

Ectoparasites of hedgehogs: From flea mite phoresy to their role as vectors of pathogens

Marcos Antônio Bezerra-Santos^a, Giovanni Sgroi^a, Jairo Alfonso Mendoza-Roldan^a, Javad Khedri^b, Antonio Camarda^{a,c}, Roberta Iatta^a, Alireza Sazmand^{d,e}, Domenico Otranto^{a,d,*}

^a Department of Veterinary Medicine, University of Bari, Valenzano, 70010, Bari, Italy

^b Department of Pathobiology, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran

^c Osservatorio Faunistico Regionale - Wildlife Rescue Center of Apulia, Bitetto, 70020, Bari, Italy

^d Department of Pathobiology, Faculty of Veterinary Science, Bu-Ali Sina University, 6517658978, Hamedan, Iran

^e Zoonotic Diseases Research Center, School of Public Health, Shahid Sadoughi University of Medical Sciences, 8915173160, Yazd, Iran

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ABSTRACT

Hedgehogs are synanthropic mammals, reservoirs of several vector-borne pathogens and hosts of ectoparasites. Arthropod-borne pathogens (i.e., *Rickettsia* spp., *Borrelia* spp., and Anaplasmataceae) were molecularly investigated in ectoparasites collected on hedgehogs ($n = 213$) from Iran (161 *Hemichinus auritus*, 5 *Erinaceus concolor*) and Italy (47 *Erinaceus europaeus*). In Iran, most animals examined ($n = 153$; 92.2%) were infested by ticks (*Rhipicephalus turanicus*, *Hyalomma dromedarii*), and 7 (4.2%) by fleas (*Archeopsylla erinacei*, *Ctenocephalides felis*). Of the hedgehogs infested by arthropods in Italy (i.e., 44.7%), 18 (38.3%) were infested by fleas (*Ar. erinacei*), 7 (14.9%) by ticks (*Haemaphysalis erinacei*, *Rh. turanicus*, *Rhipicephalus sanguineus* sensu lato), and 6 (12.8%) by mites (*Caparinia tripilis*, *Acarus nidicolous*, *Ornithonyssus* spp.). Phoretic behavior of *C. tripilis* on *Ar. erinacei* was detected in two flea specimens from Italy. At the molecular analysis *Rickettsia* spp. was detected in 93.3% of the fleas of Italy. In Iran, *Rickettsia* spp. was detected in 8.0% out of 212 *Rh. turanicus* ticks, and in 85.7% of the *Ar. erinacei* fleas examined. The 16S rRNA gene for *Ehrlichia/Anaplasma* spp. was amplified in 4.2% of the 212 *Rh. turanicus* ticks. All sequences of *Rickettsia* spp. from fleas presented 100% nucleotide identity with *Rickettsia asebonensis*, whereas *Rickettsia* spp. from *Rh. turanicus* presented 99.84%–100% nucleotide identity with *Rickettsia slovacica*, except for one sequence, identical to *Rickettsia massiliae*. The sequences of the 16S rRNA gene revealed 99.57%–100% nucleotide identity with *Anaplasma* spp., except for one, identical to *Ehrlichia* spp. A new phoretic association between *C. tripilis* mites and *Ar. erinacei* fleas has been herein reported, which could be an important route for the spreading of this mite through hedgehog populations. Additionally, spotted fever group rickettsiae were herein detected in ticks and fleas, and *Anaplasma/Ehrlichia* spp. in ticks, suggesting that hedgehogs play a role as reservoirs for these vector-borne pathogens.

1. Introduction

Wild animals, mainly those presenting synanthropic behavior, are regarded as important reservoirs of pathogens of zoonotic concern (Simpson, 2002; Hassell et al., 2017). For example, hedgehogs thrive in urban, rural, and natural settings, therefore sharing the same environments with domestic animals as well as humans (Skuballa et al., 2007). Among infectious agents, vector-borne pathogens associated with hedgehogs are of major importance, since they are transmitted by ticks, fleas, and mites blood feeding on these animal species as well as on many

other mammalian hosts, including humans (Goz et al., 2016).

Beyond their vector role, ectoparasites of hedgehogs may present ecological interactions (i.e., phoresy) which may influence their distribution within mammal hosts. For example, mites may rely on phoretic association with ticks and fleas to spread among vertebrate hosts (Baumann, 2018), also considering their small body size and scant ability to cover large distances. This phenomenon is often described as a form of commensalism (Hodgkin et al., 2010) or even mutualism (Houck and Cohen, 1995); though, arthropod hosts may be negatively influenced by their phoretic companions (Karbowiak et al., 2013), which

* Corresponding author. Department of Veterinary Medicine, University of Bari, Valenzano, 70010, Bari, Italy.

E-mail address: domenico.otranto@uniba.it (D. Otranto).

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may impair their usual behaviors in moving, feeding and in reproduction (Blackman and Evans, 1994). Parasitic mites perpetuate in a wide range of mammals, including rats, hamsters, squirrels, marmots, bats, badgers and hedgehogs (Balashov, 2006; Karbowski et al., 2013). These arthropods are commonly regarded as causative agents of dermatitis on their hosts (Fischer and Walton, 2014). For example, some species such as *Caparinia tripilis* is known to infest hedgehogs causing skin injuries, especially in conjunction with other infections (Kim et al., 2012). Scabies lesions due to *C. tripilis* infestation are located in different anatomical sites (i.e., head, ears, abdominal regions and between the limbs) causing skin irritation, inflammation and pruritus, which lead to self-injuries, secondary infections, and even death (Kim et al., 2012; Garcés et al., 2020). In addition, some mite species such as *Leptotrombidium* spp. and *Liponyssoides sanguineus* are vectors of zoonotic infectious agents such as *Orientia tsutsugamushi*, *Bartonella tamiae*, *Rickettsia akari*, and Hantaan virus (Houck et al., 2004; Kabeya et al., 2010; Fischer and Walton, 2014).

Likewise, hedgehogs are commonly infested by ticks and fleas (Iacob and Iftinca, 2018; Khodadadi et al., 2021), which are regarded as vectors of zoonotic pathogens, such as *Rickettsia*, *Borrelia*, *Ehrlichia*, *Anaplasma* and *Bartonella* species (Regnery et al., 1992; Bouyer et al., 2001; De Sousa et al., 2017; Millán et al., 2019; Julian et al., 2020). Meanwhile, hedgehogs have been suggested as possible reservoirs of zoonotic vector-borne pathogens such as *Borrelia burgdorferi* sensu lato (s.l.), *Anaplasma phagocytophilum*, *Rickettsia helvetica*, *Leishmania major* and tick-borne encephalitis virus (Skuballa et al., 2012; Speck et al., 2013; Krawczyk et al., 2015; Pourmohammadi and Mohammadi-Azni, 2019; Greco et al., 2021). Thus, investigations on the role that ectoparasites of hedgehogs have in spreading arthropod-borne pathogens, as well as on the ecological interactions among them (i.e., phoresy) are required. This study reports the occurrence of *C. tripilis* in hedgehogs and its phoretic association with *Archaeopsylla erinacei* fleas, as well as the detection of arthropod-borne pathogens in ticks, fleas and mites collected on these host species in Iran and Italy.

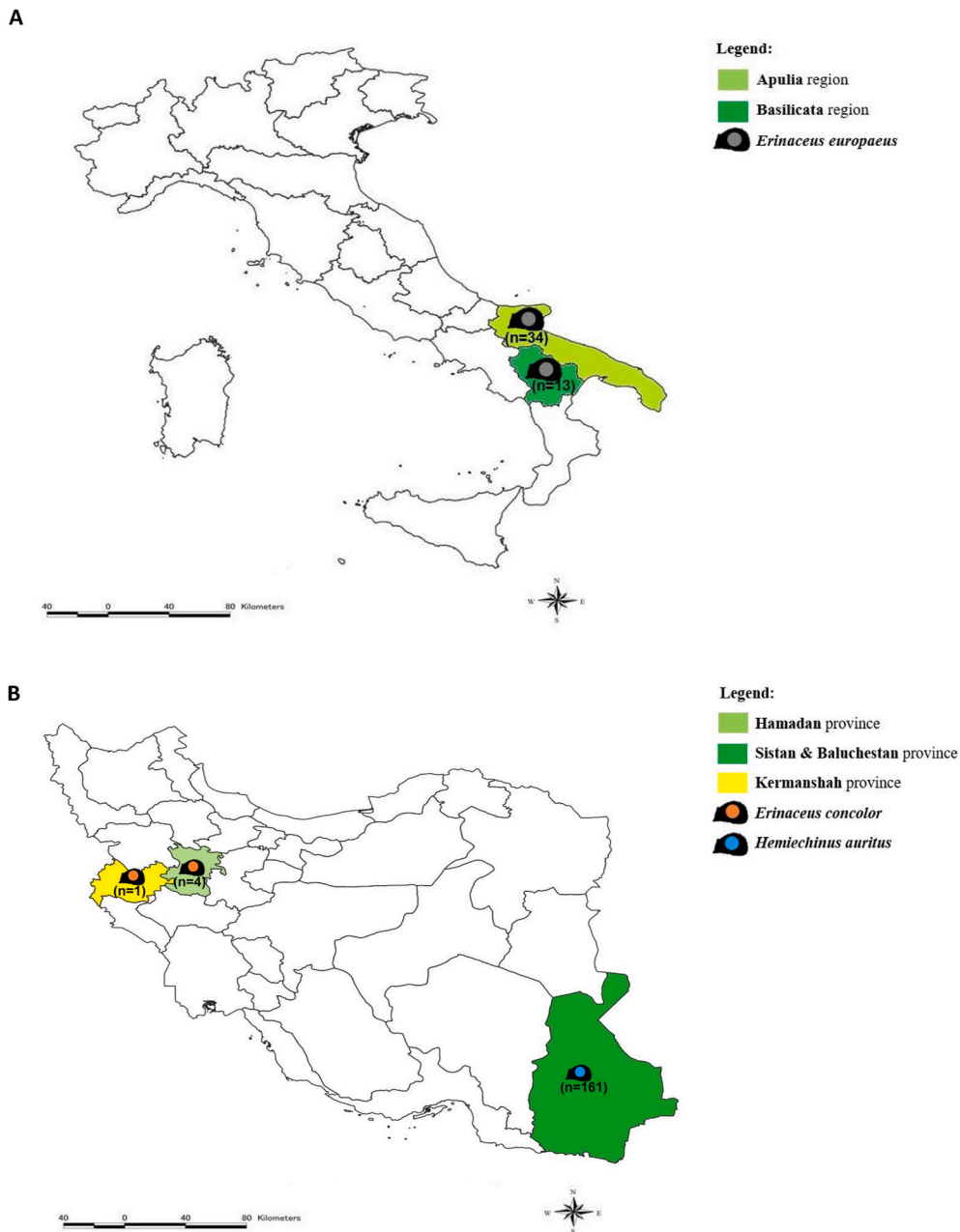


Fig. 1. Map of the study area where hedgehogs were captured. A. Italy; B. Iran.

2. Material and methods

2.1. Study area and sampling

Animals ($n = 213$) were captured by hands in different regions of Iran and Italy during 2019/2020 (Fig. 1). Animals from Italy were prevented from the clinical activities of the Recovery Center for Wildlife (CRAS), Puglia, Italy. The examined hedgehogs in Iran were road-killed animals. The hedgehogs were individually inspected for the presence of ectoparasites, which were collected with entomological forceps and placed in 1.5 ml tubes (Eppendorf, Aptaca Spa, Canelli, Italy) containing 70% alcohol. Ectoparasites were individualized, and 20% of them were molecularly processed per infested animal when the total number exceeded three, otherwise all ectoparasites collected on each individual were screened.

2.2. Ectoparasite species identification

Ectoparasites were separated by sex and life stage, and morphologically identified with a stereo microscope (Leica MS5) using dichotomous keys for ticks (Manilla, 1998; Estrada-Peña et al., 2004), and fleas (Smit, 1957). For the identification of mites, selected samples were mounted in Amman's Lactophenol on microscope slides and examined at $10 \times$, $20 \times$, and $40 \times$ magnification with an optical microscope (Leica DM LB2). Dichotomous keys for hematophagous mites (Baker et al., 1956), and phoretic mites (Baker et al., 1956; Fain and Portús, 1979) were used for genus and species identification. Additionally, ticks and fleas were carefully checked for the presence of phoretic mites.

2.3. Molecular detection of pathogens and ectoparasite species

Genomic DNA of ectoparasites was extracted using an in-house protocol previously described (Ramos et al., 2015). The detection of arthropod-borne pathogens was performed through conventional polymerase chain reaction (PCR), using primers that amplify DNA of *Rickettsia* spp., *Borrelia* spp., and Anaplasmataceae (Table 1). For *Rickettsia* spp. all samples were firstly screened using primers for the *gltA* gene, and those positives were tested for the *ompA* gene to further characterize spotted fever group rickettsiae (SFG). DNA of *Ehrlichia canis*, *Rickettsia slovaca*, and *B. burgdorferi* s.l. were used as positive controls for each PCR reaction. Morphological identification of ticks was further confirmed by PCR using the primers forward 16S+1 (5'-CTGCTCAATGATTTTTTAAATTGCTGTGG-3') and reverse Tick16S-2 (5'-TTACGCTGTTATCCCTAGAG-3'), which amplify a 460 base pair-sized (bp) fragment of the mitochondrial 16S rRNA gene (Black and Piesman, 1994), and for fleas with the primers forward LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and reverse HCO02198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3'), which amplify 710 bp of the *cox1* gene of metazoan invertebrates (Folmer et al., 1994).

2.4. Phylogenetic analysis

PCR products were purified and sequenced in both directions using the same forward and reverse primers, employing the Big Dye

Terminator v.3.1 chemistry in a 3130 Genetic analyzer (Applied Biosystems, California, USA) in an automated sequencer (ABI-PRISM 377). Nucleotide sequences were edited, aligned, and analyzed using MEGA7 software and compared with sequences available on GenBank through the BLAST search tool. For phylogenetic analysis, *ompA* and *gltA* gene sequences of *Rickettsia* spp., and 16S rRNA gene sequences of *Ehrlichia/Anaplasma* spp. from this study were included along with those available in the GenBank database. Phylogenetic relationships were inferred using the maximum likelihood (ML) method after selecting the best-fitting substitution model. Evolutionary analyses were conducted with 1000 bootstrap replications using MEGA7 software (Kumar et al., 2016). *Rickettsia prowazekii* (DQ926853), *R. akari* (L01461), and *Ehrlichia risticii* (AF206300) sequences were used as outgroups for the *gltA*, *ompA*, and 16S rRNA phylogenetic trees, respectively.

2.5. Statistical analysis

Descriptive statistics were used to calculate relative and absolute frequencies, as well as mean intensity and mean abundance of ectoparasites. Exact binomial 95% confidence intervals (CIs) were established for proportions using the QP 2.0 software.

3. Results

3.1. Hedgehogs and ectoparasites collection

Of 213 hedgehogs examined (i.e., $n = 166$ from Iran, and $n = 47$ from Italy) the majority were *Hemiechinus auritus* ($n = 161$) followed by *Erinaceus concolor* ($n = 5$) in Iran, whereas in Italy all ($n = 47$) were identified as *Erinaceus europaeus*.

In Iran, most animals examined (92.2%; 153/166; 95% CI: 0.87–0.96) scored positive for tick infestation, with the majority being infested by the species *Rhipicephalus turanicus* (91.6%; 152/166; 95% CI: 0.86–0.94), and one individual infested by the species *Hyalomma dromedarii* (Table 2). Additionally, seven (4.2%; 95% CI: 0.02–0.09) individuals presented co-infestation by ticks and fleas (Table 3).

In Italy 21 out of 47 individuals (44.7%; 95% CI: 0.31–0.60) were positive for ectoparasites (Table 2), predominantly fleas, which were all identified as *Ar. erinacei*. Co-infestation by fleas and mites, and by fleas and ticks, was detected in six (12.8%; 95% CI: 0.06–0.25) and four (8.5%; 95% CI: 0.03–0.20) animals, respectively, with one hedgehog presenting simultaneous infestation by fleas, ticks, and mites (Table 3). Phoretic behavior of *C. tripilis* mites on *Ar. erinacei* was detected in two female flea specimens from one animal in Italy with mites observed on their legs and head (Fig. 2).

Molecular analysis confirmed the morphological identification for ticks and fleas, with nucleotide identity of 100% for *Rh. turanicus* (Accession number: MT229198) and *Rhipicephalus sanguineus* sensu lato (Accession number: MN944863), 99.62% for *Haemaphysalis erinacei* (Accession number: KX237633), and 100% for *Ar. erinacei* (Accession number: KM890990). Sequences for *Hy. dromedarii* and *Ctenocephalides felis* were not obtained in the present study.

Table 1
Primers for the detection of arthropod-borne pathogens.

Pathogen	Gene	Amplicon size (bp)	Primer sequences	Reference
<i>Rickettsia</i> spp.	<i>gltA</i>	401	5'GCAAGTATCGGTGAGGATGTAAT3' 5'GCTTCCTTAAAATTCAATAAATCAGGAT3'	Labruna et al. (2004)
<i>Rickettsia</i> spp.	<i>ompA</i>	632	5'ATGGCGAATATTTCTCCAAA3' 5'AGTGCAGCATTGCTCCCCCT3'	Regnery et al. (1991)
<i>Borrelia</i> spp.	<i>fla</i>	482	5'AGAGCAACTTACAGACGAAATTAAT3' 5'CAAGTCTATTTTGAAAGCACCTAA3'	Skotarczak et al. (2002)
Anaplasmataceae	16S	345	5'GGTACCYACAGAAGATCC3' 5'TAGCACTCATCGTTTACAGC3'	Parola et al. (2000)

Table 2

Ectoparasites found on *Hemiechinus auritus*, *Erinaceus concolor* (Iran), and *Erinaceus europaeus* (Italy) hedgehogs.

Country/ Ectoparasite	Total	Infested animals	^a Mean abundance	^b Mean intensity	RF %	AF/ N
Iran						
<i>Hemiechinus auritus</i> (n = 161)						
Fleas (n = 6)						
<i>Archaeopsylla erinacei</i>	6 (3 M; 3 F)	6	6	6	3.7	6/161
Ticks (n = 256)						
<i>Hyalomma dromedarii</i>	2 (M)	1	0.01	2	0.6	1/161
<i>Rhipicephalus turanicus</i>	254 (146 M; 107 F; 1Ny)	152	1.58	1.67	94.4	152/161
Iran						
<i>Erinaceus concolor</i> (n = 5)						
Fleas (n = 4)						
<i>Archaeopsylla erinacei</i>	1 (F)	1	0.2	1	20.0	1/5
<i>Ctenocephalides felis</i>	3 (F)	1	0.6	3	20.0	1/5
Tick (n = 1)						
<i>Rhipicephalus turanicus</i>	1 (L)	1	0.2	1	20.0	1/5
Italy						
<i>Erinaceus europaeus</i> (n = 47)						
Fleas (n = 265)						
<i>Archaeopsylla erinacei</i>	265 (80 M; 175 F)	18	5.64	14.72	38.3	18/47
Mites (n = 104)						
<i>Caparinia tripilis</i>	10	1	0.21	10	2.1	1/47
<i>Acarus nidicolous</i>	4	3	0.09	1.33	6.4	3/47
<i>Ornithonyssus</i> spp.	90	2	1.91	45	4.3	2/47
Ticks (n = 13)						
<i>Haemaphysalis erinacei</i>	3 (M)	2	0.06	1.5	4.3	2/47
<i>Rhipicephalus turanicus</i>	8 (1 M; 6 F; 1Ny)	4	0.17	2	8.5	4/47
<i>Rhipicephalus sanguineus</i> s.l.	2 (L)	1	0.04	2	2.1	1/47

^a Number of ectoparasites per total of examined animals.

^b Number of ectoparasites per total of infested animals. RF – relative frequency; AF – absolute frequency; N – number of captured animals; M – male; F – female; Ny – nymph; L – Larvae.

3.2. Vector-borne pathogens detection

The molecular analysis for vector-borne pathogens detected *Rickettsia* spp. in 93.3% (n = 42/45; 95% CI: 0.81–0.98) of the fleas from Italy, but not in ticks and mites. In Iran, *Rickettsia* spp. was detected in 8.0% (n = 17/212; 95% CI: 0.05–0.12) of the *Rh. turanicus* ticks, and in 85.7% (n = 6/7; 95% CI: 0.44–0.99) of *Ar. erinacei* fleas. In addition, the 16S rRNA gene for *Ehrlichia* spp. and *Anaplasma* spp. was amplified in 4.2% (n = 9/212; 95% CI: 0.02–0.08) of the *Rh. turanicus* ticks. DNA of *Borrelia* spp. was not detected in the examined ectoparasites.

According to BLAST analysis, all *gltA* sequences of *Rickettsia* spp. detected in fleas presented 100% identity with *Rickettsia asembonensis* sequences available in the GenBank database (Accession numbers: MT499370; MT499369; KY445723). Whereas *Rickettsia* spp. *ompA* sequences from ticks presented from 99.84% to 100% nucleotide identity with *R. slovaca* (Accession numbers: MF379311; HM161769), except for

Table 3

Co-infestation by ectoparasites on hedgehogs from Iran and Italy.

Hedgehog species (infested/total)	Country	Tick species	Flea species	Mite species
<i>Hemiechinus auritus</i> (6/161)	Iran	<i>Rhipicephalus turanicus</i>	<i>Archaeopsylla erinacei</i>	–
<i>Erinaceus concolor</i> (1/5)	Iran	<i>Rhipicephalus turanicus</i>	<i>Archaeopsylla erinacei</i> ; <i>Ctenocephalides felis</i>	–
<i>Erinaceus europaeus</i> (1/47)	Italy	<i>Rhipicephalus turanicus</i>	<i>Archaeopsylla erinacei</i>	–
<i>Erinaceus europaeus</i> (1/47)	Italy	<i>Rhipicephalus sanguineus</i> sensu lato	<i>Archaeopsylla erinacei</i>	–
<i>Erinaceus europaeus</i> (1/47)	Italy	<i>Haemaphysalis erinacei</i>	<i>Archaeopsylla erinacei</i>	–
<i>Erinaceus europaeus</i> (1/47)	Italy	<i>Haemaphysalis erinacei</i>	<i>Archaeopsylla erinacei</i>	<i>Ornithonyssus</i> spp.
<i>Erinaceus europaeus</i> (2/47)	Italy	–	<i>Archaeopsylla erinacei</i>	<i>Ornithonyssus</i> spp.
<i>Erinaceus europaeus</i> (2/47)	Italy	–	<i>Archaeopsylla erinacei</i>	<i>Acarus nidicolous</i>
<i>Erinaceus europaeus</i> (1/47)	Italy	–	<i>Archaeopsylla erinacei</i> ^a	<i>Caparinia tripilis</i> ^a

^a Phoresy.



Fig. 2. *Caparinia tripilis* mites in phoretic association with *Archaeopsylla erinacei* flea.

one sample that was 99.84% identical to *Rickettsia massiliae* (Accession number: KR401143). The phylogenetic tree based on the partial *gltA* gene sequences showed that *R. asembonensis* from *Ar. erinacei* fleas from Italy and Iran assembled in a well-supported sister cluster that includes *Rickettsia felis* and other *R. felis*-like organisms (Fig. 3). The phylogenetic analysis of the *ompA* gene revealed that *R. slovaca* sequences from this study clustered with those from China, Turkey, and Italy (Fig. 4), and *R. massiliae* with those from China and Spain, as well as with *Rickettsia rhipicephali* from France (Fig. 4).

The sequences of the 16S rRNA gene revealed 99.57%–100% identity with *Anaplasma* spp. (Accession numbers: MN193068; KP642755; MH020202; MN700237; MW368828), except for one which was

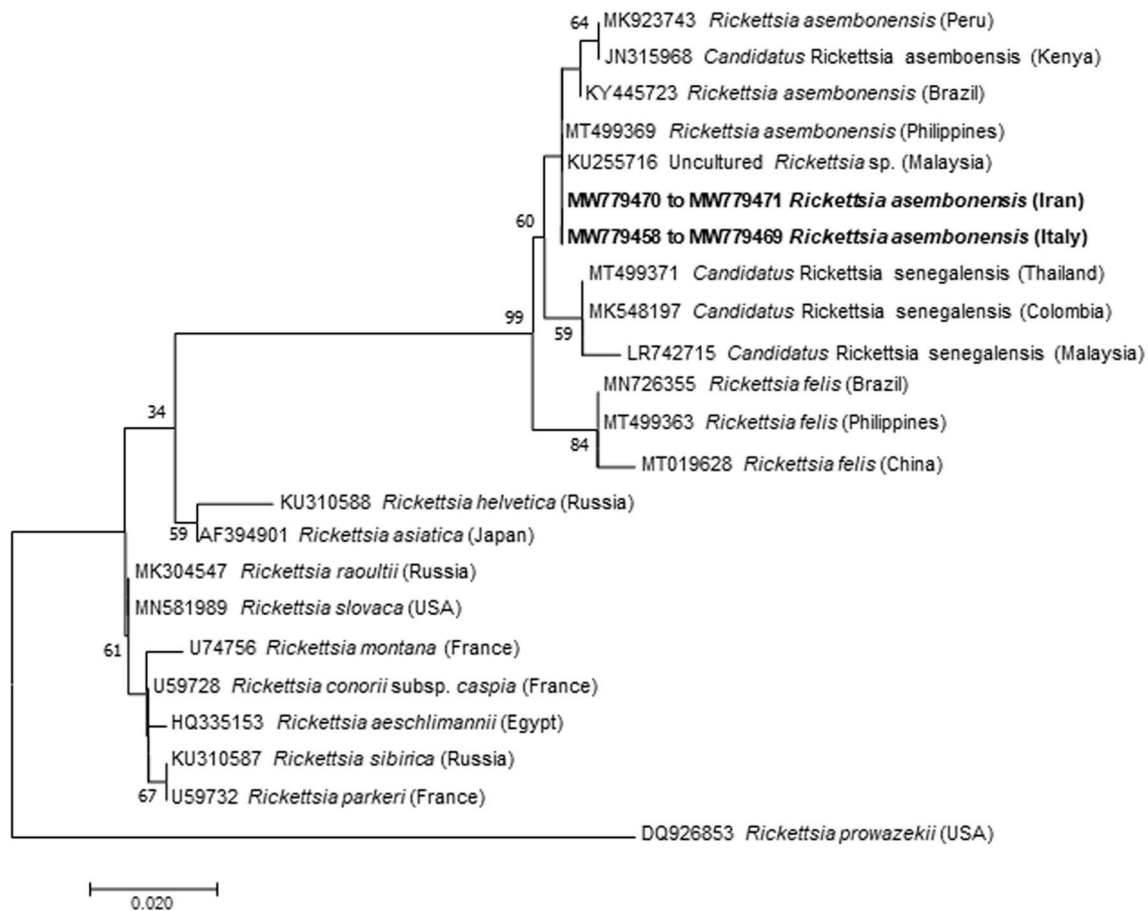


Fig. 3. Phylogenetic analysis of the *gltA* gene (345 bp) of *Rickettsia asemonensis* detected in this study (Bold) and relationship with other *Rickettsia* spp. The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura 3-parameter model (Tamura, 1992). A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories [+G, parameter = 0.2157]). GenBank accession number and country of origin are presented for each sequence.

identical to *Ehrlichia* spp. (Accession numbers: MN148616; AB074459). The phylogenetic analysis of the 16S rRNA gene sequences showed that five sequence types (i.e., ST1 to ST5) of *Anaplasma* spp. were closely related with *Anaplasma* spp. of veterinary and public health concern (Fig. 5). The sequence from *Ehrlichia* sp. herein detected assembled with *Ehrlichia ewingii* from China and USA, *Candidatus Ehrlichia shimanensis* from USA, and *Ehrlichia* sp. Yanaguni206 from Japan (Fig. 5).

Sequences herein obtained were submitted to GenBank under the accession numbers: MW779458 to MW779472 for *R. asemonensis*; MW779473 to MW779484 for *R. slovacica*; MW779485 for *R. massiliae*; MW772368, MW772370, MW772371, MW772372 and MW772373 for *Anaplasma* spp.; and MW772369 for *Ehrlichia* sp.

4. Discussion

This study assessed the occurrence of phoresy of *C. tripilis* mites on *Ar. erinacei* fleas in Italy, as well as of SFG rickettsiae, *Ehrlichia* sp., and *Anaplasma* spp. in the ectoparasite fauna of hedgehogs from Iran and Italy. Of major interest is the high prevalence of *R. asemonensis* in fleas collected from hedgehogs. To the best of our knowledge, no data is available regarding the phoretic association of *C. tripilis* mites on *Ar. erinacei* fleas infesting *E. europaeus*, with potential implications to animal health due to the host-parasite association these arthropods have with the European hedgehog. Indeed, *C. tripilis* mites are commonly detected on African hedgehogs, *Atelerix albiventris* (Kim et al., 2012; Moreira et al., 2013; Jacob and Iftinca, 2018), whereas *E. europaeus* is rarely reported harboring this mite species (Keymer et al., 1991). In

general, animals affected by *C. tripilis* present dermatitis characterized by skin inflammation, scabs, crusting, hair loss and self-injuries in consequence of pruritus (Kim et al., 2012; Jacob and Iftinca, 2018; Garcês et al., 2020). Animals herein evaluated were apparently healthy, and due to the small size of *C. tripilis* mite, it was not possible to determine whether the individuals from which the phoresy was detected also presented mites on their body.

Phoresy of mites has been widely recorded on arthropods such as ants, bees (Joharchi et al., 2019), ticks (Karbowski et al., 2013), psyllids (Liu et al., 2016), termites (Khaustov et al., 2017, 2019) and fleas (Karbowski et al., 2013). These interactions have different implications according to the biology of the species involved, and might be beneficial, costly, or may present no effect on the arthropod host (Hodgkin et al., 2010). Studies on this phenomenon between mites and fleas are scant, and up to date none of them identified the degree to which phoretic mites affect fleas (Britt and Molyneux, 1983; Schwan, 1993; Karbowski et al., 2013; Hastriter and Bush, 2014). Additionally, most studies on the phoretic behavior of mites have been performed on Coleoptera, Diptera and Hymenoptera (Norton, 1980; Khaustov and Frolov, 2018; Paraschiv et al., 2018; Durkin et al., 2019; Revainera et al., 2019). For example, phoresy of the mite species (i.e., *Acarus farris*, *Acarus siro*, *Acarus nidicolous*, *Caloglyphus rhizoglyphoides*, *Histioglyphus feroniarum* and *Tyrophagus putrescentiae*) was recorded in ticks (i.e., *Ixodes hexagonus*) and fleas (i.e., *Megabothris walkeri*, *Megabothris turbidus*, *Hystrichopsylla orientalis*, *Ctenophthalmus agyrtes*) collected on small mammals from Poland (Karbowski et al., 2013). In *E. europaeus* hedgehogs, this association was previously recorded in England for the mite species *A. nidicolous* on *Ar.*

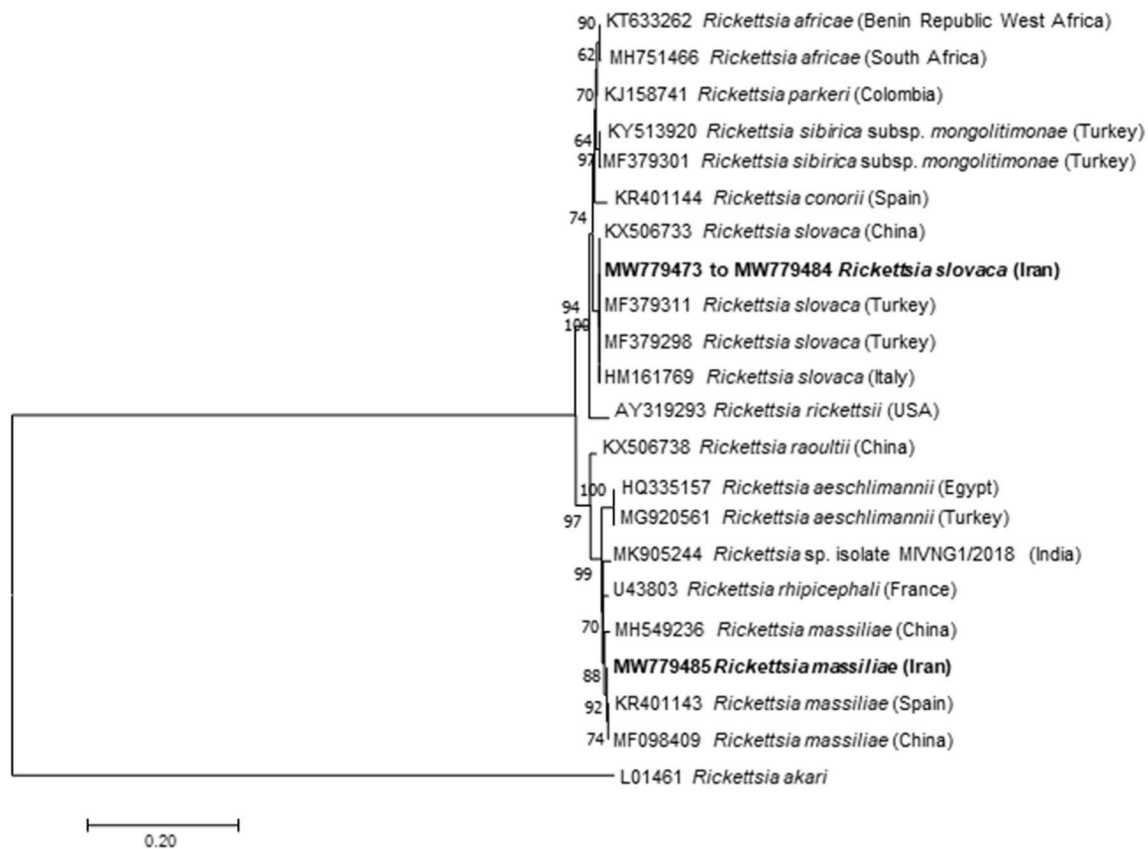


Fig. 4. Phylogenetic analysis of the *ompA* gene (579 bp) of *Rickettsia slovaca* and *Rickettsia massiliae* detected in this study (Bold) and relationship with other *Rickettsia* spp. The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura 3-parameter model (Tamura, 1992). Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The rate variation model allowed for some sites to be evolutionarily invariable ([+I], 20.90% sites). GenBank accession number and country of origin are presented for each sequence.

erinacei (Britt and Molyneux, 1983). However, in the present study no phoretic association was observed for this mite species. The high prevalence of *Ar. erinacei* in hedgehogs may favor the spreading of phoretic mites such as the pathogenic species, *C. tripilis*. Nevertheless, whether this association is harmful for the animal's health deserves further investigation, as it has been previously suggested that these two arthropod species may compete for the same sites on the host skin, and the absence of fleas may facilitate the establishment of this mite infestation on hedgehogs (Brookie, 1974). Infestation by *Ornithonyssus* spp. mites on *E. europaeus* hedgehogs has been also herein recorded for the first-time in two animals. Among the species of this genus, only the zoonotic tropical rat mite, *Ornithonyssus bacoti*, has been reported on the African hedgehog, *A. albiventris* (d'Ovidio et al., 2018).

Hedgehogs captured in Iran were rarely infested by *Ar. erinacei* fleas whereas those from Italy presented a high flea infestation rate. This flea species is commonly found infesting hedgehogs (Goz et al., 2016; Zurita et al., 2018), and has been associated with zoonotic pathogens such as *Rickettsia* spp. and *Bartonella henselae* (Hornok et al., 2014). In addition, despite being considered the hedgehog flea, this insect also infests other hosts such as cats, dogs (Gilles et al., 2008), foxes (Marié et al., 2012) and even humans (Greigert et al., 2020). The lower prevalence of fleas in the animals from Iran could be related to the fact that the examined hedgehogs were road-killed, which may cause the ectoparasites to abandon the host soon after the animal dies. In addition, climate factors could be also associated with this low prevalence, since the hot weather may reduce the overall abundance of fleas (Russell et al., 2018). Conversely, ticks (i.e., *Rh. turanicus*, *Hy. dromedarii*), especially *Rh. turanicus* were detected with high prevalence (i.e., 92.2%) in animals from Iran, whereas in Italy the prevalence of ticks (i.e., *Rh. turanicus*, *Rh.*

sanguineus s.l., *Ha. erinacei*) was low (i.e., 14.9%). These differences in prevalence of ectoparasites in the animals collected in Italy and Iran could be related to climate factors (arid and semi-arid climate in Iran, and temperate climate in Italy), and to the hedgehog species captured, which diverged between these two countries. The tick species above are regarded as potential vectors of pathogens (Khaldi et al., 2012; Wei et al., 2015; Orkun et al., 2019). For example, *Rickettsia aeschlimannii*, *R. massiliae* and *Anaplasma marginale* have been detected in *Rh. turanicus* (Wei et al., 2015; Khodadadi et al., 2021), SFG rickettsiae and *Babesia* sp. in *Ha. erinacei* (Khaldi et al., 2012; Orkun et al., 2019), and *Coxiella burnetii* and *Rickettsia* spp. in *Hy. dromedarii* (Loftis et al., 2006; Kernif et al., 2012).

The high prevalence of *R. asembonensis* (i.e., 93.3%) in *Ar. erinacei* fleas recorded in the present study is confirmed by its retrieval in fleas worldwide (Loyola et al., 2018; Maina et al., 2019; Nguyen et al., 2020). A *R. felis*-like organism presenting 100% nucleotide identity with *R. asembonensis* has been previously reported with high prevalence (i.e., 96.0%) in *Ar. erinacei* fleas in Germany, as uncultured *Rickettsia* sp. (Gilles et al., 2009), and in Portugal with prevalence of 47.0% (Barradas et al., 2021). In fact, *R. asembonensis* is a *R. felis*-like bacterium detected in fleas collected on domestic animals and from human dwellings in Kenya (Jiang et al., 2013), and recently characterized as a novel *Rickettsia* species from *C. felis* (Maina et al., 2016). Despite being closely related to *R. felis*, the pathogenicity of *R. asembonensis* to humans is still unknown (Jiang et al., 2013; Loyola et al., 2018). To the best of our knowledge, this is the first report of *R. asembonensis* in Italy and the high prevalence herein detected in fleas collected from hedgehogs is particularly important as this animal species could act as reservoir for this bacterium in the studied area. However, further investigations are

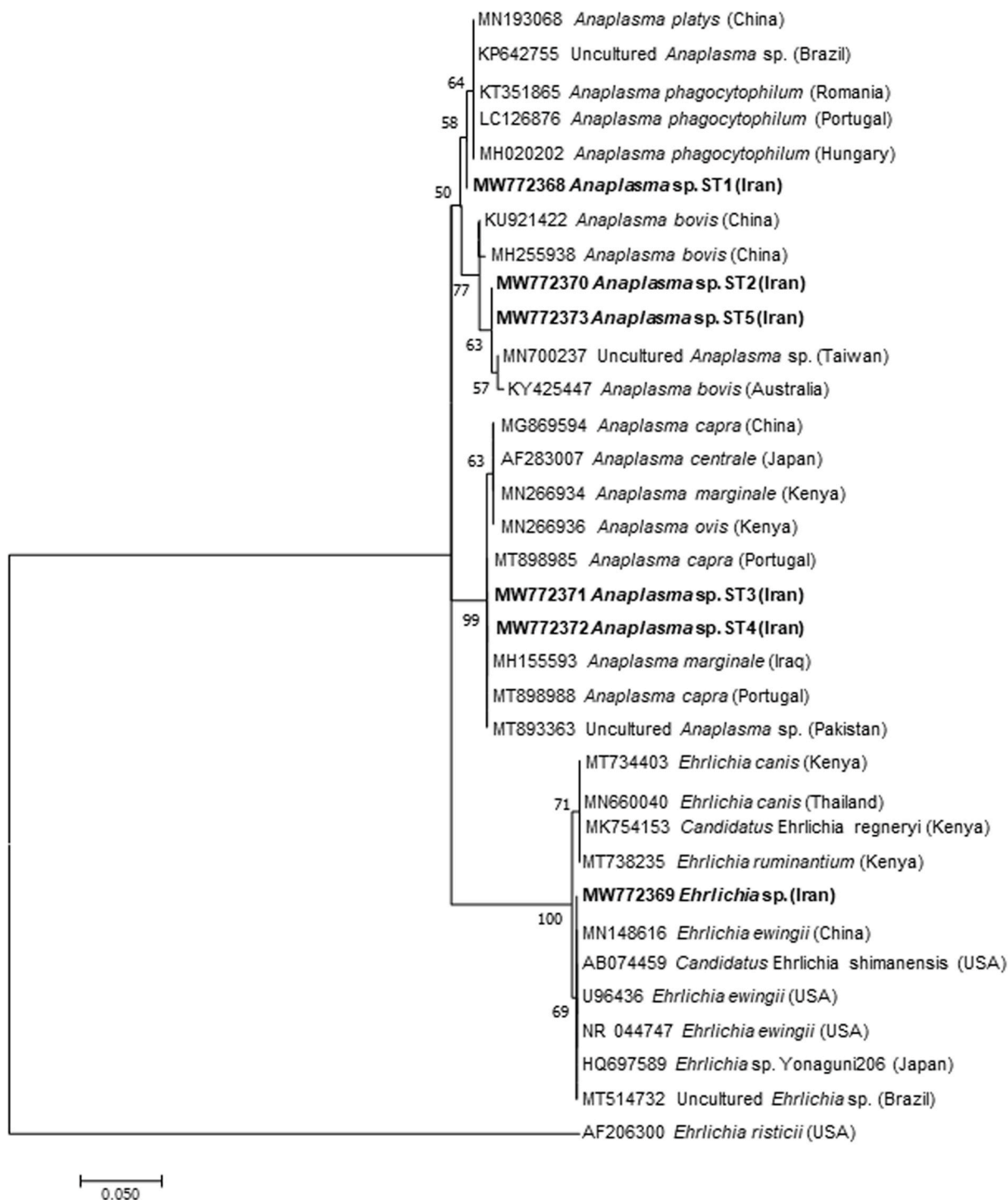


Fig. 5. Phylogenetic analysis of the 16S rRNA gene (281 bp) of *Ehrlichia* and *Anaplasma* spp. detected in this study (Bold) and relationship with other *Ehrlichia*/*Anaplasma* spp. The evolutionary history was inferred by using the Maximum Likelihood method based on the Kimura 2-parameter model (Kimura, 1980). Initial tree (s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The rate variation model allowed for some sites to be evolutionarily invariable ([+I], 37.72% sites). GenBank accession number and country of origin are presented for each sequence.

required to confirm the role of this mammal species in the epidemiology of *R. asembonensis*.

Rickettsia slovaca has been herein detected for the first time in *Rh. turanicus* ticks collected from *H. auritus* in Iran. This zoonotic spotted fever group bacterium is the main etiological agent of SENLAT (Scalp Eschar and Neck Lymphadenopathy after tick bite) in humans, an illness characterized by the enlargement of neck lymph nodes and scalp eschar

after a tick bite (Hocquart et al., 2019; Barlozzari et al., 2021). In addition, apart from humans, *R. slovaca* has been reported in other vertebrate host species including domestic animals (e.g., dogs; Iatta et al., 2021), and wildlife (e.g., rodents, wild boars; Martello et al., 2019; Sgroi et al., 2020). Ticks of the genus *Dermacentor* (e.g., *Dermacentor marginatus*, *Dermacentor reticulatus*) are the main vectors of this bacterium (Portillo et al., 2015); however, it has also been associated with

other tick species such as *Ixodes ricinus*, *Rhipicephalus bursa*, and *Hyalomma* spp. (Kachrimanidou et al., 2010; Orkun et al., 2019; Remesar et al., 2019). The detection of *R. slovaca* in *Rh. turanicus* collected from hedgehogs suggests that this tick species, as well as hedgehogs, could be involved in the transmission cycle of this bacterium in Iran. Moreover, this tick species has also been collected on humans (Eremeeva and Stromdahl, 2011), advocating further studies on the epidemiology of zoonotic SFG rickettsiae and the sympatric occurrence of *Rh. turanicus* ticks, hedgehogs, humans, and domestic animals.

Rickettsia massiliae was also herein detected in a specimen of *Rh. turanicus* female tick in Iran. This bacterium has been suggested to be one of the causative agents of the Mediterranean spotted fever (Portillo et al., 2015), and it has been associated with human cases of SENLAT in Romania (Zaharia et al., 2016). Ticks of the genus *Rhipicephalus* are suggested as the main vectors of *R. massiliae*, with transovarial and transstadial transmission being experimentally proved in *Rh. turanicus* (Matsumoto et al., 2005). Additionally, this bacterium has been recently detected in *Rh. sanguineus* s.l. ticks collected on *E. europaeus* hedgehogs (Barradas et al., 2021), again demonstrating that hedgehogs may play an important role in the epidemiology of vector-borne diseases.

Rhipicephalus turanicus ticks from Iran were also positive for *Anaplasma* and *Ehrlichia* spp., with five sequence types being detected for *Anaplasma* spp. and one for *Ehrlichia* sp. The phylogenetic analysis of the 16S rRNA gene demonstrated that the pathogens herein detected in *Rh. turanicus* ticks were closely related to species infecting humans (e.g., *A. phagocytophilum*, *E. ewingii*), and domestic animals (e.g., *Anaplasma platys*, *A. phagocytophilum*, *Anaplasma bovis*, *Anaplasma capra*, *A. marginale*). Previous studies have reported the presence of Anaplasmataceae in hedgehogs and their ticks (Silaghi et al., 2012; Khodadadi et al., 2021). For example, *A. marginale* has been detected in *H. auritus* and its *Rh. turanicus* ticks in southeastern Iran (Khodadadi et al., 2021), and *A. phagocytophilum* in *E. europaeus* and its *Ixodes hexagonus* and *I. ricinus* ticks in Germany (Silaghi et al., 2012). Our results confirm the presence of bacterial DNA of *Anaplasma* spp. and *Ehrlichia* sp. in *Rh. turanicus* ticks collected on hedgehogs in Iran, which deserves further investigation to assess the circulation of these pathogens among hedgehogs, domestic animals, and humans.

Finally, the absence of *Borrelia* spp. in the ectoparasites examined in the present study could be related to the vector competence of the arthropod species herein detected, as the main vectors for *B. burgdorferi* s.l. are ticks of the *I. ricinus* complex (Remesar et al., 2019), which have not been herein detected. Nevertheless, hedgehogs have already been suggested as reservoirs for these bacteria in Europe (Skuballa et al., 2007, 2012).

5. Conclusion

Data herein presented demonstrated a new phoretic association between *C. tripilis* mites and *Ar. erinacei* fleas collected on European hedgehogs. This could be a strategy this mite species uses to spread among hedgehog populations given the close host-parasite relationship between *Ar. erinacei* and *E. europaeus*. Additionally, we report the presence of SFG rickettsiae in ticks and fleas, and *Anaplasma* spp. and *Ehrlichia* sp. in ticks, with *Ar. erinacei* fleas presenting a high prevalence of *R. asembonensis*, a *R. felis*-like organism detected in many arthropods worldwide, suggesting that hedgehogs may play a role as a reservoir host for these vector-borne pathogens. In this aspect, due to the widespread presence of hedgehogs in rural and urban environments, they should be considered a source of ectoparasites in these areas, which is epidemiologically relevant for the circulation of arthropod-borne infectious agents among hedgehogs, domestic animals, and humans.

Ethical standards

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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