



Ticks in the box: *Argas persicus* occurrence in nest boxes of secondary cavity-nesting bird species in Italy

Flavio Monti^{1,2} · Mariella Baratti³ · Andrea Viviano^{4,5,6} · Emiliano Mori^{4,7}

Received: 12 September 2022 / Revised: 28 January 2023 / Accepted: 31 January 2023 / Published online: 9 February 2023
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Abstract

Migratory organisms can be vectors of parasitic host organisms that can then be transported along seasonal migratory journeys and spread across continents. Ornithophilic nidicolous ticks (e.g. soft ticks) include species responsible for the transmission of pathogens and bacteria, thus representing a health problem not only for wild species that are directly parasitized, but also for those that share the same environments or reproductive sites with them. In this regard, artificial nests for birds may turn out to be site-sources of parasites. Here, we document the occurrence of different life stages of *Argas persicus* ticks in nest boxes of wild birds in a natural area (not associated with poultry activities) of central coastal Italy (Maremma Regional Park, Tuscany). Between 2018 and 2022, 168 ticks were collected from nest boxes occupied by different secondary cavity-nesting birds, such as European rollers *Coracias garrulus* and scops owl *Otus scops*. Ticks were analysed morphologically, and selected specimens were also identified by mitochondrial ribosomal 16S (16S) subunit gene to ascertain their taxonomic status. All ticks were identified as *Argas persicus*. This finding not only suggests that this tick species has formed a viable population in this Italian region, but also further confirms the previously doubtful natural origin of the species at country level and sheds new light on its underestimated and little investigated distribution. Possible pathways of introduction and its potential impacts on local avian community are discussed.

Keywords Nest box · Soft ticks · *Argas* spp. · Cavity-nesting birds · Ectoparasite · Birds

Introduction

Migratory organisms are known to play an important role as transporters of other organisms eventually promoting their dispersal over large distances along seasonal migratory journeys (Viana et al. 2016; Hall et al. 2022). Birds, thanks to their enormous dispersive potential associated with flight, are among the organisms that most facilitate the passive spread of other organisms, including parasitic species such as ticks (Loss et al. 2016). Ticks from different genera (e.g. *Ixodes*, *Hyalomma*, *Haemaphysalis* and *Rhipicephalus*) may infest birds with consequences on their health, fitness and survival (e.g. Hoodless et al. 2003; Hornok et al. 2022). Ornithophilic nidicolous ticks (e.g. soft ticks) are known to be responsible for the transmission of pathogens and bacteria affecting various species through different development stages, sometimes including humans as final/accidental host (Hoogstraal 1985). Despite their importance also for medical and veterinary interest, some ticks species have been historically subject to incomplete taxonomic description or incorrect attribution, preventing an adequate knowledge of their natural history, distribution and consequently of the risks associated with their

✉ Flavio Monti
flaviomonti00@gmail.com

- 1 Department of Physical Sciences, Earth and Environment, University of Siena, Via Mattioli 4, Siena 53100, Italy
- 2 Maremma Natural History Museum, Strada Corsini 5, Grosseto 58100, Italy
- 3 Istituto di Bioscienze e Biorisorse, Consiglio Nazionale Delle Ricerche, Via Madonna del Piano 10, Sesto Fiorentino, FI 50019, Italy
- 4 Consiglio Nazionale delle Ricerche, Istituto di Ricerca Sugli Ecosistemi Terrestri, Via Madonna del Piano 10, Sesto Fiorentino, FI 50019, Italy
- 5 Consiglio Nazionale delle Ricerche, Istituto Di Ricerca Sugli Ecosistemi Terrestri, Via Traversa La Crucca 3, Li Punti, Sassari 07100, Italy
- 6 Dipartimento di Scienze E Tecnologie Agrarie, Alimentari, Ambientali e Forestali, Università Degli Studi di Firenze, Via delle Cascine 5, Florence 50144, Italy
- 7 National Biodiversity Future Center, Palermo 90133, Italy

vector viruses or bacterial agents (Muñoz-Leal et al. 2018). Furthermore, the fact that some species share morphological features but have not been molecularly examined for long time in the past (Palomar et al. 2021), where at the basis of incorrect taxonomic attribution or confusion, further complicating the fields of investigation at various levels. Soft ticks of the genus *Argas*, and in particular *Argas persicus*, are a clear example of such mismatch: the interpretation of this taxon has changed dramatically in the last decades (Pantaleoni et al. 2010), with the discovery of closely related species on the one hand, and technological advances (e.g. genetic analyses) on the other hand, which have allowed more reliable taxonomic recognition and led to reconsider past doubtful records/data. *Argas persicus* is considered native to the areas of Central Asia, as an ectoparasite of arboreal nesting birds (Pantaleoni et al. 2010). However, in association with human activities related to poultry farming in the areas of origin, the species has adapted to parasitize domestic fowl (e.g. chickens, turkeys, pigeons, ducks and geese: Kohls et al. 1970; Hoogstraal 1985; Alzahrani and Edrees 2019; Zahid et al. 2021), with documented negative effects on chicken productivity and transmitting diseases such as avian borreliosis implicated in the circulation of West Nile virus and infections of *Salmonella* spp. (Hoogstraal 1985; Muñoz-Leal et al. 2018). This has favoured, in a relatively short-time period, it spreads over the continents due to the modern transportation for the poultry trade (Muñoz-Leal et al. 2018). Currently, *Argas persicus* can be found almost all over the world, always in association with domestic flow from poultry production (Kohls et al. 1970; Muñoz-Leal et al. 2018; Alzahrani and Edrees 2019). Considerations on its distribution in the Western Palearctic and in the Mediterranean region have been reported by Pantaleoni et al. (2010). Consequently to this spread, records of parasitism on wild bird species have been documented in several countries (e.g. Palomar et al. 2021; Sándor et al. 2021). In particular, it was found that not only migratory species were directly parasitized, but also local sedentary species that shared the same environments or reproductive sites with the former. The use and the re-use even in different years of common feeding sites (especially if on the ground; e.g. common pheasant *Phasianus colchicus*; Hoodless et al. 2003) and nesting sites by different individuals and/or species represents an optimal situation for the transfer of ticks from one host to another, including the transmission of associated microorganisms (Palomar et al. 2021). In this regard, natural cavities and namely artificial nest boxes provided for birds may turn out to be site sources of parasites (Veiga and Valera 2020). Thus, the study of the nests of hole-breeding birds (including nest boxes) facilitates the investigation of the ectoparasitic community as well as of the species habitat requirements and different life stages (Veiga and Valera 2020). For example, different species of Argasidae were found on Eurasian eagle owls (*Bubo bubo*) nesting on the ground in southeastern Bulgaria (Sándor et al. 2021).

Similarly, Argasidae ticks collected from nest boxes used by European rollers (*Coracias garrulus*), and little owls (*Athene noctua*) have been reported from Southeastern and Central Spain (e.g. Veiga and Valera 2020; Palomar et al. 2021).

In Italy, due to the incorrect taxonomic attributions of the past, identity and distribution of this poultry-associated soft tick have been historically overshadowed: there is hence no sure record, excluding one case from Trieste, in North-eastern Italy (Iori et al. 2005), and a more recent finding from two sites of the province of Trapani (Western Sicily) (Pantaleoni et al. 2010). All other past records from Italy were considered doubtful and had not been validated (Pantaleoni et al. 2010). While the record from Trieste concerns samples collected from a poultry-pen site (Iori et al. 2005), the latter from Sicily reports the finding of some overwintering specimens found under the bark of *Eucalyptus camaldulensis* Dehnh. at two different semi-natural sites, not associated with poultry activities (Pantaleoni et al. 2010). Authors of this latter record consider improbable a tick passage from domestic to wild birds in the area, thus excluding a human-induced spread and suggesting the presence of the species in Sicily to be natural (Pantaleoni et al. 2010). However, at the country level, possible pathways of introduction remain blurred, making the origin of the species doubtful and debated. It is in fact possible that the species arrived both due to accidental human-mediated introductions (associated with the poultry) in some areas and naturally via migratory bird species elsewhere in line with other studies documenting tick-infested birds during migration (Toma et al. 2021; Hornok et al. 2022). Its distribution is partially known and probably still highly underestimated.

For this reason, each new data represents an important and noteworthy record, as it is aimed at contributing to the improvement of knowledge on the distribution of this species on a national scale and inform on habitat preferences and potential interaction with wild bird species.

Here, we report a new record of *Argas persicus* from a coastal area of Central Italy (Maremma Regional Park, Tuscany region). The present study aimed to assess the occurrence, development stage and phylogenetic position of *Argas persicus* ticks infesting artificial nests for birds. This finding updates the previous species' distribution and sheds new light on the doubtful natural origin of the species at country level (Pantaleoni et al. 2010).

Materials and methods

Study area

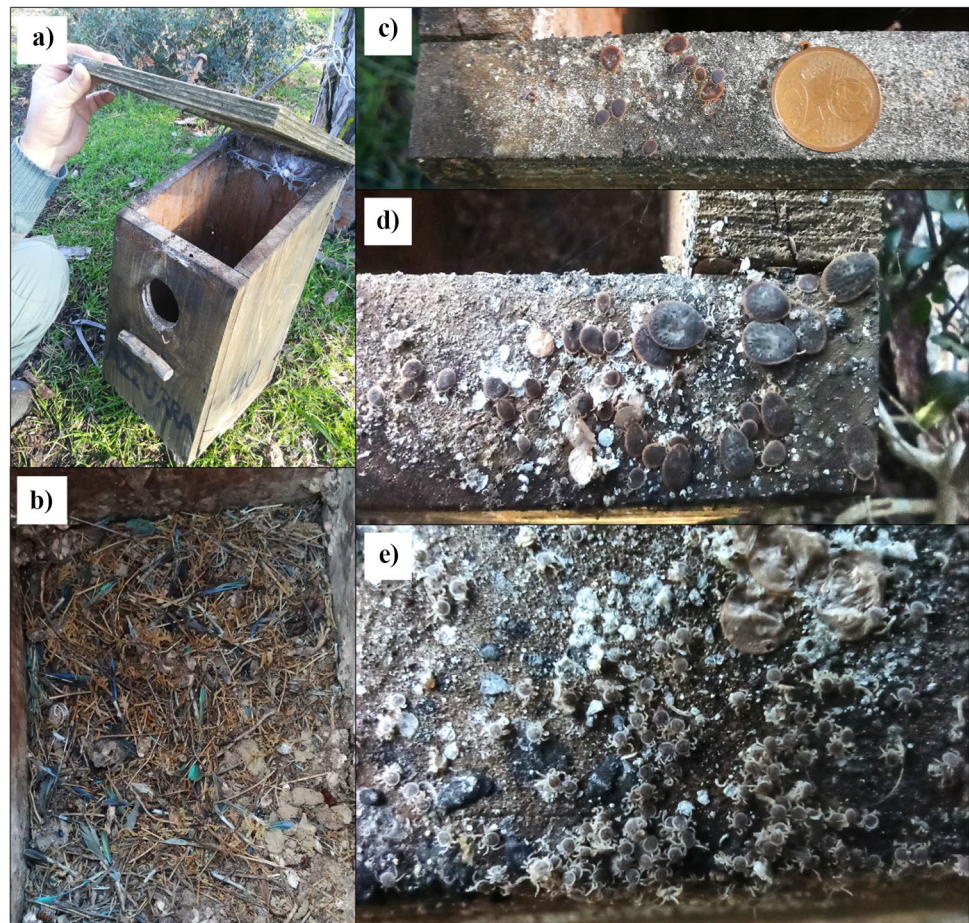
The survey was carried out in the coastal pinewood of the Maremma Regional Park (Tuscany, Central Italy; 42°39'N, 11°05'E). The local climate is typically Mediterranean. The entire pinewood is within the protected area, and it has been

declared as a Site of Community Importance, under the Habitats Directive (92/43/EEC). The pine forest, of anthropogenic origin with the planting of domestic pines *Pinus pinea* L. began at the end of 1700, extends for about 600 ha. The undergrowth is characterized by essences typical of the Mediterranean scrub such as: *Erica multiflora* L., *Pistacia lentiscus* L., *Juniperus* spp., *Rhamnus alaternus* L., *Myrtus communis* L., *Phillyrea angustifolia* L. and *Cistus* spp. Some areas of the pinewood are subject to flooding during rainy periods, while reclamation channels that drain the waters are present. Feral cattle *Bos taurus* and horses *Equus caballus* are moved through parts of pinewood during the year and several wild ungulate species (e.g. *Dama dama*, *Capreolus capreolus* and *Sus scrofa*) are present.

In the framework of a long-term project for the conservation of the European roller in the park, specifically designed nest boxes have been installed on pine trees, in order to improve nest site-availability for the species (Anselmi 1998; Pezzo and Cianchi 2014). The first installations of nest boxes date back to the 1980s (5 wooden nests installed in 1984; Anselmi 1998). Subsequently other 21 wooden nest boxes were installed in the early years of the 90s (Pezzo and Cianchi 2014). Starting from 2009,

the number of nest boxes has been significantly increased, including the installation of several wood-concrete nest boxes (Pezzo and Cianchi 2014). The nest boxes have been monitored every year, for 8 years between 2007 and 2014 (Pezzo and Cianchi 2014) and then in 2018 and 2019 (Monti 2019). The number of nests varied over the years, due to the fall of old nests and the repositioning of new ones. In 2019, year of the last standardized monitoring, 37 nest boxes (of which 54% wood and 46% concrete) were found. Nest boxes typology was hence not homogeneous; those made of wood (base dimensions 25 × 25 cm approximately) had an entrance hole of approximately 6 cm (Fig. 1a). A wooden roof, with a slight inclination at 30° to favour the outflow of rainwater, covered the upper part of nest boxes. Wood-concrete nest boxes had a diameter of entrance hole 6.5 cm and external dimensions 25 × 30 cm (model Schwegler 5KL; Schorndorf, Germany). All nest boxes were mounted on the trunks of the pines at a height of about 4–8 m from the ground. Each nest box was regularly checked (i.e. one per week) from May to August, although different monitoring protocols were adopted during years (e.g. Dottori 2008; Pezzo and Cianchi 2014; Monti 2019). During years, nest boxes

Fig. 1 *Argas persicus* found within nest boxes of secondary cavity-nesting bird species in the Maremma Regional Park (Tuscany, Italy), between 2018 and 2022: **a** picture representing a wood nest box specifically designed for rollers: base dimensions 25 × 25 cm and entrance hole of approximately 6 cm. Upper part of nest boxes were covered by a wooden roof, with a slight inclination at 30° to favour the outflow of rainwater; **b** internal view of a nest box previously occupied by breeding European rollers. The rest of the body feathers of roller chicks are visible in the bottom of the nest; **c** specimens of *Argas persicus* of various sizes (a 2-cent coin is reported for size comparison) found in the fissure that divides the upper part of the cover and the rest of the structure of the nest; **d–e** specimens of *Argas persicus* at different development stages, including nymph, found in different nest boxes. Pictures are by Flavio Monti and Giuseppe Anselmi



were used by different bird species such as European roller (Fig. 1b), scops owl (*Otus scops*), little owl (*Athene noctua*), Eurasian starling *Sturnus vulgaris* and great tit *Parus major* (Pezzo and Cianchi 2014; Monti 2019).

Tick collection, morphological and molecular identification

Ticks were opportunistically collected from nest boxes only once in 2018 and again in 2022. More specifically, in March 2018, during a survey on the field aimed at ascertaining the state of conservation of the old artificial nest boxes, specimens of a soft tick were observed, photographed and collected to run a first morphological analysis. The survey was repeated in February 2022 to ascertain their presence over the years. Overall, 168 ticks (48 adults and 120 nymphs) were opportunistically collected for morphological analyses, while two adult individuals were randomly selected to be used for molecular investigation to confirm the species taxonomic status.

Collected ticks were preserved in absolute ethanol until laboratory analyses. Ticks were first morphologically

identified following Pérez-Eid (2007) and Guglielmo et al. (2010), by using an optical microscope ($\times 400$). Following the morphological identification of samples, two individuals were analysed from the genetic point of view. The molecular analysis was carried out in order to confirm the attribution to *A. persicus* of the specimens. Genomic DNA was extracted from entire specimens preserved in absolute ethanol using the Qiamp Tissue kit (Qiagen Inc., USA), following the manufacturer's protocol. DNA quality and concentration were tested by both agarose gel electrophoresis and spectrophotometry at 260–280 nm. 16S rDNA PCR amplifications and sequencing were performed using primers and conditions, as previously described in Petney et al. (2004). Chromatograms obtained from forward and reversal sequences were visualized by CHROMAS software v. 2.01 (Technelysium Pty. Ltd.). The sequences of the ticks were blasted in the GenBank database (<https://www.ncbi.nlm.nih.gov/>) to determine its affinities with tick taxa for published 16S sequences (e.g. Pantaleoni et al. 2010; Zahid et al. 2021); this approach allowed the comparison of obtained amplicons using basic local alignment search tool (BLAST) to scan for homologous sequences (e.g. Zahid et al. 2021). We looked at phylogenetic relationships by

Fig. 2 Specimens of *Argas persicus*: **a** view of the ventral and **b** dorsal part of three adult individuals collected in nest boxes of the Maremma Regional Park (Tuscany, Italy) between 2018 and 2022 (photo by Andrea Viviano)



neighbour-joining (NJ) (Saitou and Nei 1987) analysis using MEGA software (Tamura et al. 2021) and the GTR distance model (Tavaré 1986), corrected for rate heterogeneity among sites with a gamma distribution (Yang 1993) selected by jModelTest (Darriba et al. 2012).

Results and discussion

Ticks were found in four different nest boxes made of wood and located in the pinewood of the protected area. Ticks were found using only the fissure that divides the upper part of the cover and the rest of the structure of the nest, as a shelter (Fig. 1c). This is in line with what already observed by Veiga and Valera (2020) who had found Argasidae ticks under the lid of nest boxes in Southeastern Spain. Ticks were found to be active and in a variable number from one nest to another, spanning from ca 10 individuals when only adults were present to more than 50–100 if individuals at other development stages were also present. Indeed, in two nest boxes, the occurrence of different development stages (Fig. 1c–e), including nymphs (Fig. 1e), was confirmed.

The morphological characteristics of all the adult and nymphs collected specimens were in agreement with the descriptions of the *Argas persicus* (Fig. 2), according to the

dichotomous keys (Pérez-Eid 2007; Guglielmone et al. 2010; and see also: Hoskins 1991; Kakarsulemankhel 2010). A single haplotype was obtained from the 16S of the two individuals, indicating that the individuals belonged to the same species. Sequences from Tuscany were deposited in GenBank under the accession number OP278958. The haplotype confirmed the identity of the species. By BLAST analyses we confirmed previous morphological diagnoses, since partial mitochondrial 16S sequences (437 bps) obtained were highly similar to *Argas persicus* sequences from GenBank (Fig. 3). The clade of *A. persicus* is highly supported (100) confirming that the samples analysed in this study belong to the species (Fig. 3).

Our work reveals the occurrence of different life stages of *Argas persicus* ticks in nest boxes provided for wild birds in the Maremma Regional Park (Tuscany) between 2018 and 2022, suggesting that this tick species has formed a viable population in this Italian region. In this sense, our results provide support to Pantaleoni et al. (2010), who reported that the species found overwintering in Sicily could be of a natural origin. This suggestion is corroborated by the fact that these records are not associated to poultry industry or human landscapes, but detected in natural landscapes. Moreover, we suspect that its current distribution is highly underestimated and that

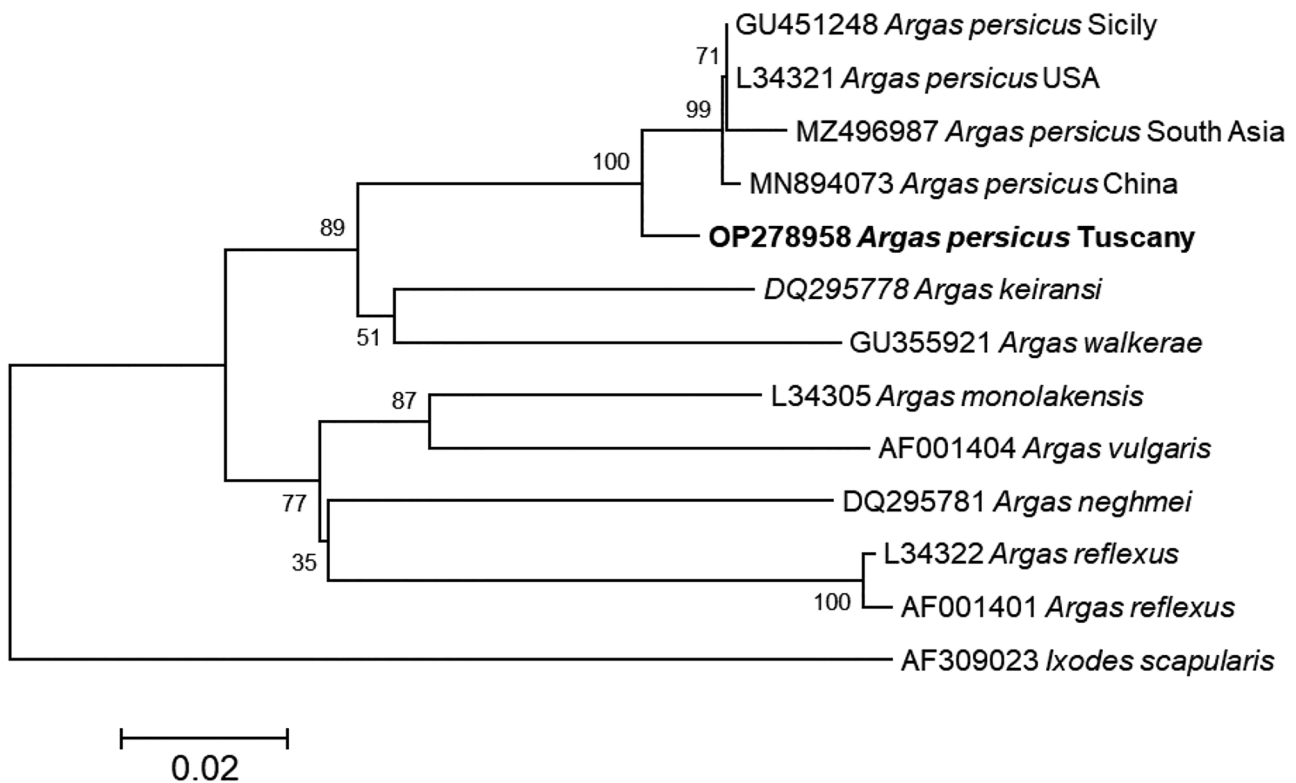


Fig. 3 Neighbour-joining tree obtained with 16S sequences of *Argas* species retrieved by GenBank and from the present study (in bold). *Ixodes scapularis* was chosen as outgroup taxon

the species, despite being well adapted and able to colonize different habitats (see its cosmopolitan distribution), could prefer particular conditions at local scale, which are still little studied/investigated. For example, in a nest box monitoring program on the European roller in another region of central Italy (Latium), where similar wood nest boxes were installed on high voltage pylons in agricultural landscapes, and where the monitoring is regularly carried out since 2010 (Monti et al. 2019), the presence of this Agaridae species has never been recorded. Yet, the fact that ticks were found occupying only a specific section of the nest structure (see also Veiga and Valera 2020), lead to think that microhabitat conditions and parameters play an important role in driving site selection. In this sense, if from one side nest boxes are a common conservation tool used to improve nest-site availability for species of conservation concern and have helped to reverse declines in many endangered bird populations, on the other site they can also function as site-sources of parasites with potential negative effects on breeding performance and productivity for parasitized species. At the local scale, this finding represents a new documented species to be included in the checklist of the park and that would deserve a more in-depth monitoring. For instance, a new standardized monitoring focused on investigating the proportion of nest boxes infested by ticks and their abundance with respect to the occupier bird species identity would allow: (a) to improve the knowledge on the ecology of ornithophilic ticks for free-ranging birds as well as (b) to suggest ecological links between tick presence and micro-climatic conditions at avian cavities. In addition, further studies are encouraged to investigate possible tick effects on local breeding avian community reproducing in nest boxes, as per its potential effects related to the transmission of pathogens of high health concern. At national scale, conducting similar surveys at other localities where nest box programs are in progress would help gather a more comprehensive and updated distribution map of the species occurrence.

Acknowledgements We are grateful to Maremma Regional Park Agency and in particular to Dr. Laura Tonelli (technical sector) for technical and logistical support and to the ranger Giuseppe Anselmi for providing support during field survey and assistance in the collection of ticks. We also thank Dr. Francesco Pezzo (ISPRA) for useful insights on the nest box monitoring program in the park and to Dr. Tiziana Di Lorenzo (CNR-IRET) who allowed us to use the microscope for the morphological determination of the ticks.

Author contribution FM conceived the idea, collected the field data and wrote most of the manuscript; AV and EM conducted the morphological analyses; MB, EM and AV conducted genetic analyses; MB carried out phylogeographic analyses. All the authors contributed critically to the manuscript and gave the final approval for publication.

Funding Open access funding provided by Università degli Studi di Siena within the CRUI-CARE Agreement.

Data availability Genetic sequences are available on GenBank.

Code availability Not applicable.

Declarations

Animal handling and use All the parasites used in this study were detected in the nest boxes. No live bird was used.

Competing interests The authors declare no competing interests.

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