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Original article

Description of all the stages of *Ixodes inopinatus* n. sp. (Acari: Ixodidae)

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ABSTRACT

All of the parasitic stages of *Ixodes inopinatus* n. sp. are described from specimens collected by flagging and on lizards and foxes. The new species replaces *I. ricinus* in dry areas of the Mediterranean region in Spain, Portugal, Morocco, Algeria and Tunisia. It has also been collected in areas of western Germany in sympatry with *I. ricinus*, far of its known distribution range and on an unusual host. The females of the new species can be separated from *I. ricinus* by the relative dimensions and punctations of the scutum, the length of the idiosomal setae, the size of the auriculae, and the aspect of the porose areas. Nymphs of *I. inopinatus* can be easily separated from *I. ricinus* by a combination of scutal dimensions, the relative size of scutal and alloscutal setae, and the relative size of the spurs on coxa I. The larvae of the new species have a broader than long scutum and unusually long Md1 to Md3 idiosomal setae. The new species is allopatric with *I. ricinus* in Spain and Portugal. It is hypothesized that it has been historically overlooked and reported as *I. ricinus* at least in northern Africa, southern Spain and parts of south-western Portugal. The existence of a new species in the *I. ricinus* complex makes necessary the critical assessment of its complete distribution, its abiotic preferences and seasonal activity, as well as its hosts and implications for the transmission of pathogens.

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Introduction

Ixodes ricinus (Linnaeus) has traditionally been considered an extreme generalist tick (Medlock et al., 2013). Like other species of ticks, its range is restricted mainly by environmental constraints rather than host-associated interactions (e.g., Randolph et al., 2002). The area of distribution of *I. ricinus*, as currently known, includes a variety of environments in Alpine, Boreal, Nemoral, Continental, Atlantic, Lusitanian and Mediterranean domains (Estrada-Peña et al., 2006, according to the classification of European landscapes by Metzger et al., 2005). Being a tick involved in the transmission of a wide variety of pathogens with considerable impact on human and animal health (Gray et al., 2009), there is a substantial interest in understanding the factors driving its distribution and its fitness under a wide range of environmental conditions. Studies have been aimed at tracking *I. ricinus*'s changes in altitude (e.g., Materna et al., 2005) and latitude (Jaenson et al., 2012; Jore et al., 2011), to capture its climate niche (Estrada-Peña et al., 2006) and to summarize the driving forces resulting in its spatial distribution (Medlock et al., 2013). Such understanding is necessary to determine the spatial and temporal dimensions of the exposure of humans to this important vector (Estrada-Peña et al., 2013).

A further complication is whether *I. ricinus* is an environmental generalist, adapted to a very wide range of climatic conditions, or whether it exists as a series of populations, each adapted to local prevailing conditions and displaying a certain degree of geographical population structure. The only phenotypic analysis carried out on populations of this species involved the use of cuticular hydrocarbon composition to characterize the chemical profile (Estrada-Peña et al., 1996, 1998). This demonstrated the presence of well-defined phenotypic groups of ticks collected within each of the main climate zones of Europe. Studies of allozyme data or DNA sequences of *I. ricinus* in Europe led to contradictory results. Some studies reported a lack of genetic structure in the species (Delaye et al., 1997; Casati et al., 2008), or to a sex-biased genetic structure of the populations (de Meeûs et al., 2002), or to the existence of "races" driven by host associations (Kempf et al., 2011). Most important in this context is the reported lack of molecular consistency between the European and the African populations of *I. ricinus* (Noureddine et al., 2011). These authors observed low levels of nucleotide diversity for the local, regional and Eurasian populations of *I. ricinus* without clear differences between these scales. The phylogenetic trees showed evidence of weak genetic

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structure among Eurasian *I. ricinus*. Conversely, a strong and significant genetic differentiation was observed between the Eurasian and North-African ticks, with the latter forming a genetically divergent clade. This suggests that the North-African ticks evolved independently (Noureddine et al., 2011). The study of de Meeûs et al. (2002) using microsatellite loci showed similar results, with Swiss and Tunisian ticks being strongly differentiated. However, due to the species-specific mutation rate of microsatellite loci, the limited number of populations sampled and the absence of intermediate populations between Switzerland and Tunisia, it was difficult to accurately compare the level of genetic divergence (de Meeûs et al., 2002).

Field collections have revealed the existence of a new species of tick. This species thrives in Mediterranean habitats and so far has been collected on lizards and foxes. It seems to have been historically confused with and reported as *I. ricinus* in zones of Spain, Portugal and Northern Africa. Herein we describe all of the parasitic stages of this species and explicitly compare it with related species. We also provide molecular data for the new species. This description should stimulate new studies on the *I. ricinus–I. persulcatus* complex of species aimed at re-examining current taxonomic concepts that no longer seem to be valid, and to evaluate their main relationships with pathogens, a topic not yet addressed.

Materials and methods

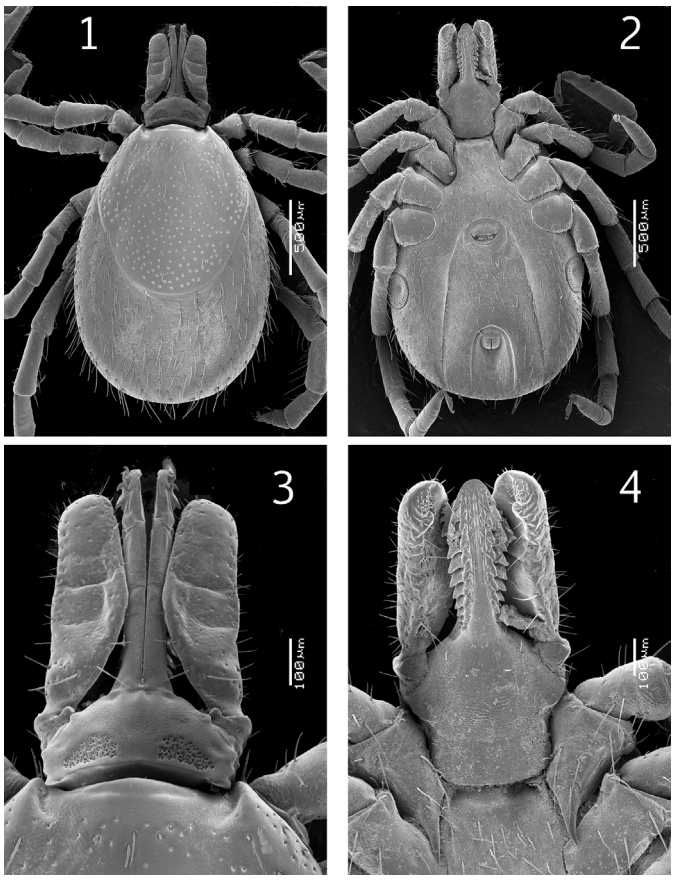
Immature specimens were collected by flagging and from lizards, while adults were collected by flagging, and from foxes and sheep (only two specimens). Table 1 includes the complete list of localities, the number of specimens collected and their specific identity. For morphological examination, individual immatures were cleared and mounted in Hoyer's medium and measurements were recorded using a Nikon SMZ1500 stereomicroscope with digital recording of images.

DNA was extracted from representative specimens of the new species from Spain and Tunisia, and from specimens of *I. ricinus* from Norway, France, Serbia and Italy (Table 1). Samples were processed by polymerase chain reaction (PCR) following the methodology described by Mangold et al. (1998). The DNA was then used to amplify a 420-bp fragment of the mitochondrial 16S rDNA gene using the primers designed by Mangold et al. (1998). Each of the sequences was aligned with each other and with the corresponding 16S rDNA sequences of *I. ricinus* published by Noureddine et al. (2011). In addition, sequences of other *Ixodes* species available in GenBank were included. The sequences were edited using BioEdit Sequence Alignment Editor (Hall, 1999), with manual editing whenever it was necessary, and aligned with the program Clustal W (Thompson et al., 1994). Phylogenetic analyses were carried out using maximum-likelihood (ML) method with the program MEGA 5 (Tamura et al., 2011). The ML tree was generated with the GTR model by using a discrete Gamma-distribution (+G). The best-fitting substitution models were determined with the Bayesian Information Criterion using the ML model test implemented in MEGA 5 (Tamura et al., 2011). Support for the topologies was tested by bootstrapping over 1000 replications, and gaps were excluded.

Description

Ixodes inopinatus Estrada-Peña, Nava and Petney, new sp.: description (all the measurements are in millimeters; included are the range and the mean).

Female (Figs. 1–4, nine specimens measured). Length from scapular apices to posterior idiosomal margin 3.23–4.03 (3.63), breadth 1.81–2.02 (1.90), ratio idiosomal length/width 1.74–1.96 (1.79). Scutum (Fig. 1) outline broadly rounded, 1.25–1.37 (1.34) long, 1.12–1.21 (1.18) broad (ratio length/width scutum: 1.11–1.13). Cervical grooves and lateral carinae absent or, at most, faintly indicated by integumental indentations when viewed obliquely. Punctations scattered and more abundant centrally, less numerous laterally, largest and deepest all over median and posterior scutal surfaces. Setae abundant, long, randomly distributed.



Figs. 1–4. *Ixodes inopinatus*, female. Fig. 1: dorsal. Fig. 2: ventral. Fig. 3: gnathosoma, dorsal. Fig. 4: gnathosoma, ventral.

Marginal groove not evident even in flat specimens. Central scutal setae 0.112–0.114, central alloscutal setae 0.145–0.199 (0.178), lateral alloscutal setae 0.188–0.211 (0.201). Genital aperture at level of coxae IV. Spiracular plates sub-circular, 0.37 long, 0.41 wide (ratio length/width: 0.89). Gnathosoma: length from palpal apices to posterior margin of basis 0.54–0.59 (0.57). Basis capituli dorsally (Figs. 3 and 4) 0.41–0.47 (0.43) broad; ratio gnathosomal length to basis capituli width 1.25–1.31 (1.27) posterior margin broadly concave, cornua small. Porose areas irregularly triangular, anterior margin broadly rounded, posterior margin essentially straight and well delineated, separated by distance slightly smaller than the breadth of each area. Pores in the porose areas irregularly placed. Basis capituli ventrally (Fig. 4) with posterior margin slightly convex; transverse suture unapparent; auriculae indicated by clear rounded lateral ridges, without hooks. Palpi 0.46–0.54 (0.49) long, 0.15–0.18 (0.16) broad (ratio length/width palpi: 3–3.1), and length of segments in descending order: 2, 3, 1, 4. Hypostome (Fig. 4) 0.43–0.46 (0.44) long, 0.15–0.16 (0.16) broad, ratio length/width: 2.86. Apex bluntly pointed, corona of many fine denticles, dental formula 4/4 for the anterior half, then 3/3 and very basal 2 rows of 2/2. Medial basal teeth smaller, all denticles sharply pointed.

Legs moderately long, black colored, slender, coxae I-IV (Fig. 2) each with a very small external spur, that of coxa IV not evident in a few specimens. Coxa I with a long tapering pointed internal spur, slightly curved outwardly, reaching coxa II. Internal spur absent on

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