Ticks and Tick-borne Diseases 11 (2020) 101379

Contents lists available at ScienceDirect

Ticks and Tick-borne Diseases

journal homepage: www.elsevier.com/locate/ttbdis

First records and molecular-phylogenetic analyses of three tick species (*Ixodes kaiseri*, *Hyalomma lusitanicum* and *Ornithodoros coniceps*) from Malta

Sándor Hornok[a,*], Andrea Grima[b], Nóra Takács[a], Sándor Szekeres[a], Jenő Kontschán[c]

[a] Department of Parasitology and Zoology, University of Veterinary Medicine, Budapest, Hungary
[b] APH Veterinary Hospital, Attard, Malta
[c] Plant Protection Institute, Centre for Agricultural Research, Hungarian Academy of Sciences, Budapest, Hungary

ARTICLE INFO

Keywords:
Ixodidae
Argasidae
cox1
16S rRNA
Host-association
Island

ABSTRACT

The Maltese islands are situated south of mainland Europe and north of Africa, therefore are expected to share tick species and tick-borne pathogens with both continents. This situation highlights the importance of studying ticks in this country. Nevertheless, the tick fauna of Malta appears to be a seldom investigated issue, with hitherto only five tick species reported in the country. Here, as part of a tick collection campaign continuing since 2016 in Malta, three tick species new to the country are reported and analyzed in comparison with GenBank data. *Ixodes kaiseri* (collected from North African hedgehog in Malta) had unique cytochrome *c* oxidase subunit I (*cox1*) and 16S rRNA gene haplotypes (with 98.1–99.3 % sequence identity to *I. kaiseri* from Europe and China). Phylogenetically, these haplotypes from Malta clustered separately from other, mainland-associated haplotypes, with high support. On the other hand, *Ornithodoros coniceps* (collected from domestic chicken in Malta) had identical or nearly identical *cox1* and 16S rRNA gene haplotypes with soft ticks reported from France, northern Africa and western African islands, similarly to *Hyalomma lusitanicum* (collected from rabbit and cat in Malta) in comparison with conspecific ticks in Spain and Portugal. These results are most likely related to differences in host associations and corresponding translocality of these three tick species. Taken together, results of the present study add three new tick species to those five already known to be present in Malta.

1. Introduction

Ticks (Acari: Ixodida) are among the most studied and economically most important ectoparasites (Jongejan and Uilenberg, 2004). Ticks remain long enough on their terrestrial vertebrate hosts to be transported over large geographical distances (Hornok et al., 2016a), but also have a significant part of their life cycle off host, in the environment, establishing local populations. Consequently, host preference will define the extent to which conspecific tick populations are isolated or connected in a geographical sense. For instance, association of ticks with various bird species may promote local tick diversity on islands predominated with natural habitats (Gómez-Díaz et al., 2012). On the other hand, human activities obscure the genetic pattern that would normally be found in free-living tick populations (Araya-Anchetta et al., 2015), which is a likely explanation for the genetic homogeneity observed in the case of a cosmopolitan tick species, *Rhipicephalus sanguineus* sensu lato on the Maltese islands (Hornok et al., 2018).

The Maltese archipelago, occupying an area of 316 km^2, consists of the densely inhabited islands of Malta and Gozo, as well as a number of uninhabited islets and rocks (Schembri, 1993). These islands are situated south of mainland Europe and north of Africa, thus representing "stepping stones" for the dispersal of ticks between Europe and Africa. Such events would inevitably depend on the host preference of relevant tick species, once again emphasizing the importance of studying the tick fauna on these islands.

Up to now only five tick species have been reported from Malta, i.e. *Ixodes ricinus* and *I. frontalis* from birds (Sultana and Gauci, 1977-1978), *Hyalomma marginatum* from rabbit (Pfliegler et al., 2017), *H. rufipes* (host unknown; Apanaskevich and Horak, 2008), and *R. sanguineus* s. l. from dogs (Hornok et al., 2017a; Licari et al., 2017) and other hosts (Hornok et al., 2018). Concerning the latter, recently it has been demonstrated that samples of *R. sanguineus* s. l. from Malta have 100 % sequence identity with each other and with samples from Italy, most likely as a consequence of the cosmopolitan nature and frequent transportation of its preferred host species, dogs (Hornok et al., 2018). However, to the best of our knowledge, other tick species from Malta have not been evaluated in the same context, particularly not those which have differences in the translocality (local vs migratory habit) of

* Corresponding author.
E-mail address: hornok.sandor@univet.hu (S. Hornok).

https://doi.org/10.1016/j.ttbdis.2020.101379
Received 6 June 2019; Received in revised form 9 January 2020; Accepted 19 January 2020
Available online 21 January 2020
1877-959X/

their typical host species (as exemplified by ticks from hedgehogs and birds in Hornok et al., 2016b). Here we report on the identification of three tick species for the first time from Malta. These tick species have been analyzed with molecular-phylogenetic methods, and the results are interpreted according to host species.

2. Materials and methods

2.1. Sample collection and identification

During a tick collection campaign initiated in 2016 and involving multiple host species at 20 locations in Malta (Hornok et al., 2018), all ticks other than *R. sanguineus* s. l. were retained in 96 % ethanol. These included three hard ticks: a female *Ixodes* (*Pholeoixodes*) sp. from a rescued North African hedgehog, *Atelerix algirus* (collected in March, 2018); one *Hyalomma* nymph from a rabbit (collected in July, 2016) and a *Hyalomma* male from a cat (collected in October, 2018). In addition, seven soft tick (*Ornithodoros* sp.) larvae were removed from a domestic chicken (in October, 2018 at Mosta). Their species were identified according to Hornok et al. (2017b), Apanaskevich et al. (2008) and Hoogstraal et al. (1970, 1976, 1979) (Supplementary Materials and Methods; Supplementary Table 1). Measurements of four soft tick larvae were performed under a VHX-5000 digital microscope (Keyence Co., Osaka, Japan).

2.2. Molecular taxonomy and phylogenetic analyses

DNA was extracted from one leg (of *I. kaiseri* and both *H. lusitanicum*) or the whole body (of *O. coniceps*, n = 3) individually with the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instruction, including an overnight digestion in tissue lysis buffer and 6.6 % Proteinase-K at 56 °C. A 710-bp-long fragment of the cytochrome *c* oxidase subunit I (*cox1*) gene was the primary target to identify tick species (Lv et al., 2014). The primer pair HCO2198 (5′-TAA ACT TCA GGG TGA CCA AAA AAT CA-3′) and LCO1490 (5′-GGT CAA CAA ATC ATA AAG ATA TTG G-3′) (Folmer et al., 1994) was used as reported (Hornok et al., 2017a), except for *O. coniceps*, in the case of which a new primer set was designed based on published sequence of *O. capensis* (AB075953): Cc.cox1.fw (5′-TTA GGA GCA TGA TCC ATA ATA GTA-3′) and Cc.cox1.rev (5′-AAT AAA TGT TGA TAT AAG ATT GG-3′). Thermal conditions were the same as above (Hornok et al., 2017a). In addition, an approx. 460-bp-long fragment of the 16S rDNA gene was amplified (Black and Piesman, 1994), with the primers 16S + 1 (5′-CTG CTC AAT GAT TTT TTA AAT TGC TGT GG-3′) and 16S-1 (5′-CCG GTC TGA ACT CAG ATC AAG T-3′) and annealing at 51 °C. Sequencing and phylogenetic analyses are described in Supplementary Materials and Methods.

3. Results

3.1. Molecular and phylogenetic analyses of I. kaiseri

The female hard tick from a North African hedgehog was identified as *I. kaiseri*. The *cox1* sequence of *I. kaiseri* from Malta (MK946448) showed 98.1–99 % (619–625/631 bp) identity with conspecific specimens from mainland Europe (central Europe: e.g. KY962011 ; the Balkans: e.g. KY962028) and China (MH279561). The 16S rRNA gene of this tick (MK946451) had slightly higher, i.e. 99–99.3 % (398–399/402 bp) sequence identity in the same geographical context (in comparison with KY962054, KY962066 and MG656445 from central Europe, the Balkans and China, respectively). These differences were well-reflected by the phylogenetic analyses, because the *I. kaiseri* specimen from Malta occupied a basal position to the phylogenetic group of other conspecific isolates (Fig. 1). This separation received a strong (99 %) and a moderately strong (84 %) bootstrap support in the *cox1* and 16S rRNA gene phylogenetic analyses, respectively (Fig. 1).

3.2. Molecular and phylogenetic analyses of H. lusitanicum

Two other hard ticks (a nymph from a rabbit and a male from a cat) were identified as *H. lusitanicum*. Both specimens of *H. lusitanicum* had 100 % identical *cox1* and 16S rRNA gene sequences to each other. The *cox1* gene sequence of these ticks from Malta (from rabbit: MG855655, from cat: MK946446) showed 99.2–100 % (626–631/631 bp) identity with conspecific isolates from the western Mediterranean Basin, i.e. from Portugal and Spain (GenBank: EU827698, EU827719). The amplified part of the 16S rRNA gene (from rabbit: MG855659, from cat: MK946449) had similar, 99.5–99.7 % (397/399 to 377/378 bp) rates of sequence identities with western Mediterranean isolates (GenBank: KU130444, Z97881). In the phylogenetic analyses *H. lusitanicum* from Malta clustered together with conspecific ticks from the western Mediterranean region (Supplementary Fig. 1).

3.3. Molecular and phylogenetic analyses of O. coniceps

Soft tick larvae collected from the chicken were identified as *O. coniceps* (Fig. 2, Supplementary Table 1). All three *O. coniceps* sequences obtained here were 100 % identical to each other. Considering closely related species of *O. capensis* s. l., only two shorter *cox1* sequences (from France) were available in GenBank for comparison (KX826016 and KX826017). These had 97.2–97.5 % (387–388/398 bp) identity with *O. coniceps* from Malta (MK946447). At the same time, the *cox1* sequence of *O. coniceps* from Malta had only 82.9 % (525/633 bp) sequence identity with that of *O. capensis* sensu stricto (from Japan: AB075953).

The 16S rRNA gene sequences of *O. capensis* s. l. in GenBank (KX825959 from France and KX825970 from the Canary Islands) were also shorter than that obtained here, and showed 99.7 % (346/347 bp with KX825959) identity with the corresponding sequence of *O. coniceps* from Malta (MK946450). In addition, the 16S rRNA gene sequence of *O. coniceps* from Malta had high level of sequence identities with several additional GenBank entries belonging to *O. capensis* s. l., namely: 99.5 % (427/429 bp) identity with one sequence from northern Africa (Algeria: KP776644) and 98–100 % (397–405/405 bp) identity with others from Cape Verde Islands near western Africa (JQ824323 and JQ824297). However, in comparison with isolates of *O. capensis* s. s. (e.g. from southern Africa: KR907245) the level of sequence identity was considerably lower, i.e. 91.8 % (393/428 bp).

According to the 16S rRNA phylogenetic analysis, *O. coniceps* from Malta clustered within the *O. capensis* group, including *O. capensis* and *O. sawaii* (Supplementary Fig. 2).

4. Discussion

Geographical isolation and connection play important roles in the genetic diversification of parasites (Koop et al., 2014). Thus, molecular-phylogenetic analyses of ticks from islands offer the opportunity to compare them on the local scale, as well as in a broader geographical context. Considering the latter, it was within the scope of this study (reporting three new records of tick species from Malta) to compare the mitochondrial markers of these "island-collected" ticks with more or less distant conspecific populations, depending on their preferences for tick-dispersing (flying or frequently transported) vs local host species. However, due to the rare occurrence and consequent limited availability of these three tick species in Malta, no similar molecular and phylogenetic comparisons could be performed on the local scale.

This is the first report of *I. kaiseri* from Malta. The known geographical range of *I. kaiseri* extends from northern Africa (Arthur, 1957) to the Middle East (Theodor and Costa, 1967) and Turkey (Orkun and Karaer, 2018), central-eastern Europe (Hornok et al., 2017b) and China (Sheng et al., 2019). The hosts of this species are usually carnivores (Filippova and Uspenskaya, 1973), but also *Erinaceus* sp. hedgehogs (Arthur, 1965). However, to the best of our knowledge, this is the first report of *I. kaiseri* from the North African hedgehog (*Atelerix algirus*).

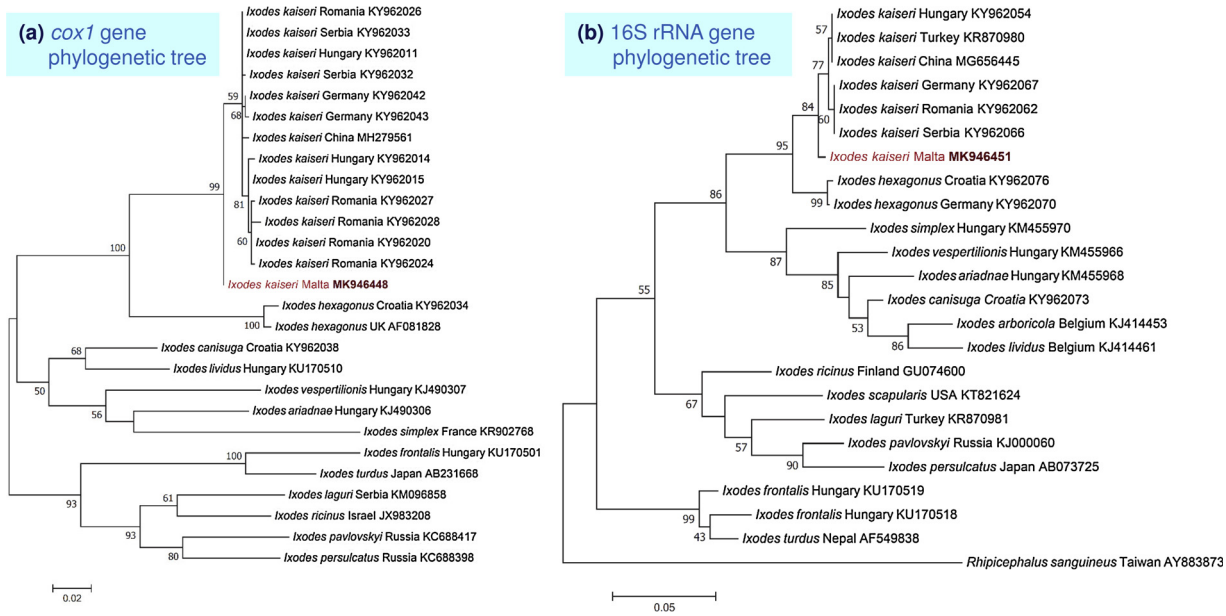

Fig. 1. Maximum Likelihood phylogenetic trees of *Ixodes* species based on (a) *cox1* and (b) 16S rRNA gene sequences. *Ixodes kaiseri* from Malta is highlighted with red color and bold accession number. After each species name the country of origin and the relevant GenBank accession number are shown. The scale-bar indicates the number of substitutions per site.

Interestingly, the *cox1* sequence divergence of *I. kaiseri* was more prominent between the sample collected in Malta and those from mainland Europe, than between *I. kaiseri* from mainland Europe and China. In addition, both the *cox1* and the 16S rRNA gene phylogenetic trees reflected a basal position of the Maltese specimen within the phylogenetic group of *I. kaiseri*, and this separation received strong support. These differences are most likely due to the hosts of *I. kaiseri* in Malta, such as wild carnivores and insectivores, as exemplified by the North African hedgehog in this study. These hosts are "localized" (i.e., neither migratory nor frequently transported), thus preventing or reducing the chances of genetic exchange between island- and mainland-inhabiting tick populations.

On the other hand, *O. coniceps*, which is also reported here for the first time from Malta, uses domestic or wild pigeons as typical hosts (Hoogstraal et al., 1979), therefore it is expected to show relative intraspecific genetic homogeneity over large geographical distances while associated with these volant, migratory host species. This is confirmed by the present molecular and phylogenetic data, because *O. coniceps* from Malta had identical sequences with some of the soft ticks reported from the west African islands of Cape Verde (Gómez-Díaz et al., 2012) and nearly identical sequences with others from the Canary Islands, France (Dupraz et al., 2016) and Algeria (Baziz-Neffah et al., 2015).

This also implies that these completely or nearly identical *cox1* or 16S rRNA gene sequences between *O. coniceps* from Malta and those from Cape Verde, Canary Islands and France belong to conspecific soft ticks, because these values (sequence divergence below 2.8 % in the *cox1* gene and below 2 % in the 16S rRNA gene) were within the reported mean values of species boundaries (*cox1*: 6.1 %, 16S rRNA gene: 5.3 % – Lv et al., 2014). Nevertheless, these closely related specimens were not identified as *O. coniceps* (only as *O. capensis* s. l.: Gómez-Díaz et al., 2012; Dupraz et al., 2016; although in the latter some of the sequences were labelled as "presumed tick species" *O. maritimus*). These data indicate difficulties in species-level identification within the *O. capensis* group and suggest that *O. coniceps* has a broader geographical distribution than previously thought (Hoogstraal et al., 1979). In particular, *O. coniceps* has long been known to occur in the western and middle Palaearctic (with a range extending from France to India, now including Malta), as well as in northern and eastern Africa (Hoogstraal et al., 1979). However, according to the comparison of *O. coniceps* sequences obtained here with others of the *O. capensis* group (see above) this species is also present on islands west of Africa. This maybe partly explained by the association of *O. coniceps* with marine bird species as hosts (Hoogstraal et al., 1979).

Morphological characteristics excluded the possibility that the examined ticks were *O. maritimus*. In addition, *O. maritimus* is almost always associated with marine birds (Hoogstraal et al., 1976) and to the best of our knowledge has never been reported from domestic chickens. Therefore, because soft tick larvae in the present study were collected from domestic chicken and *O. coniceps* is known to infect *Gallus gallus* var. *domesticus* (Hoogstraal et al., 1979), this host association is in line with the identity of the soft tick species investigated here as *O. coniceps*. To the best of our knowledge, *O. coniceps* (identified to the species level) has not been molecularly and/or phylogenetically analyzed prior to this study, thus increasing the significance of the results. These data confirmed that it belongs to the group of *O. capensis*, as it has long been postulated on a morphological basis.

Taken together, sequencing of additional molecular markers may be useful in future studies, especially for those species within the *O. capensis* group, in the case of which no molecular data are available from specimens identified morphologically to the species level. In this respect, molecular tools have the advantage to help identification of larvae and nymphs (e.g. in comparison with sequences obtained from adults), because their morphological characters may sometimes be misleading.

Hyalomma lusitanicum was the third tick species identified for the first time in Malta (on two independent occasions) during the present study. Rabbits are among the main hosts of larvae and nymphs of this tick species (Apanaskevich et al., 2008), consistently with the first of its two findings in Malta. However, adults of *H. lusitanicum* typically infest domestic and wild ungulates, sometimes dogs, but cats are usually not reported as hosts for this stage (Apanaskevich et al., 2008) unlike in the present case. Based on the high degree of genetic identity between isolates from the central and western Mediterranean regions as shown here (i.e., from Malta vs Spain/Portugal), trading of domestic ungulates may account for the observed homogeneity.

While the distribution of *H. lusitanicum* is restricted to the western part of the Mediterranean subregion of the Palaearctic zoogeographic region (Europe: France, Italy, Portugal and Spain including Canary

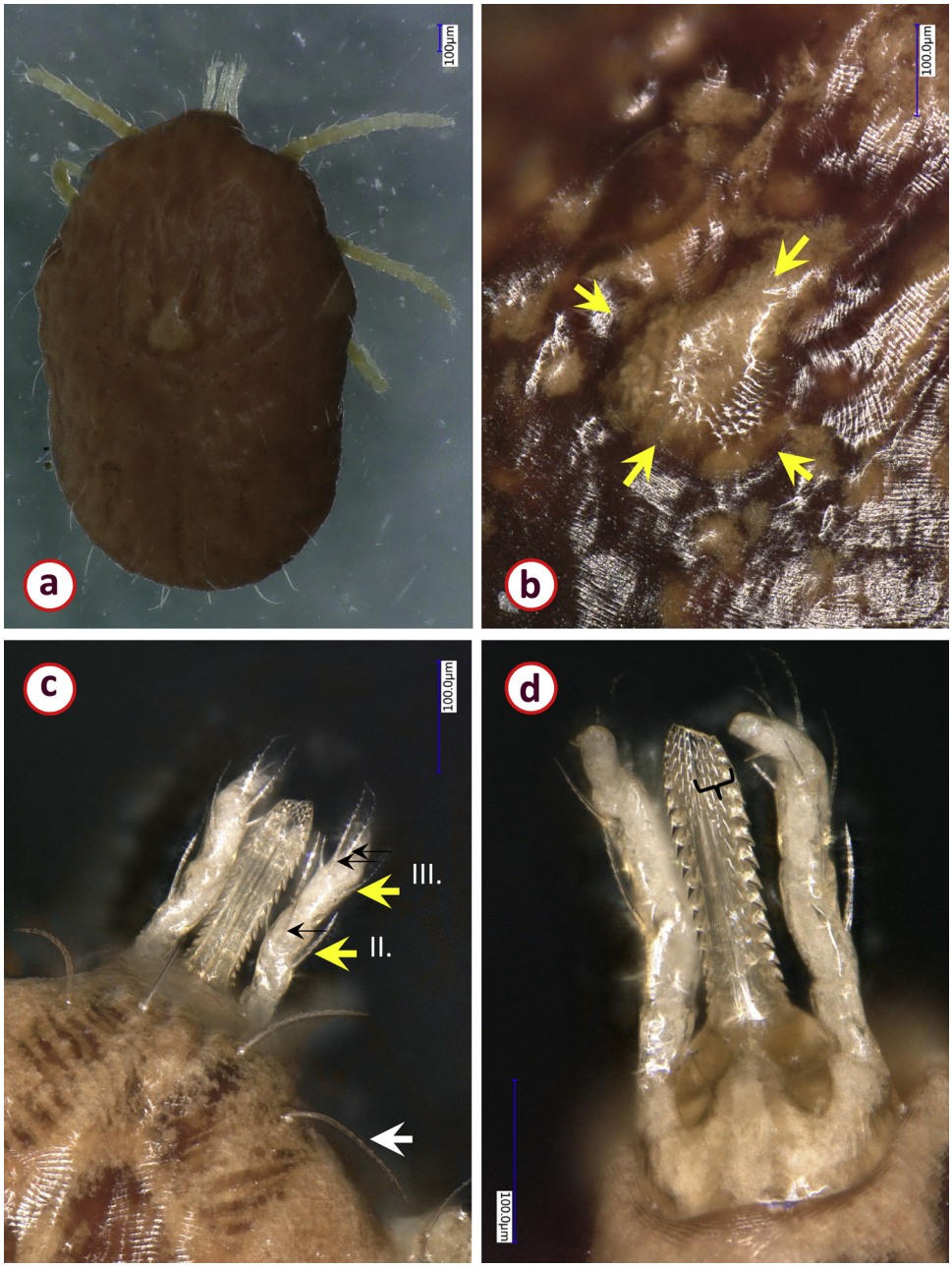

Fig. 2. Diagnostic morphological characters of *Ornithodoros coniceps* larvae: (a) habitus, showing 15 pairs of dorsal setae; (b) dorsal plate is pyriform, with an approx. 1:1 length to breadth ratio (its anterior, posterior and lateral margins marked with yellow arrows; (c) dorsal view of gnathosoma, showing two and three dorsal setae on II. and III. palpal segments (yellow and black arrows), as well as strongly fringed long dorsal seta (white arrow); (d) ventral view of gnathosoma, with 4/4 dentition formula of the hypostome anteriorly (indicated with black squared braces).

Islands; Africa: Algeria and Morocco) (Apanaskevich et al., 2008), the present results add Malta to its geographical range. Because this tick species is known as a vector of *Theileria annulata*, the causative agent of tropical theileriosis (Apanaskevich et al., 2008), the epidemiological risks associated with its first time recognition in Malta should deserve attention in the future.

CRediT authorship contribution statement

Sándor Hornok: Conceptualization, Writing - review & editing, Methodology. **Andrea Grima:** Conceptualization, Data curation, Investigation. **Nóra Takács:** Methodology. **Sándor Szekeres:** Methodology, Software. **Jenő Kontschán:** Methodology, Supervision.

Acknowledgement

The study received funding from NKFIH130216.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.ttbdis.2020.101379.

References

Apanaskevich, D.A., Horak, I.G., 2008. The genus *Hyalomma* Koch, 1844: V. Re-evaluation of the taxonomic rank of taxa comprising the *H.* (*Euhyalomma*) *marginatum* Koch complex of species (Acari: Ixodidae) with redescription of all parasitic stages and notes on biology. Int. J. Acarol. 34, 13–42.

Apanaskevich, D.A., Santos-Silva, M.M., Horak, I.G., 2008. The genus *Hyalomma* Koch, 1844. IV. Redescription of all parasitic stages of *H.* (*Euhyalomma*) *lusitanicum* Koch, 1844 and the adults of *H.* (*E.*) *franchinii* Tonelli Rondelli, 1932 (Acari: ixodidae) with a first description of its immature stages. Folia Parasitol. (Praha) 55, 61–74.

Araya-Anchetta, A., Busch, J.D., Scoles, G.A., Wagner, D.M., 2015. Thirty years of tick population genetics: a comprehensive review. Infect. Genet. Evol. 29, 164–179.

Arthur, D.R., 1957. Two North African *Ixodes* ticks. *I. Kaiseri* sp. Nov. From Egyptian desert fox cubs. A redescription of the female and a description of the male of *I. festai,* Rondelli, 1926 (Ixodoidea, Ixodidae). J. Parasitol. 43, 578–585.

Arthur, D.R., 1965. Ticks of the Genus *Ixodes* in Africa. Athlone Press, London, pp. 13–21.

Baziz-Neffah, F., Bitam, I., Kernif, T., Beneldjouzi, A., Boutellis, A., Berenger, J.-M., Zenia, S., Doumandji, S., 2015. Contribution à la connaissance des ectoparasites d'oiseaux en Algerie. Bull. Soc. Zool. France 140, 81–98 [in French].

Black, W.C., Piesman, J., 1994. Phylogeny of hard and soft-tick taxa (Acari: ixodida) based on mitochondrial 16s rDNA sequences. Proc. Nat. Acad. Sci. U. S. A. 91, 10034–10038.

Dupraz, M., Toty, C., Noël, V., Estrada-Peña, A., González-Solís, J., Boulinier, T., Dujardin, J.P., McCoy, K.D., 2016. Linking morphometric and genetic divergence with host use in the tick complex, *Ornithodoros capensis* sensu lato. Infect. Genet. Evol. 46, 12–22.

Filippova, N.A., Uspenskaya, I.G., 1973. *Ixodes kaiseri* Arthur 1957 (Ixodidae) species taxonomic status. Parazitologiya 7, 297–306 [In Russian].

Folmer, O., Black, M., Hoeh, W., Lutz, R., Vrijenhoek, R., 1994. DNA primers for amplification of mitochondrial cytochrome C oxidase subunit I from diverse metazoan invertebrates. Mel. Mar. Biol. Biot. 3, 294–299.

Gómez-Díaz, E., Morris-Pocock, J.A., González-Solís, J., McCoy, K.D., 2012. Trans-oceanic host dispersal explains high seabird tick diversity on Cape Verde islands. Biol. Lett. 8, 616–619.

Hoogstraal, H., Oliver, R.M., Guirgis, S.S., 1970. Larva, nymph and life cycle of *Ornithodoros* (*Alectorobius*) *muesebecki* (Ixoidea: argasidae), a virus-infected parasite of birds and petroleum industry employees in the Arabian Gulf. Ann. Entomol. Soc. Am. 63, 1762–1769.

Hoogstraal, H., Clifford, C.M., Keirans, J.E., Kaiser, M.N., Evans, D.E., 1976. The *Ornithodoros* (*Alectorobius*) *capensis* group (Acarina: ixodoidea: argasidae) of the palearctic and oriental regions. *O.* (*A.*) *maritimus*: identity, marine bird hosts, virus infections, and distribution in western Europe and northwestern Africa. J. Parasitol. 62, 799–810.

Hoogstraal, H., Clifford, C.M., Keirans, J.E., 1979. The *Ornithodoros* (*Alectorobius*) *capensis* group (Acarina: ixodoidea: argasidae) of the palearctic and oriental regions. *O.* (*A.*) *coniceps* identity, bird and mammal hosts, virus infections, and distribution in Europe, Africa, and Asia. J. Parasitol. 65, 395–407.

Hornok, S., Flaisz, B., Takács, N., Kontschán, J., Csörgő, T., Csipak, Á., Jaksa, B.R., Kováts, D., 2016a. Bird ticks in Hungary reflect western, southern, eastern flyway connections and two genetic lineages of *Ixodes frontalis* and *Haemaphysalis concinna*. Parasit. Vectors 9, 101.

Hornok, S., Wang, Y., Otranto, D., Keskin, A., Lia, R.P., Kontschán, J., Takács, N., Farkas, R., Sándor, A.D., 2016b. Phylogenetic analysis of *haemaphysalis erinacei* Pavesi, 1884 (Acari: ixodidae) from China, Turkey, Italy and Romania. Parasit. Vectors 9, 643.

Hornok, S., Sándor, A.D., Tomanović, S., Beck, R., D'Amico, G., Kontschán, J., Takács, N., Görföl, T., Bendjeddou, M.L., Földvári, G., Farkas, R., 2017a. East and west separation of *Rhipicephalus sanguineus* mitochondrial lineages in the Mediterranean Basin. Parasit. Vectors 10, 39.

Hornok, S., Sándor, A.D., Beck, R., Farkas, R., Beati, L., Kontschán, J., Takács, N., Földvári, G., Silaghi, C., Meyer-Kayser, E., Hodžić, A., Tomanović, S., Abdullah, S., Wall, R., Estrada-Peña, A., Duscher, G.G., Plantard, O., 2017b. Contributions to the phylogeny of *Ixodes* (*Pholeoixodes*) *canisuga*, *I.* (*Ph.*) *kaiseri*, *I.* (*Ph.*) *hexagonus* and a simple pictorial key for the identification of their females. Parasit. Vectors 10, 545.

Hornok, S., Grima, A., Takács, N., Kontschán, J., 2018. Infestation of *Rhipicephalus sanguineus* sensu lato on cats in Malta. Ticks Tick Borne Dis. 9, 1120–1124.

Jongejan, F., Uilenberg, G., 2004. The global importance of ticks. Parasitology 129 (Suppl), S3–S14.

Koop, J.A., DeMatteo, K.E., Parker, P.G., Whiteman, N.K., 2014. Birds are islands for parasites. Biol. Lett. 10 pii: 20140255.

Licari, E., Takács, N., Solymosi, N., Farkas, R., 2017. First detection of tick-borne pathogens of dogs from Malta. Ticks Tick Borne Dis. 8, 396–399.

Lv, J., Wu, S., Zhang, Y., Chen, Y., Feng, C., Yuan, X., Jia, G., Deng, J., Wang, C., Wang, Q., Mei, L., Lin, X., 2014. Assessment of four DNA fragments (COI, 16S rDNA, ITS2, 12S rDNA) for species identification of the Ixodida (Acari: ixodida). Parasit. Vectors 7, 93.

Orkun, Ö., Karaer, Z., 2018. First record of the tick *Ixodes* (*Pholeoixodes*) *kaiseri* in Turkey. Exp. Appl. Acarol. 74, 201–205.

Pfliegler, W.P., Schönhofer, A., Niedbała, W., Vella, P., Sciberras, A., Vella, A., 2017. New records of mites (Acari) and harvestmen (Opiliones) from Malta with a preliminary checklist of Maltese Arachnida. Soil Org. 89, 85–110.

Schembri, P.J., 1993. Physical geography and ecology of the Maltese Islands: a brief overview. In: Busuttil, S., Lerin, F., Mizzi, L. (Eds.), Malta: Food, Agriculture, Fisheries and the Environment. Montpellier. CIHEAM, pp. 27–39.

Sheng, J., Jiang, M., Yang, M., Bo, X., Zhao, S., Zhang, Y., Wureli, H., Wang, B., Tu, C., Wang, Y., 2019. Tick distribution in border regions of Northwestern China. Ticks Tick Borne Dis. 10, 665–669.

Sultana, J., Gauci, C., 1977. Ectoparasites (other than Hippoboscidae) collected from birds. -1978. Il-Merill (Malta) 19, 21.

Theodor, O., Costa, M., 1967. A survey of the parasites of wild mammals and birds in Israel. Part one. Ectoparasites. The Israel Academy of Sciences and Humanity, Jerusalem 119 p.