

Consistently with morphological identification (Figs. 1–3) the sequences with the highest identity in BOLD were obtained from Mexican *A. scutatum* specimens (sequence identifications: MXBAC087-11, MXBAC088-11). The obtained *COI* sequences were mutually identical and showed 97.86% identity with the 2

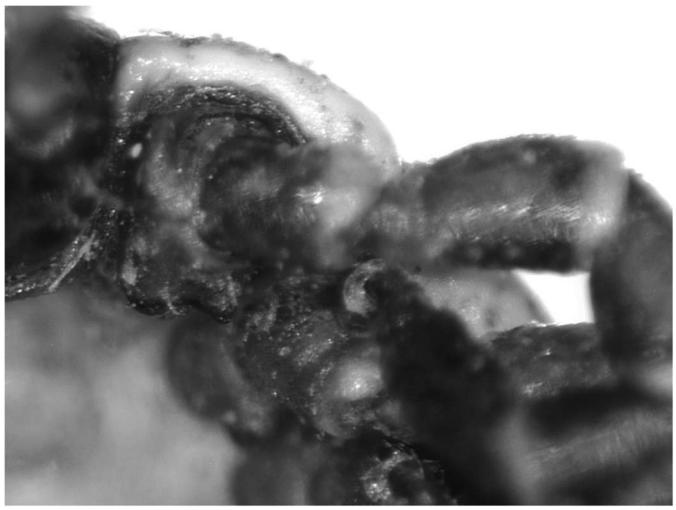

Figure 3. Coxa 1 *Amblyomma scutatum* male.

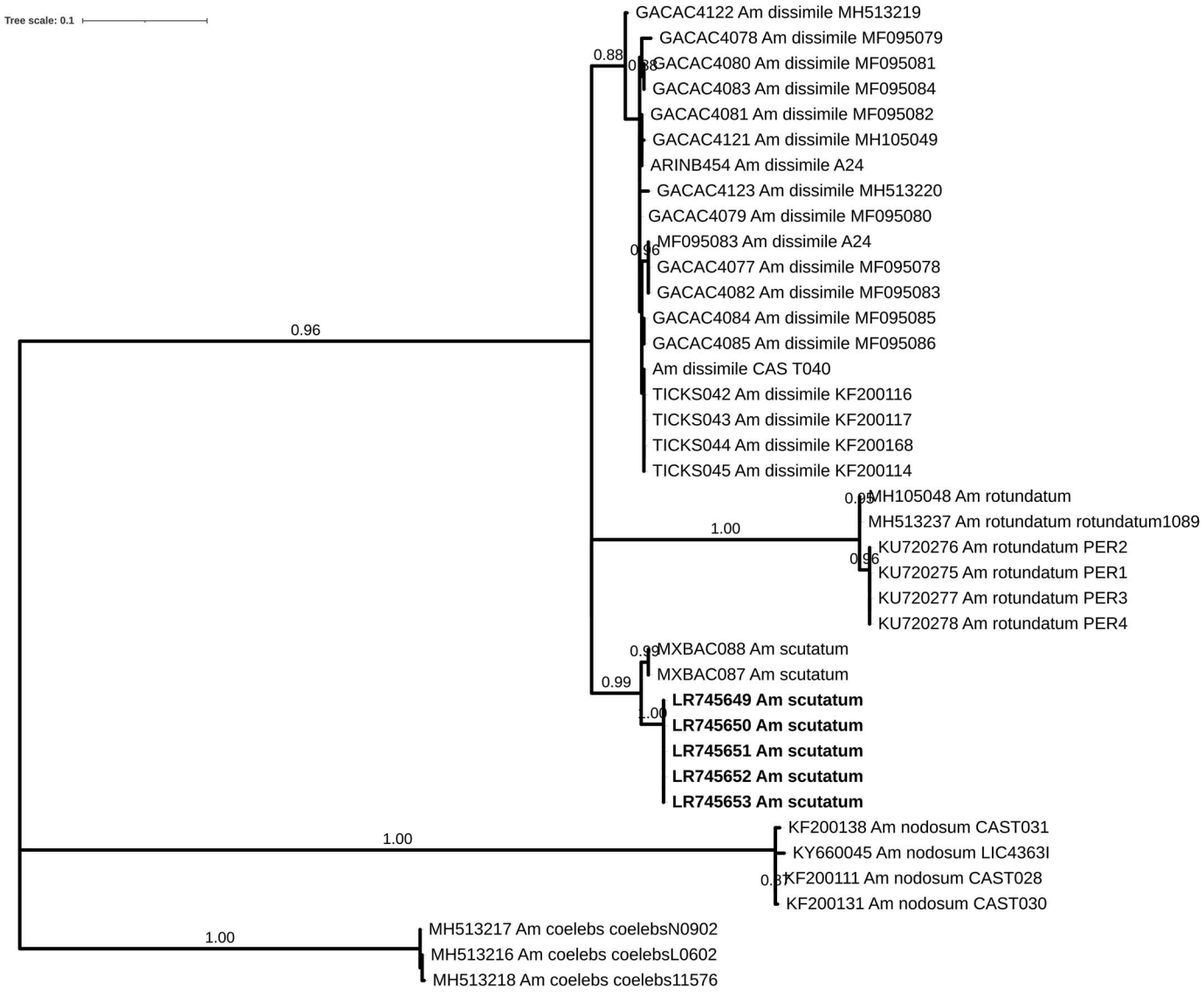

Figure 4. Tree inferred by maximum likelihood method by MEGA X software. GenBank or barcode of life database identifications are reported near the names; sequences recorded in this work are reported in bold. The bootstrap values (1,000 replicates) are shown next to the nodes if >80%.

BOLD sequences of *A. scutatum* collected in Mexico (BIN ABY3129). Sequences of *A. scutatum* were not available in GenBank. The obtained ML tree shows the sequences of our individuals grouped into a well-supported clade with other sequences of *A. scutatum*, whereas sequences from the other 4 species (*A. dissimile, Amblyomma rotundatum, Amblyomma nodosum, Amblyomma coelebs*) are represented by distinct and well-supported clades (Fig. 4). Thus, the topology of the tree confirmed the morphological identification.

There are 138 species of the genus *Amblyomma* worldwide, representing 19% of Ixodidae (Santiago et al., 2017), of which the great majority is recorded in the Neotropical region. The host preference is peculiar since they are more prone to feed on reptiles rather than other ixodids (Santiago et al., 2017). Occasionally, *A. scutatum* has been collected from mammalian hosts and birds (Burridge and Simmons, 2003). A few reports of ticks imported on reptiles in European countries (Italy, Poland, Spain, the Netherlands, Belgium, Slovenia, and the U.K.) have been published. Most of these ticks were reported to come mainly from Africa, followed by U.S.A. and Asia (Mihalca, 2015).

Data show that the importation of reptiles in 2016 represented 2.26% of the total live animal importation in Italy (Donato et al., 2016). These data represent just a part of the actual number since the number of accidentally imported animals is unknown/not included in it. Italy could provide a suitable environment for some species of these ticks. Pasucci et al. (2007) proposed a model for the risk of survival and establishment for *Amblyomma variegatum* and *Amblyomma hebraeum* on the basis of environmental characteristics. For these 2 species of ticks, they found a high risk of survival and a medium risk of establishment in many Italian areas. Moreover, the prospect of climate change, with milder winters and drier summers, can increase the risk of their survival and establishment in Europe. Although other species of *Amblyomma* can parasitize humans (Duckworth et al, 1985; Isohisa et al., 2011), this has not yet been established for

A. scutatum (Guglielomone et al., 2014) and thus, this risk should be addressed and further investigated. Custom controls on imported animals and live plants should be increased to avoid the possible entry and establishment of exotic species.

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