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Tick species from Africa by migratory birds: a 3-year study in Italy

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Abstract
The role of resident or migratory birds in dispersal of tick species and tick-borne pathogens is still poorly known in Italy. We report here the results of a 3-year project based on sampling ticks from migratory birds, as well as from the vegetation at three stop-over sites for migrants, namely the islands of Ventotene (Latium), Asinara (Sardinia) and Ustica (Sicily). During the spring seasons from 2017–2019, in total 2681 ticks were collected, 2344 of which were sampled from migratory birds and 337 from the vegetation. Ticks were identified by morphology or by molecular tools when necessary. In total, 16 tick species were identified among which the following were exclusively found on birds: *Hyalomma rufipes* (43.3%), *Hy. truncatum* (0.1%), *Ixodes frontalis* (11.8%), *Ix. inopinatus* (0.2%), *Ix. ricinus* (3%), *Haemaphysalis punctata* (0.08%), *Hae. erinacei* (0.1%), *Amblyomma variegatum* (0.08%) and *Argas vulgaris* 0.1%), whereas five species were exclusively collected from the vegetation: *Rhipicephalus bursa* (10.5%), *Rh. turanicus* (5.9%), *Rh. sanguineus* sensu lato (2%), *Rh. pusillus* (2.4%), *Hae. sulcata* (0.08%). *Hy. marginatum* (10.3%) and *Ix. ventalloi* (9.3%) were found both on birds and on the vegetation on the island Ustica. It is worth noting that the search for ticks on the vegetation did not detect allochthonous tick species. Although we found several interesting local species and allochthonous ticks like *Hy. rufipes*, *Am. variegatum* and *Ar. vulgaris* on birds, further investigations are needed to better define the possible role of migratory birds in the introduction of ticks and tick-borne diseases in Italy, above all after the evidence of imported ticks positive to Crimean Congo hemorrhagic fever (CCHF) virus in several European countries.

Keywords Ticks · Migratory birds · Italy

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Introduction

Ticks are known to be competent vectors of viral, bacterial, and protozoan agents responsible for emerging diseases worldwide. The increase of human mobility, along with global warming, represents a key factor for the introduction and spread of vector-borne diseases in Europe (Khasnis and Nettleman 2005). In addition to human activities, passive transport by migratory birds may also favor the spread of tick species and of the consequent risk of tick-borne diseases, across countries and continents (Hildebrandt et al. 2010). Tick-bird association contributes to the dissemination of ticks and related pathogens over long distances but also in urbanized areas that are less accessible to medium-sized or large mammals (de la Fuente et al. 2015; Heylen et al. 2019). In Europe, similarly to other parts of the globe, migratory birds play an important role in transportation of ixodid ticks (Acari: Ixodidae) and tick-borne pathogens (Hasle 2013). In this way, traveling over long distances and across geographical barriers, such as oceans, seas and deserts, these avian species may be implicated in the spread of zoonoses as reservoirs and could be hosts or carriers of infected ectoparasites, causing water-borne, tick-borne, and insect-borne diseases (Georgopoulou and Tsiouris 2008). Moreover, birds are recognized as reservoirs of Usutu virus (USUV) and several different West Nile virus (WNV) genetic lineages and clades (Monaco et al. 2015; Savini et al. 2008; Sotelo et al. 2009) in the Mediterranean basin and this is attributable to the movement of birds in areas with abundance of mosquitoes and ticks (Kolodziejek et al. 2014; Lwande et al. 2014). Migratory birds could also carry infected ticks potentially implicated in the Crimean Congo hemorrhagic fever virus (CCHFV) spread. In this regard, an emerging perspective within the scientific community is that future CCHFV research should be based on understanding the epidemiological and pathogenic properties of strains circulating within the Mediterranean region, as well as considering the role of birds in dispersing infected ticks (Spengler et al. 2018).

The role of birds in the potential spread of the virus was confirmed by CCHFV detection in ticks collected from migratory birds in Greece in 2009 (Lindeborg et al. 2012) and Morocco in 2011 (Palomar et al. 2013). Some species of bacteria, as well as viruses, should be mentioned as relevant pathogens involved in tick-borne diseases with birds as the main reservoirs, such as for example *Borrelia garinii* (Norte et al. 2019). In spring, migratory birds reach Europe mainly from sub-Saharan Africa (trans-Saharan or long-distance migrants), or from northern African countries (intra-Palearctic or short distance migrants) but not much is known about the diversity and ecology of the tick species using this way of dispersion (Laakkonen et al. 2009; Hornok et al. 2016). For this reason, the studies on ticks hosted by migratory birds constitute a valid occasion to collect data not only on the possible pathogens associated but also about the potential dispersion of tick species from remote regions.

In Italy, pathogens implicated in tick-borne disease transmission cycles and the role of resident and migratory birds in the maintenance and dispersal of these pathogens are still poorly known (Toma et al. 2014; De Liberato et al. 2018; Pascucci et al. 2019). In order to fill this knowledge gap and assess the risk of introduction of CCHF in Italy, an extensive acarological survey was conducted on ticks on three Italian islands in 2017–2019, based on morphological and molecular analyses of a large number of specimens. The study consisted in collecting ticks from spring migrants staging at three stopover sites namely islands of Ventotene (Latium), Asinara (Sardinia) and Ustica (Sicily) (Fig. 1) during 2017–2019. Sampled ticks were molecularly analyzed under the virological aspect, after a nymph of *Hyalomma rufipes*, from a Whinchat (*Saxicola rubetra*) on Ventotene, was positive for

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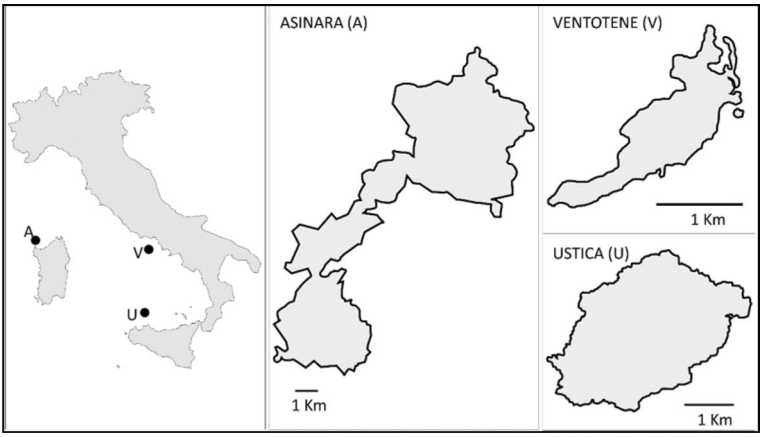

Fig. 1 Map of the study area. Location and outline of the three islands in the Tyrrhenian Sea, Italy: A = Asinara (50.9 km^2, ca. 2 km from Capo Falcone, Sardinia), V = Ventotene (1.89 km^2, 51.4 km from the mainland, Port of Formia) and U = Ustica (8 km^2, 66 km from Port of Palermo, Sicily)

CCHFV in 2017, resulting as the first case of infected tick carried by migratory birds in Western Europe (Mancuso et al. 2019).

Although the tick dispersal by migratory birds does not imply the establishment of the different species in new territories, global warming could move the northern limit of their original distribution range, together with possible new risks of pathogen introduction (Pascucci et al. 2019).

With this project it was possible to improve our knowledge on species composition, taxonomy, biology and behavior of tick species moving through such passive dispersal and evaluate the risk of introduction of exotic ticks. Here we report the results of this survey.

Materials and methods

Sample collection

Fieldwork activities were conducted in 2017 and 2018, from March to May, on the three islands where ringing stations usually operate. In 2019 sampling activities were carried out only on Ventotene from April to May, in order to focus attention on long-distance migratory species at the site with highest numbers of captures. During spring migration, Ventotene is characterized by the passage of huge numbers mainly of songbirds that reach the island after a non-stop flight from northern African (Spina et al. 1993). On the island, birds spend a variable lapse of time, from a few hours to a few days, depending on weather and body conditions (Goymann et al. 2010) resting or feeding on insects or nectar (Cecere et al. 2010) before resuming their northbound journey (Tenan and Spina 2010).

Birds were caught by experienced ringers. In the three study seasons, in total ca. 48,500 birds were identified at species level, sexed, aged, measured, marked with metal rings according to standard ringing procedures and inspected for the presence of tick parasites. Ticks were removed from each bird by using a tick twister or tweezers and stored in Eppendorf tubes containing 70% ethanol. On Ventotene, Asinara and Ustica,

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two operators have also randomly collected ticks nearby the sites where the ringing activities were being carried out, from 8:00 a.m. to 12:00, by dragging two 1-m^2 clear blankets on the vegetation. On Ustica, the collection was carried out also in the outskirt of a farm hosting poultry, goats, pigs and donkeys, nearby a pinewood.

Tick identification

All ticks were analyzed morphologically using published keys (Manilla 1998; Iori et al. 2005). Ticks collected on wild birds are generally immature and their morphological identification at species level is challenging (Pascucci et al. 2019); for this reason, the identification of most ticks was limited at the genus level.

After morphological identification, ticks were singularly subjected to DNA and RNA extraction for subsequent laboratory analyses aimed at the arboviruses detection on all collected ticks (IZS—Animal Health Institute of Teramo) and species identification on a selected sample of individual ticks (ISS—National Public Health Institute in Rome, Italy). Based on the morphological and molecular results obtained in 2017, only a part of immature ticks, mainly *Hyalomma* spp. and *Ixodes* spp., were identified by molecular tools in the following 2 years. The specimens to be molecularly processed were selected by choosing one tick from each bird (given we usually observed that only one genus of tick occurs on one a same bird), considering half of the total number of parasitized birds, in accordance with the distribution of catches during the ringing period. In addition, all specimens of dubious identification were molecularly analyzed for species determination.

Ticks were homogenized using Tissue-lyser (Qiagen, Germany) and nucleic acids were extracted with Maxwell 16 LEV simply RNA blood kit (Promega, USA) for specimens of 2017 and with BioSprint 96 One-For-All Vet Kit (Qiagen) for all the following ones, according to each manufacturer's protocol. These kits allow to purify both DNA and RNA. Quality control was performed by the Nanodrop spectrophotometer ND1000 (ThermoFisher Scientific, USA). Different molecular markers were amplified namely 12S, 16S, 18S, COI and TROSPA, depending on the tick genus examined (Table 1). A small region of the 12S rRNA was amplified and sequenced to identify the species (Toma et al. 2014) according to previously described procedures (Beati and Keirans 2001). Moreover, for the characterization of the species belonging to *Ixodes* genus, mitochondrial 16S rRNA was used. The molecular identification of *Ixodes inopinatus* specimens was obtained by using 16S molecular target and confirmed by sequencing of partial region of TROSPA gene, as described in Noureddine et al. (2011). Occasionally, 18S and cytochrome c oxidase subunit 1 (COI) genes were characterized.

All PCR products were sent to Eurofins Genomics Company (Germany) for sequencing. The identified representative sequences were submitted to GenBank. The accession numbers are as follows: MW173319-MW173322 and MW173326-173331 (*Ixodes frontalis*), MW173332-MW173335 and MW173340-MW173341 (*Ixodes ricinus*), MW173359-MW173366, MW173465-MW173482 and MW175443 (*Ixodes ventalloi*), MW173342 (*Ixodes inopinatus*), MW175438-MW175442 (*Hyalomma rufipes*), MW175434-MW175437 and MW172439 (*Hyalomma marginatum*), MW172502 (*Hyalomma truncatum*), MW171249 (*Haemaphysalis punctata*), MW172218 (*Rhipicephalus pusillus*), and MW172416 (*Rhipicephalus sanguineus*).

Table 1 Molecular markers employed in this study

Tick genus or species	Target gene	Primers	Sequence (5′-3′)	References
Ixodes,	16S	16S-F	CCGGTCTGAACTCAGATCAAGT	Black and Piesman (1994)
Hyalomma,		16S-R	GCTCAATGATTTTTTAAATTGCTGT	
Rhipicephalus,				
Haemaphysalis,				
Argas,				
Amblyomma				
Hy. rufipes,	12S	T2A	AAAGAGTGACGGGCGATATGT	Beati and Keirans (2001)
Hy. marginatum,		T1B	AAACTAGGATTAGATACCCT	
Haemaphysalis				
Ix. ricinus/inopinatus	TROSPA	Trospa-F2	TATGGACACGGCGTCGCTGTC	Noureddine et al. (2011)
		Trospa-R2	GCCCAAGCGCATAAATAAGAAGCGG	
Ix. frontalis/ventalloi,	COI	HCO1490	GGTCAACAAATCATAAAGATATTGG	Folmer et al. (1994)
Amblyomma		LCO2198	TAAACTTCAGGGTGACCAAAAATCA	
Argas	18S	18S-A	AACCTGGTTGATCCTGCCA	Mangold et al. (1998)

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Results

Morphological species identification

Most ticks were collected on Ventotene (n = 2040) where we found ticks belonging to five genera but in particular to the genus *Hyalomma* (n = 1623; 79.6%) and *Ixodes* (n = 396; 19.4%) (Table 2). On Ustica (n = 145) and Asinara (n = 159), ticks sampled on birds belonged only to the genera *Hyalomma* and *Ixodes*, whereas on Asinara most of the sample was represented by *Ixodes* (n = 116; 73%), which is different from the other two islands (Table 2).

In 2017 and 2018, in total 337 ticks were collected by dragging on the vegetation of Ustica (n = 167) and Asinara (n = 170), mainly belonging to *Ixodes* and *Rhipicephalus* genera, as summarized in Table 3. No ticks were found on the vegetation on Ventotene.

Molecular species identification

For a complete view of the species composition, all ticks collected in 2017 were molecularly analyzed and successfully sequenced (Tables 3 and 4). In 2018 we performed the molecular identification of 243 specimens (21.3%) out of the annual sample. Within this subsample 191 ticks were from Ventotene, 16 (20.5%) from Ustica, and 36 (50%) from Asinara. In 2019 we proceeded with the molecular identification of 125 specimens (25.7%) of the total sample of the year. *Hyalomma* ticks collected in Ventotene during the 3 years (Table 3) showed that they were constituted by predominant *Hy. rufipes* (n = 409) followed by *Hy. marginatum* (n = 93), and *Hy. truncatum* (n = 2). The 156 *Ixodes* were identified as *Ix. frontalis* (n = 117), *Ix. ventalloi* (n = 22), *Ix. ricinus* (n = 14), and *Ix. inopinatus* (n = 3). In addition, one nymph of *Hae. punctata* and two larvae of *Argas vulgaris* were identified along with two females of *Haemaphysalis erinacei*, confirming for this last species the morphological determination.

From 2017 to 2019, 14 immature specimens of *Amblyomma* were found (Table 2). All these specimens were molecularly analyzed using four molecular markers (16S, 18S, COI and 12S). Only 12S was informative by identifying one specimen of *Amblyomma variegatum* collected in 2017, showing 100% identity with sequences (MH781752 and MF668118) available in GenBank (Table 3). The remaining specimens showed an identity ranging from 100 to 99.1% with an *Amblyomma* sp. A-sequence deposited in GenBank (KC817413). At Ustica the total number of ticks collected on migratory birds during 2017 and 2018 were molecularly identified as *Hy. rufipes* (n = 51), *Hy. marginatum* (n = 3), *Ix. frontalis* (n = 9), *Ix. ricinus* (n = 1), and *Ix. ventalloi* (n = 1) (Table 3). On this island, the total of 167 adult ticks caught on the vegetation were *Hy. marginatum* (n = 17), *Ix. ventalloi* (n = 82), *Rh. pusillus* (n = 27), *Rh. sanguineus* (n = 7), and *Rhipicephalus turanicus* (n = 34) (Table 4).

At Asinara the total number of ticks collected from migratory birds during 2017 and 2018 were *Hy. rufipes* (n = 29), *Hy. marginatum* (n = 2), *Ix. ricinus* (n = 19), and *Ix. frontalis* (n = 7) (Table 3). On this island, 170 adult ticks belonging to the following species were caught on the vegetation: *Hy. marginatum* (n = 1), *Rhipicephalus bursa* (n = 119), *Rh. sanguineus* (n = 16), and *Rh. turanicus* (n = 33) (Table 4).

In total 2681 ticks were collected during the three field seasons, out of which 2344 were sampled from birds and 337 were caught from the vegetation (Tables 2 and 4). Overall, ticks were found on 1079 birds of 43 species. Among infested individuals, 806 (74.7%)

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Table 2 Morphological identification of the ticks collected on birds on the three islands during the whole study period

Species	Ventotene					Ustica					Asinara				
	Larvae	Nymphs	Adults	Total	%	Larvae	Nymphs	Adults	Total	%	Larvae	Nymphs	Adults	Total	%
Hyalomma sp.	540	1082	1	1623	79.6	22	108	0	130	89.7	4	39	0	43	27.0
Ixodes sp.	241	117	38	396	19.4	8	7	0	15	10.3	94	22	0	116	73.0
Amblyomma sp.	6	8	0	14	0.7	0	0	0	0	0.0	0	0	0	0	0.0
Haemaphysalis sp.	1	2	2	5	0.2	0	0	0	0	0.0	0	0	0	0	0.0
Argas sp.	2	0	0	2	0.1	0	0	0	0	0.0	0	0	0	0	0.0
Total	791	1209	48	2040	100	30	115	0	145	100	98	61	0	159	100

Table 3 Molecular identification of the ticks collected on birds on the three islands in 2017 (19–30 March), 2018 (8 March – 29 May), and 2019 (9 April – 30 May)

Year	Species	Ventotene					Ustica					Asinara				
		Larvae	Nymphs	Adults	Total	%	Larvae	Nymphs	Adults	Total	%	Larvae	Nymphs	Adults	Total	%
2017	*Hyalomma rufipes*	46	155	0	201	32.5	8	28	0	36	50.7	2	19	0	21	80.8
	Hyalomma marginatum	21	66	1	88	14.2	0	3	0	3	4.2	0	0	0	0	0.0
	Hyalomma ND	47	50	0	97	15.7	2	16	0	18	25.4	0	0	0	0	0.0
	Ixodes frontalis	43	41	5	89	14.4	2	6	1	9	12.7	2	0	0	2	7.7
	Ixodes ventalloi	1	5	6	12	1.9	0	0	0	0	0.0	0	0	0	0	0.0
	Ixodes ricinus	0	7	0	7	1.1	0	1	0	1	1.4	0	1	0	1	3.8
	Ixodes ND	118	2	0	120	19.4	4	0	0	4	5.6	2	0	0	2	7.7
	Amblyomma ND	0	3	0	3	0.5	0	0	0	0	0.0	0	0	0	0	0.0
	Amblyomma variegatum	0	1	0	1	0.2	0	0	0	0	0.0	0	0	0	0	0.0
Total		276	330	12	618	100	16	54	1	71	100	6	20	0	26	100
2018	*Hyalomma rufipes*	39	73	0	112	58.6	3	12	0	15	93.8	2	6	0	8	22.2
	Hyalomma marginatum	2	2	0	4	2.1	0	0	0	0	0.0	0	2	0	2	5.6
	Hyalomma truncatum	0	2	0	2	1.0	0	0	0	0	0.0	0	0	0	0	0.0
	Hyalomma ND	10	13	0	23	12.0	0	0	0	0	0.0	0	1	0	1	2.8
	Ixodes frontalis	10	8	0	18	9.4	0	0	0	0	0.0	5	0	0	5	13.9
	Ixodes inopinatus	0	3	0	3	1.6	0	0	0	0	0.0	0	0	0	0	0.0
	Ixodes ventalloi	1	5	0	6	3.1	1	0	0	1	6.3	0	0	0	0	0.0
	Ixodes ricinus	0	2	0	2	1.0	0	0	0	0	0.0	14	4	0	18	50.0
	Ixodes ND	6	5	0	11	5.8	0	0	0	0	0.0	2	0	0	2	5.6
	Amblyomma ND	6	2	0	8	4.2	0	0	0	0	0.0	0	0	0	0	0.0
	Argas vulgaris	2	0	0	2	1.0	0	0	0	0	0.0	0	0	0	0	0.0
Total		76	115	0	191	100	4	12	0	16	100	0	0	0	36	100

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Table 3 (continued)

Year	Species	Ventotene					Ustica					Asinara				
		Larvae	Nymphs	Adults	Total	%	Larvae	Nymphs	Adults	Total	%	Larvae	Nymphs	Adults	Total	%
2019	*Hyalomma rufipes*	20	76	0	96	76.8										
	Hyalomma marginatum	1	0	0	1	0.8										
	Ixodes frontalis	6	3	1	10	8.0										
	Ixodes ventalloi	0	4	0	4	3.2										
	Ixodes ricinus	0	5	0	5	4.0										
	Ixodes ND	2	2	0	4	3.2										
	Amblyomma ND	0	2	0	2	1.6										
	Haemaphysalis ND	0	1	1	2	1.6										
	Haemaphysalis punctata	0	1	0	1	0.8										
Total		29	94	2	125	100										

Percentages are based on the whole numbers of analyzed specimens; 'ND' indicates the specimens for which the molecular analyses failed

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Table 4 Species composition of the ticks collected on the vegetation in Ustica and Asinara in 2017 and 2018

Year	Species	Ustica					Asinara				
		Larvae	Nymphs	Adults	Total	%	Larvae	Nymphs	Adults	Total	%
2017	*Hyalomma marginatum*	0	0	13	13	14.3	0	0	1	1	0.6
	Ixodes ventalloi	0	35	11	46	50.5	0	0	0	0	0.0
	Rhipicephalus sanguineus	0	0	3	3	3.3	0	0	16	16	9.6
	Rhipicephalus turanicus	0	0	21	21	23.1	0	0	33	33	19.8
	Rhipicephalus pusillus	0	0	8	8	8.8	0	0	0	0	0.0
	Rhipicephalus bursa	0	0	0	0	0.0	0	0	117	117	70.1
Total		0	35	56	91	100	0	0	167	167	100
2018	*Hyalomma marginatum*	0	0	4	4	5.3	0	0	0	0	0.0
	Ixodes ventalloi	0	24	12	36	48.0	0	0	0	0	0.0
	Rhipicephalus sanguineus	0	0	4	4	5.3	0	0	0	0	0.0
	Rhipicephalus turanicus	0	0	13	13	17.3	0	0	0	0	0.0
	Rhipicephalus pusillus	0	0	19	19	25.3	0	0	0	0	0.0
	Rhipicephalus bursa	0	0	0	0	0.0	0	0	0	2	66.7
	Haemaphysalis sulcata	0	0	0	0	0.0	0	0	0	1	33.3
Total		0	24	52	76	100	0	0	0	3	100

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belonging to 26 species were long-distance migrants, carrying 1728 (64.5%) ticks, whereas 266 (24.7%) birds belonging to 14 species were short-distance migrants carrying 611 (22.8%) ticks. Only seven birds (0.65%) belonging to three resident species were found parasitized by one tick each (0.26%).

In total, 16 tick species were identified: nine were exclusively picked up on birds (*Hy. rufipes* (43.3%), *Hy. truncatum* (0.1%), *Ix. frontalis* (11.8%), *Ix. inopinatus* (0.2%), *Ix. ricinus* (3%), *Hae. punctata* (0.08%), *Hae. erinacei* (0.1%), *Am. variegatum* (0.08%) and *Ar. vulgaris* 0.1%); five were exclusively collected on the vegetation—*Rh. bursa* (10.5%), *Rh. turanicus* (5.9%), *Rh. sanguineus* (2%), *Rh. pusillus* (2.4%), and *Hae. sulcata* (0.08%)–, whereas *Hy. marginatum* (10.3%) and *Ix. ventalloi* (9.3%) were found both on birds and on the vegetation in Ustica.

Discussion

This study allowed to identify ticks carried by birds belonging to the genera *Hyalomma*, *Ixodes*, *Amblyomma* and *Haemaphysalis*. The vast majority of individuals was represented by immature specimens of *Hyalomma* spp. (namely *Hy. rufipes* and *Hy. marginatum*), with *Hy. rufipes* being the most abundant species detected. The highest prevalence of this tick species, mainly diffused in sub-Saharan regions, is representative of the respective abundance of long-distance migrants among infested birds.

As widely known, the status of *Hyalomma* genus has been debated for years because of the difficulties in the identification of species within the subgenus *Euhyalomma* (Apanaskevich and Horak 2008). One of the most difficult groups from the taxonomic perspective is the *Hy. marginatum* species complex. This was considered consisting of a single and extremely polymorphic species, *Hy. marginatum*, with a distribution almost coincident with that of the entire genus, ranging from southern Europe, to Asia Minor, Arabia, central, southern and (to a limited extent) continental South-east Asia, and Africa. It was generally accepted that this species contains four subspecies, with a fifth taxon belonging to this group recently re-established as a full species in South Africa (Estrada-Peña et al. 2018). Currently, *Hy. marginatum* complex is formed by species in the Palaearctic region, namely *Hy. marginatum*, *Hy. rufipes* and *Hy. turanicum* (Estrada-Peña et al. 2018). *Hyalomma rufipes*, vector of CCHFV in humans, is widely distributed in most of Africa, being more common in drier areas; it also occasionally occurs in southern Europe and extends eastwards to central Asia, possibly introduced by migratory birds (Walker et al. 2003). Adults are generally found on cows, but also on sheep, horses and wild ungulates, whereas immature stages feed on hares as well as on vegetation-nesting birds.

Another exotic *Hyalomma* tick recorded in this study is *Hy. truncatum* with two nymphs detected on a trans-Saharan Common redstart (*Phoenicurus phoenicurus*). Although recorded in northern Sudan and Egypt infesting cattle, goats and horses, *Hy. truncatum* is endemic to the Afrotropical zoogeographical region (Walker et al. 2003). From 2017 to 2019, 14 specimens of *Amblyomma* (six larvae and eight nymphs) were found on birds on Ventotene and molecularly analyzed. One nymph collected on the trans-Saharan Tree pipit (*Anthus trivialis*) in 2017 on Ventotene was identified as *Am. variegatum* by comparing 12S sequence with those available in GenBank. Although sporadic findings in the Mediterranean region, this detection is not an infrequent event (Cicculli et al. 2019; Pintore et al. 2021), confirming how important migratory birds are in dispersing this species and genus. In a previous survey carried out on Ventotene in 2014, five unidentified *Amblyomma* ticks

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were found on four trans-Saharan migratory birds (Whinchat, Pied flycatcher, Spotted flycatcher and Common whitethroat) and molecularly analyzed for 12S, showing two haplotypes, called A and B (KC817413 and KC817417) (Toma et al. 2014). Also in this study, it was not possible to achieve species identification for the other 13 *Amblyomma* specimens, even using various molecular tools. However, the low 12S sequence variability and the high identity (99.1–100%) with KC817413 and KC817417, suggest the presence of a single species of *Amblyomma*. Similarly, in a further study carried out in Ventotene Island on migratory birds (Pascucci et al. 2019), one *Am. variegatum* nymph and four unspecified *Amblyomma* nymphs were collected on migrants.

Another exotic species of tick found in this study is represented by a specimen of *Argas* showing a high percentage of identity with *Ar. vulgaris*, a soft tick very common in Russia and Asia (Dusbabek 1976) very close to *Argas delicatus*, and so far both not reported for the European tick fauna. Currently, both names—*Ar. vulgaris* and *Ar. delicatus* – are considered valid (Guglielmone et al. 2009, 2010) until the types can be compared in the light of further data. As the genomic resources for the different *Argas* species are very scarce and ancient (Dabert et al. 1999), in general the systematics of the *Argas* genus, and especially of the '*reflexus* group', should be considered with caution.

For the first time in Italy, this species (two larvae) was collected from a Black redstart and an Icterine warbler on Ventotene in 2018, so indicating a passive transportation from long distances. Soft ticks have rarely been detected on migratory birds, because after the blood feeding these ticks drop off the host and remain close to the bird nest until the next meal. Studies on tick-bird systems have mainly focused on land birds and data about soft-ticks carried by migratory birds are limited to sea birds (Dietrich et al. 2011).

Haemaphysalis erinacei and *Hae. punctata* were collected on Black redstart (*Phoenicurus ochruros*) in 2017 and in 2019, respectively. The first species is an endophilic tick and has a three-host lifecycle, with hedgehogs, foxes, martens and weasels known to be suitable natural hosts for both its immature and mature forms (Petney et al. 2012; Liu et al. 2018; Orkun et al. 2019). Undetermined remain the two specimens of *Haemaphysalis* sp. found in 2019 on two trans-Saharan migrants, a Tree pipit (*Anthus trivialis*) and a Western yellow wagtail *(Motacilla flava*), showing only a 94% identity with 12S sequence from *Haemaphysalis bancrofti*, the Australian wallaby tick (Barker and Walker 2014).

Four *Ixodes* species belonging to the Italian and European fauna were found in this study: *Ix. frontalis*, *Ix. ricinus*, *Ix. inopinatus* and *Ix. ventalloi*, collected also on the vegetation, especially on Ustica. Known as 'rabbit tick', mainly parasitizing the European rabbit (*Oryctolagus cuniculus*), *Ix. ventalloi* is poorly known in its natural history, despite being implicated as a vector of pathogens (e.g., *Anaplasma phagocytophilum*, *Rickettsia helvetica*, *Rickettsia monacensis*, *Bartonella clarridgeiae* and Eyach virus) (Chastel et al. 1984; Santos-Silva et al. 2006; Marquez 2008; Hubálek and Rudolf 2012; Otranto et al. 2014; Pennisi et al. 2015). This tick may occasionally feed on other hosts including dogs, cats, ground-dwelling birds and humans (Santos Dias and Santos-Reis 1989; Santos-Silva et al. 2011; Otranto et al. 2014; Pennisi et al. 2015). The geographical distribution of *Ix. ventalloi* includes areas of western Mediterranean Europe (Portugal, Spain, southern France, central and southern Italy, and Cyprus) and northern Africa (Morocco and Tunisia) (Estrada-Peña et al 2018). This tick species was also documented in Great Britain (Jameson and Medlock 2011; Gillingham et al. 2020) and southwestern Germany (Petney et al. 2012). Despite being considered a species with limited potential for dispersal, *Ix. ventalloi* may actively seek for hosts at the vegetation, as confirmed by Torina et al. (2018) and our findings on Ustica, where the species may have been carried by birds. This species, not reported before for this island, represented

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about half of the sample collected on the vegetation in 2017 (50.5%) and 2018 (48%). The specimens molecularly analyzed in this study showed the same genetic profile of *Ix. ventalloi* reported by Latrofa et al. (2017).

Worthy of note is the finding, on Ventotene in 2018, of three nymphs molecularly identified as *Ix. inopinatus*, collected on a Hawfinch (*Coccothraustes coccothraustes*) and a Robin (*Erithacus rubecula*), both intra-Palearctic species. This species recently described is present in Spain and North Africa and appears morphologically very similar to *Ix. ricinus*, of which it was considered to be a form adapted to dry environments (Estrada-Peña et al. 2014). Currently *Ix. inopinatus* replaces *Ix. ricinus* in dry areas of the Mediterranean region in Spain, Portugal, Morocco, Algeria and Tunisia. This species is allopatric with *Ix. ricinus* in Spain and Portugal. It is hypothesized that it has been historically overlooked and reported as *Ix. ricinus* at least in northern Africa, southern Spain, parts of south-western Portugal in the southwestern German federal state Rhineland-Palatinate (Estrada-Peña et al. 2014). Recently, this species was found in sympatry with *Ix. ricinus* in Romania and western Austria, in the southern German federal state Bavaria (Chitimia-Dobler et al. 2018) and in in the northern German cities of Hanover and Hamburg (Hauck et al. 2019). Specific surveys made on rodents and birds at every site of collection of *Ix. inopinatus* lead to think that adults feed on these hosts whereas immatures might be restricted to lizards; differences in host-association and seasonal activity have also been recorded between the populations of northern Africa and Europe (Estrada-Peña et al. 2014).

During our study, 11 immature *Ix. ricinus* were also collected on birds. The area of distribution of this species includes a variety of environments in Alpine, Boreal, Nemoral, Continental, Atlantic, Lusitanian and Mediterranean domains (Estrada-Peña and Venzal 2006). 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 general interest in improving the knowledge of its distribution in relation with a wide range of environmental conditions.

The ornithophilic tick *Ix. frontalis* is an abundant species in Europe (Estrada-Peña et al. 2017), even if it has been poorly investigated because of the small size (difficult to detect) of the free stages (Heylen et al. 2014; Heylen et al. 2016), until in 2019 a study by Agulon et al. deeply investigated its phenology. This study carried out in western France reports a differential phenology observed in the *Ix. frontalis* life stages: nymphs and adults are sporadically present on the vegetation throughout the year, whereas larvae show a distinct peak of activity in October–November, being completely absent in summer (Agoulon et al. 2019). *Ixodes frontalis* is commonly found feeding on a great diversity of bird species that are shared with *Ix. ricinus* (Iori et al. 2005; Doby 1998; Martyn 1988; Monks et al. 2006; Norte et al. 2012) and it has also been exceptionally reported to bite humans (Gilot et al. 1997; Cull et al. 2018). In the present study *Ix. frontalis* was found on all islands, but the majority of specimens were collected on Ventotene from Robins. This bird follows a predominantly south-west to north-east spring migration route when moving from the Mediterranean region towards Central Europe. Based on the analysis of 16S rDNA, we detected two genetic lineages of *Ix. frontalis*, being around 50% each, confirming previous studies (Hornok et al. 2016; Charrier et al. 2019).

An *Ixodes* nymph found in 2019 on Ventotene, parasitizing a Whinchat (*Saxicola rubetra*), was genetically analyzed using 12S target, showing 92% identity with *Ixodes brunneus.* A similar homology was found in 2014, in an *Ixodes* tick collected on a European Robin (*E. rubecula*), on the same island (Toma et al. 2014). In this context it should be emphasized that *Ix. brunneus* is only known as a neartic species. However, other species of the *Trichotoixodes* subgenus, to which the species belongs, are known from Africa but

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for which no sequences are available in GenBank (such as *Ix. daveyi*, *Ix. theileriae* or *Ix. domerguei* from Madagascar).

The analysis of ticks collected around the ringing sites provided specimens only on Asinara and Ustica, whereas no ticks were sampled on Ventotene, likely as a consequence of the scarcity of possible hosts on the island. On Ustica, where *Ix. ventalloi* was the most abundant species collected on the vegetation, also *Hyalomma* and *Rhipicephalus* species were found. *Hyalomma marginatum* was collected on the island as questing adults in 2017 and 2018. *Rhipicephalus pusillus* was found both on birds 2017 and 2018, with about 24% of the total samples, confirming the presence of this species on the island (Manilla 1998) where it was probably introduced together with the European rabbit as its favorite host. *Rhipicephalus turanicus* and *Rh. sanguineus* sensu lato were also found almost with the same abundance of *Rh. pusillus*. *Rhipicephalus bursa* was found in 70% of the samples collected in Ustica in 2017, whereas it was not recorded in 2018. On Asinara, the environment is different from Ustica, as the island is a natural reserve, wild and uninhabited and the cattle (sheep, goats and donkeys) are free to roam around. Here in 2017 we found *Rh. bursa* (70%) and *Rh. turanicus* to be the most frequent species on the vegetation, followed by *Rh. sanguineus* and *Hy. marginatum*. We did not find ticks on Asinara in 2018, with the exception of two males of *Rh. bursa* and one male of *Hae. sulcata*, another species common on ovine, goats and cattle in such environment. This is a possible consequence of the rainy and windy climate that determined a general decrease of the sampled specimens on both islands, and especially on Asinara. Obviously, as some species found on the vegetation were also collected on the caught birds, we cannot exclude that some of these were get on the birds during the stop on the studied islands.

Interestingly, no exotic ticks were sampled during our field activities. Although the surveys on the islands were not carried out in the months after the migration events, they allow to collect specimens on the vegetation during the ringing activities from March to May period in which the molting by ticks dropping off the birds was likely. It is reasonable to think that the establishment of a new tick species in an area, although considered a rare event, is made possible when it can feed on livestock, as previously observed in the UK (Jameson and Medlock 2011), Hungary (Hornok and Horváth 2012) and in the Czech Republic (Hubálek et al. 2020) for *Hy. rufipes*. This hypothesis is also supported by the record of an adult of *Am. variegatum* in Sicily in 1971 (Albanese et al. 1971), the recent reports of an adult of males *Am. variegatum* in Sardinia (Pintore et al. 2021) and Corsica (Cicculli et al. 2019) and the very recent one of *Hy. marginatum* and *Hy. rufipes* found on humans and on animals in Sweden (Grandi et al. 2020). These records reported in various studies carried out in Europe, show that ticks hosted by migratory birds incoming from Africa, or however from the southern Mediterranean basin, can arrive molting in the next stage after feeding during the days spent in migration. These data confirm this aspect is worth of investigation also within the perspective of ongoing climate change.

Conclusion

Our data confirm that migratory birds disperse ticks from Africa into Europe along their migratory routes. We observed the incoming of African tick species both in high numbers, as in the case of *Hy. rufipes*, as well as small but constant numbers, as in the case of *Amblyomma.* Moreover, the finding of two larvae of the exotic soft tick *Ar. vulgaris*, belonging to a genus scarcely reported on land birds but frequently observed on seabirds (Dietrich et al.

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2011), lead us to speculate on the complex role of various species of migrants in tick dispersal. The search for ticks on the vegetation at the ringing sites did not detect the presence of alien species. In general, the species collected around the ringing stations both on Ustica and Asinara are known to be thermophilic, with a great affinity for Mediterranean habitats and are common in Italy, even if the record of all stages of *Ix. ventalloi* in the environment is not so frequently reported.

Further investigations are necessary to better define the role of migratory birds in the introduction and circulation of tick-borne diseases in Italy and in other countries of the Mediterranean basin, above all after the evidence of imported ticks positive to CCHF virus.

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Compliance with ethical standards

Conflict of interest The Authors declare they have no conflicts of interest.

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