



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

Annalisa Grisendi, Mattia Calzolari, Francesco Defilippo, Deborah Torri, Katia Marzani, Michele Dottori, Paolo Bonilauri, and Giulia Maioli

Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia-Romagna Bruno Umbertini, Sezione di Reggio Emilia, via Pitagora 2, 42124 Reggio Emilia, Italy.

Correspondence should be sent to Annalisa Grisendi (<https://orcid.org/0000-0001-9150-9141>) at: annalisa.grisendi@izsler.it

KEY WORDS ABSTRACT

Exotic ticks	Eight ticks were found in Comacchio (FE), Italy parasitizing a young black iguana (<i>Ctenosaura similis</i>) that had been accidentally transported in a commercial plant container from Costa Rica. Specimens were identified morphologically as <i>Amblyomma scutatum</i> and then confirmed by the barcoding of the mitochondrial cytochrome c oxidase subunit 1 gene. <i>Amblyomma scutatum</i> 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.
Imported reptiles	
<i>Amblyomma scutatum</i>	
Italy	
Morphological identification	
Sequencing	

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 (Guglielmo 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 (Guglielmo 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 (Guglielmo 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



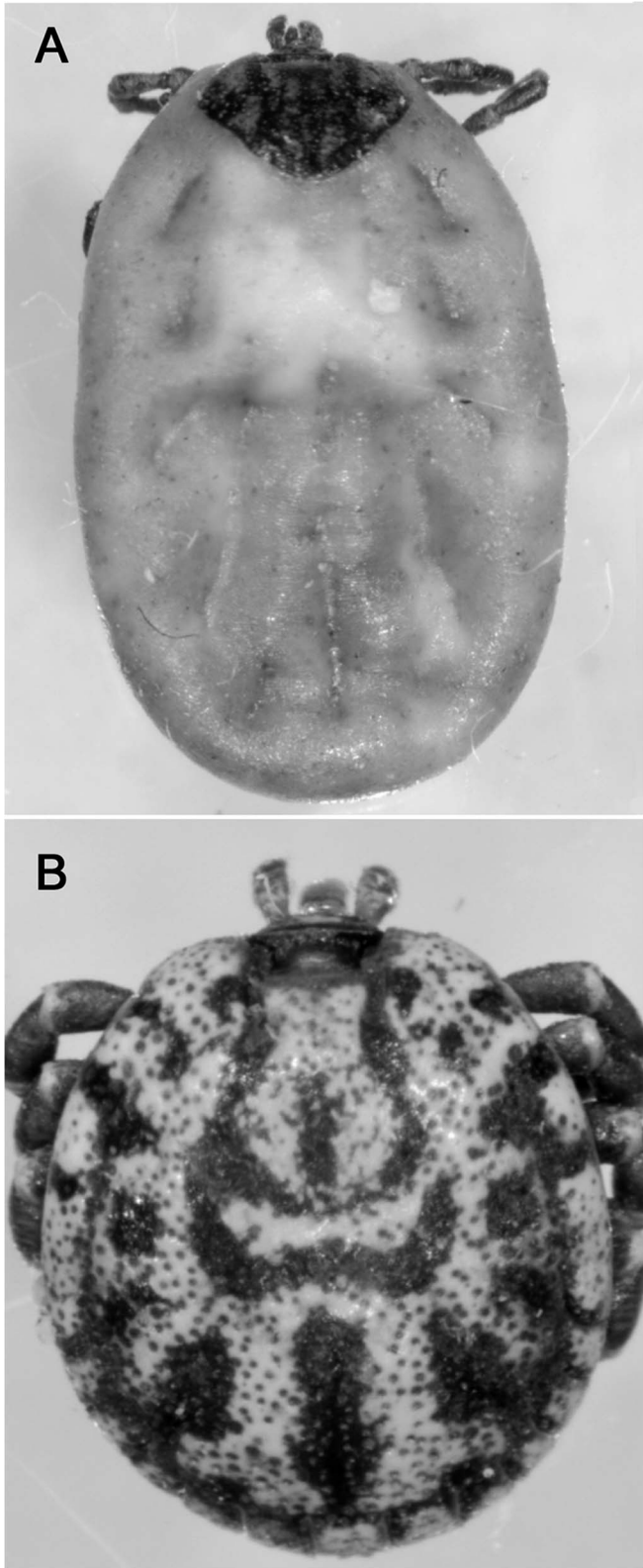


Figure 1. Dorsal view of female (A) and male (B) of two of the *Amblyomma scutatum* specimens collected from the iguana (*Ctenosaura similis*) in Comacchio, Italy.



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 I *Amblyomma scutatum* male.

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.

LITERATURE CITED

- BARRÉ, N., AND G. UILENBERG. 2010. Spread of parasites transported with their hosts: Case study of two species of cattle tick. *Revue Scientifique et Technique* 29: 149–160.
- BURRIDGE, M. J., AND L. A. SIMMONS. 2003. Exotic ticks introduced into the United States on imported reptiles from 1962 to 2001 and their potential roles in international dissemination of diseases. *Veterinary Parasitology* 113: 289–320.
- COSTELLO, C., AND C. MCAUSLAND. 2003. Protectionism, trade, and measure of damage from exotic species introduction. *American Journal of Agricultural Economics* 85: 964–975.
- DONATO, A., G. ATTANZIO, P. BROCCOLO, C. FARINA, G. IZZI, L. BRESUTTI, AND M. PRINCIPESSA. 2016. L'attività dei posti di ispezione frontiera e uffici veterinari per gli adempimenti comunitari. Ministero della salute, via G. ribotta, 5 00144–Roma EUR. Available at: http://www.salute.gov.it/imgs/C_17_pubblicazioni_2614_allegato.pdf. Accessed 29 April 2020.
- DUCKWORTH JR., P. F., G. F. HAYDEN, AND C. N. REED. 1985. Human infestation by *Amblyomma americanum* larvae (“seed ticks”). *Southern Medical Journal* 78: 751–753.
- ENRÍQUEZ, S., R. GUERRERO, J. ARRIVILLAGA-HENRÍQUEZ, P. ARAUJO, E. VILACRÉS, A. ENRÍQUEZ, AND W. BENÍTEZ-ORTÍZ. 2020. New records of ticks of genus *Amblyomma* Koch, 1844 (Acari: Ixodidae) for Ecuador. *Acta Parasitologica* 65: 430–440.
- FARGNOLI, L., C. FERNANDEZ, AND L. D. MONJE. 2020. Novel *Ehrlichia* strain infecting cattle tick *Amblyomma neumanni*, Argentina, 2018. *Emerging Infectious Diseases* 26: 1027–1030.
- GUGLIELMONE, A. A., G. R. ROBBINS, D. A. APANASKEVICH, T. N. PETNEY, A. ESTRADA-PEÑA, AND I. G. HORAK. 2014. *Hard Ticks of the World*. Springer, Dordrecht, the Netherlands, 495 p.
- GUZMÁN-CORNEJO, C., R. G. ROBBINS, A. A. GUGLIELMONE, G. MONTIEL-PARRA, AND T. M. PÉREZ. 2011. The *Amblyomma* (Acari: Ixodida: Ixodidae) of Mexico: Identification keys, distribution and hosts. *Zootaxa* 2998: 16–38.
- ISOHISA, T., N. NAKAI, Y. OKUZAWA, M. YAMADA, N. ARIZONO, AND N. KATOH. 2011. Case of tick bite with infestation of an extraordinary number of larval *Amblyomma testudinarium* ticks. *Journal of Dermatology* 38: 1110–1112.
- JONGEJAN, F., AND G. UILENBERG. 2004. The global importance of ticks. *Parasitology* 129(Suppl. 1.): S3–S14.
- KEIRANS, J. E., AND L. A. DURDEN. 2001. Invasion: Exotic ticks (Acari: Argasidae, Ixodidae) imported into the United States. A review and new records. *Journal of Medical Entomology* 38: 850–861.
- KUMAR, S., G. STECHER, M. LI, C. KNYAZ, AND K. TAMURA. 2018. MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution* 35: 1547–1549.
- MIHALCA, A. D. 2015. Ticks imported to Europe with exotic reptiles. *Veterinary Parasitology* 213: 67–71.
- NEUMANN, L. G. 1899. Révision de la famille des ixodidés (3e mémoire). *Mémoires de la Société Zoologique de France* 12: 107–294.
- NOWAK, M. 2010. The international trade in reptiles (Reptilia)—The cause of the transfer of exotic ticks (Acari: Ixodida) to Poland. *Veterinary Parasitology* 169: 373–381.
- PARK, D. S., R. FOOTIT, E. MAW, AND P. D. N. HEBERT. 2011. Barcoding bugs: DNA-based identification of the true bugs (Insecta: Hemiptera: Heteroptera). *PLoS ONE* 6: e18749. doi:10.1371/journal.pone.0018749.
- PASUCCI, I., A. CONTE, AND M. SCACCHIA. 2007. Use of geographic information systems to identify areas at risk of introducing *Amblyomma variegatum* and *A. hebraeum* to Italy. *Veterinaria Italiana* 43: 655–661.
- PIETZSCH, M. E., R. QUEST, P. D. HILLIARD, J. M. MEDLOCK, AND S. LEACH. 2006. Importation of exotic ticks into the United Kingdom via the international trade in reptiles. *Experimental and Applied Acarology* 38: 59–65.
- RATNASINGHAM S., AND P. D. N. HEBERT. 2007. BOLD: The barcode of life data system (<http://www.barcodinglife.org>). *Molecular Ecology Notes* 7: 355–364.
- ROBINSON, L. E., 1926. *Ticks: A Monograph of the Ixodoidea, Part IV: The Genus Amblyomma*. Oxford University Press, Oxfordshire, U.K., 103 p.
- SANTIAGO, N., J. M. VENZAL, AND A. ALBERTO. 2017. *Ticks of the Southern Cone of America. Diagnosis, Distribution, and Hosts with Taxonomy, Ecology and Sanitary Importance*. Academic Press, Cambridge, Massachusetts, 372 p.
- SUMRANDEE, C., S. HIRUNKANOKPUN, K. DOORNBOS, S. KITTHAWEE, V. BAIMAI, L. GRUBHOFFER, W. TRINACHARTVANIT, AND A. AHANTARIG, 2014. Molecular detection of *Rickettsia* species in *Amblyomma* ticks collected from snakes in Thailand. *Ticks and Tick-borne Diseases* 6: 632–640.
- VOLTZIT, O. V. 2007. A review of Neotropical *Amblyomma* species (Acari: Ixodidae). *Acarina* 15: 3–134.