

**Supplementary table 1.** Transects where tick samplings were performed in the Natural Protected Area of Doñana. Dates in which each transect was sampled is marked in grey.

Transect*	Province	Municipal boundary	Area	Coordinate		Habitat	Sampling dates						
				X	Y		S-1 Apr-18	S-2 May-18	S-3 Aug-18	S-4 Oct-18	S-5 Nov-18	S-6 Feb-19	S-7 Mar-19
D1	Huelva	Almonte	Laguna de las pajas	181187	4106049	Grassland on the shore of the lake							
D2	Huelva	Almonte	Laguna huerto Bejarano	180510	4106893	The shore of the lake with Mediterranean shrub (rockrose, rosemary, lentisk, etc.)							
D3	Huelva	Bonares	Arboreto del Villar I	173551	4126174	Cork oak and Mediterranean shrub (rockrose, rosemary, lentisk, etc.)							
D4	Huelva	Bonares	Arboreto del Villar II	173085	4126562	Cork oak and Mediterranean shrub (rockrose, rosemary, lentisk, etc.)							
D5	Huelva	Almonte	Laguna de Santa Olalla	190034	4098488	Grassland on the shore of the lake and scrubs of sage-leaved rockrose							
D6	Huelva	Almonte	El Puntal-Helechal	193599	4098535	Fern under a cork oak							
D7	Huelva	Hinojos	Casa del Pichiricha (Coto del Rey)	193272	4114582	Grassland							
D8	Huelva	Hinojos	Raya de los Álamos	195412	4114499	Cork oak and Mediterranean shrub (rockrose, rosemary, lentisk, etc.)							
D9	Huelva	Almonte	Rocina Laguna	188825	4114833	Pine forest on the shore of the lake with fens and scrubs							
D10	Huelva	Almonte	Sendero Acebrón	185082	4116876	Cork oak, Mediterranean shrub and grassland with fan palms							
D11	Huelva	Almonte	Toril de Manecorro	190510	4113701	Grassland close to the lake							
D12	Huelva	Almonte	Casa Hato Villa	191804	4111984	Grassland at the edge of marshes							
D13	Huelva	Almonte	El Guaperal zona de acampada	191484	4109023	Grassland, scrub and eucalyptus							
D14	Huelva	Almonte	Toril Casa del Puntal	194058	4097193	Grassland							
D15	Huelva	Hinojos	Cuevas de Conejos.	193897	4113834	Cork oak forest and Mediterranean shrub							

\* Neighbouring areas (buffer areas) were sampled in August (n=3; D4, D6 and D8), October (n=9; D1-2, D4, D6-9 and D12-13), November (n=4; D4-7), February (n=3; D5, D7 and D10) and March (n=5; D3-D7).

**Supplementary table 2.** Transects where tick samplings were performed in the Natural Protected Area of Los Alcornocales (South Spain). Dates in which each transect was sampled is marked in grey.

Transect*	Province	Municipal boundary	Area	Coordinate		Habitat	Sampling dates							
				X	Y		S-1 Apr-18	S-2 Jun-18	S-3 Aug-18	S-4 Oct-18	S-5 Dec-18	S-6 Feb-19	S-7 Mar-19	
A1	Cádiz	Alcalá de los Gazules	Cañadas de los ratones. Conejeras	254254	4032969	Grassland								
A2	Cádiz	Alcalá de los Gazules	La Peguera	262887	4027813	Grassland at the edge of the Mediterranean scrub								
A3	Cádiz	Alcalá de los Gazules	Finca Buenas Noches	267824	4030159	Grassland at the edge of the Mediterranean scrub (rockrose, rosemary, lentisk, etc.)								
A4	Cádiz	Alcalá de los Gazules	Pradera en la Cañana de los ratones	254227	4032857	Grassland								
A5	Cádiz	Los Barrios	Pantano Monte La Torre	275161	4003948	Grassland on the shore of the lake								
A6	Cádiz	Los Barrios	Cañada Real Botafuegos-Jaramillo	271244	4010225	Grassland at the edge of the Mediterranean scrub (rockrose, rosemary, lentisk, etc.)								
A7	Cádiz	Los Barrios	Sendero de Valdeinfierno	266752	4012397	Cork oak forest and Mediterranean scrub (rockrose, heather, etc.)								
A8	Cádiz	Los Barrios	La Teja	268097	4018499	Grassland and Mediterranean scrub								
A9	Málaga	Cortes de la Frontera	Cerca de PerezGil	268478	4046307	"Bujeo" grasslands								
A10	Cádiz	Algar	Carril Finca la Atalaya	266809	4061435	"Bujeo" grasslands next to riverside								
A11	Cádiz	Jerez de la Frontera	Arroyo entrada Montes de Jerez	268611	4054374	Riverside meadows								
A12	Cádiz	Algar	Garganta Peñalpargate	266687	4061715	"Bujeo" grasslands next to riverside								
A13	Cádiz	Alcalá de los Gazules	Praderas con casas subiendo a Buenas Noches	265239	4030360	Grassland and cork oak forest								
A14	Málaga	Cortes de la Frontera	Patio de Corcha	273954	4043661	Grassland at the edge of the Mediterranean scrub								
A15	Cádiz	Jerez de la Frontera	Finca Rojitan, Embalse de los Hurones.	270293	4058386	River valley forests								
A16	Cádiz	Alcalá de los Gazules	CR Corzo, el Picacho.	262942	4046108	Cork oak forest and Mediterranean scrub (rockrose, heather, etc.)								

\*Neighbouring areas (buffer areas) were sampled in August (n=5; A5-6, A8-9 and A14), October (n=3; A9, A11 and A14), December (n=3; A9, A11 and A13) and February (n=4; A5-7 and A13).

**Supplementary table 3:** PCR primer pairs and conditions used in this study for the screening of tick-borne microorganisms.

Organisms	Target gene	Primer sequence (5' → 3')	Fragment size (bp)	References
Ticks	16S rRNA	F:CTGCTCAATGATTTTTTAAATTGCTGTGG R:CCGGTCTGAACTCAGATCAAGT	456	[1]
	TROSPA	F: GTAAACATCGGCCTAATGG R: GGAAAAAATAATGTTAAAAACACC F: GTTGTTACAGCGAATACT R: GAAAAAATATTAGAACATTAACACTC	362* 233†	[2]
<b>Borrelia spp.</b>	<i>flaB</i> (Lyme group)	F1: AARGAATTGGCAGTTCAATC R1: GCATTTTCWATTTTAGCAAGTGATG	497	[3]
		F2: ACATATTCAGATGCAGACAGAGGTTCTA R2: GAAGGTGCTGTAGCAGGTGCTGGCTGT	389	[4]
	<i>g/pQ</i> (Relapsing fever group)	F1: ATGGGTTCAAACAAAAAGTCACC R1: CATTACTGTGTGTCAGTAAAATCTGTAATATACCATCTAC	920	[5]
		F2: ATGGGTTCAAACAAAAAGTCACC R2: CCAGGGTCCAATTCCATCAGAATATTGTGCAAC	700	[6]
<b>Rickettsia spp.</b>	<i>ompA</i> (Spotted fever group)	F1: ATGGCGAATATTTCTCCAAAA R1: GTTCCGTTAATGGCAGCATCT F2: ATGGCGAATATTTCTCCAAAA R2: AGTGCAGCATTGCTCCCCCT	631 532	[7,8]
		<i>ompB</i> †	F1: GTAACCGGAAGTAATCGTTTCGTAA R1: GCTTTATAACCAGCTAAACCACC F2: GTTTAATACGTGCTGCTAACCAA R2: GGTTCGGCCATATACCATAAG	420 230
<b>Anaplasma / Ehrlichia / Neoehrlichia spp.</b>	groESL	F1: AITGGGCTGGTAITGAAAT R1: CCICCGIACIAIACCTTC F2: ATWGCWAARGAAGCATAGTC R2: CTCAACAGCAGCTCTAGTAGC	1350 1297	[10]
<b>Anaplasma phagocytophilum</b>	msp2	F1:CCAGCGTTTAGCAAGATAAGAG R1:GCCCAGTAACAACATCATAAGC	380	[11]
<b>Ehrlichia spp.</b> ‡	16S rRNA	F1: GAACGAACGCTGGCGGCAAGC R1: AGTAYCGRACCAGATAGCCGC	696	[12,13]
	<i>gltA</i>	F1:ATGTCTACTGCTGCTTGTGA R1: ATGACCAGTATATAACTGACGTG F2:ATGTCTACTGCTGCTTGTGA R2: GAGCAGACCAACCAGATGTTC	1098 1030	[13,14]
<b>Crimean-Congo haemorrhagic fever virus</b> ‡	S segment	F1: TCTCAAAGAAACACGTGCC R1: CCTTTTTGAACTCTTCAAACC Probe: ACTCAAGKAACTGTGGGCGTAAG	122	[15]
		F1: TTGTGTTCCAGATGGCCAGC R1: CTTAAGGCTGCCGTGTTTGC F2: GAAGCAACCAARTTCTGTGC R2: AAACCTATGTCTTCTCTCC	307 221	[16]
		F1: AATGTGCCGAACCTGGACAG R1: TGCGACAAGTGCAATCCCG F2: ATCAGATGGCCAGTGCAACC R2: ACTCCCTGCACCACTCAATG	593 469	[16]

F1: AARGGAAATGGACTTRTGGGA		[17]
R1: GCAAGGCCTGTWGCACAAAGTGC	605	
F2: TGGAYACYTTACAAACTCC		
R2: GCAAGGCCTGTWGCACAAAGTGC	621	

bp: base pairs; \* amplicon size corresponding to *Ixodes ricinus*; †amplicon size corresponding to *Ixodes inopinatus*; ‡Only performed to selected samples

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**Supplementary table 4.** Number of ticks captured in each transect in the Natural Protected Areas of Doñana and Los Alcornocales from April 2018 to

March 2019.

Transect <sup>1</sup>	<i>D. marginatus</i>		<i>H. punctata</i>				<i>Hy. lusitanicum</i>				<i>Hy. marginatum</i>		<i>I. acuminatus</i>				<i>I. ricinus</i>		<i>Rh. annulatus</i>		<i>Rh. bursa</i>			<i>Rh. pusillus</i>		<i>Rh. sanguineus</i>	
	M	F	L	N	M	F	L	N	M	F	L	F	M	N	M	F	L	N	L	M	F	M	F	M	F		
D1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	22	0	0	0	0	0	0	0	1	1	
D2	0	0	0	0	0	0	0	2	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	
D3	6	7	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	4	
D4	0	1	0	0	0	0	0	44	5	8	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1		
D5	0	3	0	6	0	1	21	11	4	0	33	0	0	0	0	0	0	0	24	5	2	1	0	3	1		
D6	0	0	0	1	0	0	0	3	0	0	0	0	0	0	0	0	74	0	0	11	4	0	0	2	0		
D7	8	10	0	0	0	0	0	0	5	4	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0		
D8	0	0	0	0	0	0	142	39	5	4	0	0	0	0	1	0	29	1	0	0	0	0	0	0	0		
D9	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	2		
D10	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0		
D11	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	28	0	0	0	0	0	0	1	0		
D12	0	0	0	0	0	0	0	0	3	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
D13	0	0	0	1	0	0	159	1	0	0	0	0	0	0	0	0	28	0	0	1	0	0	0	0	2		
D14	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0		
D15	2	1	0	0	0	0	0	1	2	2	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0		
FD1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0		
FD2	0	0	0	0	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
FD3	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0		
FD4	0	0	0	0	0	0	0	0	3	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
FD5	3	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	46	0	0	0	0	0	0		
FD6	0	0	0	1	0	1	18	5	0	0	2	0	0	0	0	0	1	0	0	2	1	0	0	0	1		
FD7	9	11	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
FD8	0	0	0	0	0	0	31	68	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
FD9	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
FD10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0		
FD12	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
FD13	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		

Transect <sup>1</sup>	<i>D. marginatus</i>		<i>H. punctata</i>				<i>Hy. lusitanicum</i>				<i>Hy. marginatum</i>		<i>I. acuminatus</i>				<i>I. ricinus</i>		<i>Rh. annulatus</i>		<i>Rh. bursa</i>			<i>Rh. pusillus</i>		<i>Rh. sanguineus</i>	
	M	F	L	N	M	F	L	N	M	F	L	F	M	N	M	F	L	N	L	M	F	M	F	M	F		
A1	0	1	0	0	0	0	0	17	30	19	0	0	0	0	0	0	0	0	0	0	0	2	6	0	0		
A2	1	0	0	2	0	0	0	10	13	11	0	0	0	0	1	1	0	0	26	3	1	0	0	0	0		
A3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
A4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0		
A5	0	1	0	0	0	0	2	0	0	0	0	0	1	0	0	0	0	0	21	0	0	0	0	4	3		
A6	0	1	0	0	0	0	31	9	10	3	0	0	0	0	1	0	0	1	2	1	0	0	3	4			
A7	0	0	143	0	1	1	22	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0			
A8	0	0	176	3	1	0	2	5	2	1	0	0	0	0	0	0	0	2	5	3	0	0	1	1			
A9	0	0	0	0	1	0	213	4	0	1	0	0	0	0	0	4	0	28	1	1	0	1	0	0			
A10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0			
A11	0	0	0	0	0	0	63	2	1	0	0	0	0	0	0	3	0	4	0	0	0	0	3	4			
A12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0			
A13	0	0	0	0	0	0	236	6	0	1	0	0	0	0	0	0	0	0	5	11	0	0	0	1			
A14	0	0	0	0	0	0	152	20	6	5	0	0	0	0	0	0	0	32	1	4	0	0	1	3			
A15	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0			
A16	0	0	0	49	26	17	1223	37	280	258	0	0	0	0	1	0	0	0	0	0	0	0	0	0			
FA5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	1	0	0	0	0	2			
FA6	0	0	0	0	1	0	0	0	1	5	0	0	0	0	0	0	0	2	0	0	0	0	0	0			
FA7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0			
FA8	0	0	0	0	0	0	33	0	0	0	0	0	0	0	0	0	0	0	2	1	0	0	0	0			
FA9	0	0	0	0	1	0	0	53	0	0	0	0	0	0	0	0	0	37	0	0	0	0	0	0			
FA11	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	3	0	8	0	0	0	0	0	0			
FA13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	60	0	0	0	0	0	0			
FA14	0	0	0	0	0	0	15	14	0	0	0	0	0	0	0	91	0	0	9	8	0	0	0	0			

<sup>1</sup>D1-D15: Transects in the Natural Protected Area of Doñana; FD: Buffer areas in the NPAD; A1-A16: Transects in the Natural Protected Area of Los Alcornocales; FA: Buffer areas in the NPAA; *D.*: *Dermacentor*; *H.*: *Haemaphysalis*; *Hy.*: *Hyalomma*; *I.*: *Ixodes*; *Rh.*: *Rhipicephalus*; L: Larvae; N: Nymph; M: male; F: Female

**Supplementary table 5.** Number of ticks captured in each transect in April 2018 in the Natural Protected Areas of Doñana and Los Alcornocales.

Transect1	D. marginatus		H. punctata				Hy. lusitanicum				Hy. marginatum		I. acuminatus	I. ricinus				Rh. annulatus		Rh. bursa			Rh. pusillus		Rh. sanguineus	
	M	F	L	N	M	F	L	N	M	F	L	F	M	N	M	F	L	N	L	M	F	M	F	M	F	
D1											1														1	1
D2																										
D3	2	4																							1	2
D4										1	1															
D5		1								1															1	
D6																										
D7																										
D8																										
D9																1										1
D10	1																								1	
D11																										
D12										2	2															
D13																										2
D14																										
D15																										
A1										14	7													5		
A2																										
A3																										
A4																										
A5		1																								
A6																									2	2
A7					1	1									1										1	
A8					1																					
A9																										
A10																										
A11																										
A12																										
A13																										
A14																										

<sup>1</sup>D1-D15: Transects in the Natural Protected Area of Doñana; A1-A16: Transects in the Natural Protected Area of Los Alcornocales ; *D.*: *Dermacentor*; *H.*: *Haemaphysalis*; *Hy.*: *Hyalomma*; *I.*: *Ixodes*; *Rh.*: *Rhipicephalus*; L: Larvae; N: Nymph; M: male; F: Female

**Supplementary table 6.** Number of ticks captured in each transect in May 2018 in the Natural Protected Area of Doñana and in June in the Natural Protected Area of Los Alcornocales.

Transect <sup>1</sup>	<i>D. marginatus</i>		<i>H. punctata</i>				<i>Hy. lusitanicum</i>				<i>Hy. marginatum</i>		<i>I. acuminatus</i>			<i>I. ricinus</i>			<i>Rh. annulatus</i>		<i>Rh. bursa</i>			<i>Rh. pusillus</i>		<i>Rh. sanguineus</i>	
	M	F	L	N	M	F	L	N	M	F	L	F	M	N	M	F	L	N	L	M	F	M	F	M	F		
D1																											
D2											1																
D3	2	2									1															1	
D4										4	3													1	1		
D5										2										3	2	1		1	1		
D6																					1			2			
D7										5	4										1						
D8										2	1						29										
D9																										2	
D10																											
D11										1	1													1			
D12										1	1																
D13																					1						
D14										1											1						
D15											1										1						
A1										6	3											1					
A2										12	9																
A4																									1		
A5																								4	3		
A6										8	3													1	1		
A7			143																								
A8			169				1		2	1				1						3				1	1		
A9											1										1			1			
A11									1															3	4		
A12																											
A13																				1	2					1	

<sup>1</sup>D1-D15: Transects in the Natural Protected Area of Doñana; A1-A16: Transects in the Natural Protected Area of Los Alcornocales; *D.*: *Dermacentor*; *H.*: *Haemaphysalis*; *Hy.*: *Hyalomma*; *I.*: *Ixodes*; *Rh.*: *Rhipicephalus*; L: Larvae; N: Nymph; M: male; F: Female

**Supplementary table 7.** Number of ticks captured in each transect in August 2018 in the Natural Protected Areas of Doñana and Los Alcornocales.

Transect <sup>1</sup>	<i>D. marginatus</i>		<i>H. punctata</i>				<i>Hy. lusitanicum</i>				<i>Hy. marginatum</i>		<i>I. acuminatus</i>				<i>I. ricinus</i>		<i>Rh. annulatus</i>		<i>Rh. bursa</i>			<i>Rh. pusillus</i>		<i>Rh. sanguineus</i>	
	M	F	L	N	M	F	L	N	M	F	L	F	M	N	M	F	L	N	L	M	F	M	F	M	F		
D1																	22								1	1	
D2								2									1										
D3																									1	4	
D4								3		3															1	1	
D5				6			21	11												2					3	1	
D6				1				3											11	3					2		
D7																							1				
D8							142	39	1	3								1									
D9																									1	2	
D10																									1		
D11																	28								1		
D12																											
D13				1			159	1									20									2	
D15									2	1																	
FD4																											
FD6				1			18	4												2	1						
FD8							31	67	2	2															1		
A1								17	7	7												1	1				
A2				1				7		1										3							
A5																										1	
A6									1											2	1						
A7							22																				
A8			7	3				2											2	2	3						
A9							213													1							
A11							62	1																			
A13							236			1										4	9						
A14							152														2						
A16							1067	8	134	147																	
FA5																				1							

Transect <sup>1</sup>	<i>D. marginatus</i>		<i>H. punctata</i>				<i>Hy. lusitanicum</i>				<i>Hy. marginatum</i>		<i>I. acuminatus</i>			<i>I. ricinus</i>			<i>Rh. annulatus</i>		<i>Rh. bursa</i>			<i>Rh. pusillus</i>		<i>Rh. sanguineus</i>	
	M	F	L	N	M	F	L	N	M	F	L	F	M	N	M	F	L	N	L	M	F	M	F	M	F		
FA6									1	5														1			
FA8							33													2	1			4	3		
FA9																								3	4		
FA14							15													9	8			1			

<sup>1</sup>D1-D15: Transects in the Natural Protected Area of Doñana; FD: Buffer areas in Doñana; A1-A16: Transects in the Natural Protected Area of Los Alcornocales; FA: Buffer areas in Alcornocales

*D.*: *Dermacentor*; *H.*: *Haemaphysalis*; *Hy.*: *Hyalomma*; *I.*: *Ixodes*; *Rh.*: *Rhipicephalus*; L: Larvae; N: Nymph; M: male; F: Female

**Supplementary table 8.** Number of ticks captured in each transect in October 2018 in the Natural Protected Areas of Doñana and Los Alcornocales.

Transect <sup>1</sup>	<i>D. marginatus</i>		<i>H. punctata</i>				<i>Hy. lusitanicum</i>				<i>Hy. marginatum</i>		<i>I. acuminatus</i>	<i>I. ricinus</i>				<i>Rh. annulatus</i>		<i>Rh. bursa</i>			<i>Rh. pusillus</i>		<i>Rh. sanguineus</i>	
	M	F	L	N	M	F	L	N	M	F	L	F	M	N	M	F	L	N	L	M	F	M	F	M	F	
D1																									1	1
D2																									0	0
D3																									1	4
D4								41		1															1	1
D5											33														3	1
D6																	74								2	0
D7																									0	0
D8									1																0	0
D9																									1	2
D10																									1	0
D11																									1	0
D12																									0	0
D13																									0	2
D15								1																	0	0
FD1																	1								0	0
FD2								5																	0	0
FD4									3	2															0	0
FD6								1			2						1								1	0
FD7								1																	0	0
FD8								1																	0	0
FD9								1		1															0	1
FD12									1																0	0
FD13								1																	0	0
A1									3	1															0	0
A2				1				3		1											1				0	0
A5							2																		0	0
A6							31	9																	0	0
A7								1																	0	0

Transect <sup>1</sup>	<i>D. marginatus</i>		<i>H. punctata</i>				<i>Hy. lusitanicum</i>				<i>Hy. marginatum</i>		<i>I. acuminatus</i>	<i>I. ricinus</i>			<i>Rh. annulatus</i>		<i>Rh. bursa</i>			<i>Rh. pusillus</i>		<i>Rh. sanguineus</i>	
	M	F	L	N	M	F	L	N	M	F	L	F	M	N	M	F	L	N	L	M	F	M	F	M	F
A8							1	3																0	0
A9								4									4							0	0
A11							1	1									3							1	0
A13								6																4	3
A14								20																3	4
A15				1				1																1	0
A16				10			156	29	104	83														1	1
FA9								51																0	0
FA11								2									3							0	0
FA14								14									91							3	4

<sup>1</sup>D1-D15: Transects in the Natural Protected Area of Doñana; FD: Buffer areas in Doñana; A1-A16: Transects in the Natural Protected Area of Los Alcornocales; FA: Buffer areas in Alcornocales; *D.*: *Dermacentor*; *H.*: *Haemaphysalis*; *Hy.*: *Hyalomma*; *I.*: *Ixodes*; *Rh.*: *Rhipicephalus*; L: Larvae; N: Nymph; M: male; F: Female

**Supplementary table 9.** Number of ticks captured in each transect in November 2018 in the Natural Protected Area of Doñana and in December 2018 in the Natural Protected Area of Los Alcornocales.

Transect <sup>1</sup>	<i>D. marginatus</i>		<i>H. punctata</i>				<i>Hy. lusitanicum</i>				<i>Hy. marginatum</i>		<i>I. acuminatus</i>	<i>I. ricinus</i>			<i>Rh. annulatus</i>		<i>Rh. bursa</i>			<i>Rh. pusillus</i>		<i>Rh. sanguineus</i>	
	M	F	L	N	M	F	L	N	M	F	L	F	M	N	M	F	L	N	L	M	F	M	F	M	F
D1																									
D2																									
D3																									
D4		1																							
D5		2				1													24						
D6																									
D7	8	9																							
D8																									
D9																									
D10																									
D11																									
D12																									
D13																									
D15	2	1																							
FD4										1															
FD5		1																	46						
FD6						1																			
FD7	4	6																							
A1																									
A2															1				26						
A5																			7						
A6																									
A7																									
A8																									
A9					1														28						
A11																			4						
A13																									

Transect <sup>1</sup>	<i>D. marginatus</i>		<i>H. punctata</i>				<i>Hy. lusitanicum</i>				<i>Hy. marginatum</i>		<i>I. acuminatus</i>	<i>I. ricinus</i>			<i>Rh. annulatus</i>		<i>Rh. bursa</i>			<i>Rh. pusillus</i>		<i>Rh. sanguineus</i>	
	M	F	L	N	M	F	L	N	M	F	L	F	M	N	M	F	L	N	L	M	F	M	F	M	F
A14																			5						
A15																									
A16				6	8	4			21	14				1											
FA9					1			2											37						
FA11																			8						
FA13																			57						

<sup>1</sup>D1-D15: Transects in the Natural Protected Area of Doñana; FD: Buffer areas in Doñana; A1-A16: Transects in the Natural Protected Area of Los Alcornocales; FA: Buffer areas in Alcornocales; *D.*: *Dermacentor*; *H.*: *Haemaphysalis*; *Hy.*: *Hyalomma*; *I.*: *Ixodes*; *Rh.*: *Rhipicephalus*; L: Larvae; N: Nymph; M: male; F: Female

**Supplementary table 10.** Number of ticks captured in each transect in February 2019 in the Natural Protected Areas of Doñana and Los Alcornocales.

Transect <sup>1</sup>	<i>D. marginatus</i>		<i>H. punctata</i>				<i>Hy. lusitanicum</i>				<i>Hy. marginatum</i>		<i>I. acuminatus</i>	<i>I. ricinus</i>			<i>Rh. annulatus</i>		<i>Rh. bursa</i>			<i>Rh. pusillus</i>		<i>Rh. sanguineus</i>			
	M	F	L	N	M	F	L	N	M	F	L	F	M	N	M	F	L	N	L	M	F	M	F	M	F		
D1																											
D2																											
D3	1	1																									
D4																											
D5																											
D6																											
D7		1																									
D8															1												
D9																											
D10																											
D11																											
D12																											
D13																	8										
D15																											
FD5	3	2																									
FD7	5	5																									
FD10															1												
A1		1																									
A2															1												
A5													1						14								
A6																		1									
A7																											
A8																											
A9																											
A11																											
A13																											
A14																			27								
A15																											

Transect <sup>1</sup>	<i>D. marginatus</i>		<i>H. punctata</i>				<i>Hy. lusitanicum</i>				<i>Hy. marginatum</i>		<i>I. acuminatus</i>	<i>I. ricinus</i>			<i>Rh. annulatus</i>		<i>Rh. bursa</i>			<i>Rh. pusillus</i>		<i>Rh. sanguineus</i>	
	M	F	L	N	M	F	L	N	M	F	L	F	M	N	M	F	L	N	L	M	F	M	F	M	F
A16				33	18	13			21	14															
FA5																			6						
FA6																			2						
FA7					1																				
FA13																			3						

<sup>1</sup>D1-D15: Transects in the Natural Protected Area of Doñana; FD: Buffer areas in Doñana; A1-A16: Transects in the Natural Protected Area of Los Alcornocales; FA: Buffer areas in Alcornocales;  
*D.*: *Dermacentor*; *H.*: *Haemaphysalis*; *Hy.*: *Hyalomma*; *I.*: *Ixodes*; *Rh.*: *Rhipicephalus*; L: Larvae; N: Nymph; M: male; F: Female

**Supplementary table 11.** Number of ticks captured in each transect in March 2019 in the Natural Protected Areas of Doñana and Los Alcornocales.

Transect <sup>1</sup>	<i>D. marginatus</i>		<i>H. punctata</i>				<i>Hy. lusitanicum</i>				<i>Hy. marginatum</i>		<i>I. acuminatus</i>	<i>I. ricinus</i>			<i>Rh. annulatus</i>		<i>Rh. bursa</i>			<i>Rh. pusillus</i>		<i>Rh. sanguineus</i>		
	M	F	L	N	M	F	L	N	M	F	L	F	M	N	M	F	L	N	L	M	F	M	F	M	F	
D1																										
D2																										
D3	1																									1
D4																										
D5									1																1	
D6																										
D7																										
D8									1																	
D9																										
D10																										
D11																										
D12																										
D13																										
D15																										
FD3	1																								1	
FD4										1																
FD5		1																								
FD6																										1
FD7									1																	
A1										1																
A2	1								1																	
A5																										
A6		1							1						1											1
A7																										
A8																										
A9																										
A11																										
A13																										
A14																										

Transect <sup>1</sup>	<i>D. marginatus</i>		<i>H. punctata</i>				<i>Hy. lusitanicum</i>				<i>Hy. marginatum</i>		<i>I. acuminatus</i>	<i>I. ricinus</i>			<i>Rh. annulatus</i>		<i>Rh. bursa</i>			<i>Rh. pusillus</i>		<i>Rh. sanguineus</i>		
	M	F	L	N	M	F	L	N	M	F	L	F	M	N	M	F	L	N	L	M	F	M	F	M	F	
A15																										
A16																										

<sup>1</sup>D1-D15: Transects in the Natural Protected Area of Doñana; FD: Buffer areas in Doñana; A1-A16: Transects in the Natural Protected Area of Los Alcornocales; FA: Buffer areas in Alcornocales;

*D.*: *Dermacentor*; *H.*: *Haemaphysalis*; *Hy.*: *Hyalomma*; *I.*: *Ixodes*; *Rh.*: *Rhipicephalus*; L: Larvae; N: Nymph; M: male; F: Female

**Supplementary table 12.** Tick densities in each transect from April 2018 to March 2019 in the Natural Protected Areas of Doñana and Los Alcornocales.

Transect <sup>1</sup>	Apr-18	May-18 Jun-18	Aug-18	Oct-18	Nov-18 Dec-18	Feb-19	Mar-19	Total No. of ticks	No. of samplings	Nº. of ticks per 100 m <sup>2</sup>
D1	3	0	22	0	0	0	0	25	7	3.6
D2	0	1	3	0	0	0	0	4	7	0.6
D3	9	6	0	0	0	2	2	19	7	2.7
D4	2	9	6	42	1	0	0	60	7	8.6
D5	3	10	40	33	27	0	2	115	7	16.4
D6	0	3	18	74	0	0	0	95	7	13.6
D7	0	10	1	0	17	1	0	29	7	4.1
D8	0	32	186	1	0	1	1	221	7	31.6
D9	2	2	0	0	0	0	0	4	7	0.6
D10	2	0	0	0	0	0	0	2	7	0.3
D11	0	3	28	0	0	0	0	31	7	4.4
D12	4	2	0	0	0	0	0	6	7	0.9
D13	2	1	181	0	0	8	0	192	7	27.4
D14	0	2						2	2	1.0
D15	0	2	3	1	3	0	0	9	7	1.3
FD1				1				1	1	1.0
FD2				5				5	1	5.0
FD3							2	2	1	2.0
FD4			0	5	1		1	7	4	1.8
FD5					47	5	1	53	3	17.7
FD6			26	4	1		1	32	4	8.0
FD7				1	10	10	1	22	4	5.5
FD8			102	1				103	2	51.5
FD9				2				2	1	2.0
FD10						1		1	1	1.0
FD12				1				1	1	1.0

Transect <sup>1</sup>	Apr-18	May-18 Jun-18	Aug-18	Oct-18	Nov-18 Dec-18	Feb-19	Mar-19	Total No. of ticks	No. of samplings	Nº. of ticks per 100 m <sup>2</sup>
FD13				1				1	1	1.0
A1	26	10	33	4		1	1	75	7	10.7
A2	0	21	12	6	27	1	2	69	7	9.9
A3	0							0	1	0.0
A4	0	1						1	2	0.5
A5	1	7	0	2	7	15	0	32	7	4.6
A6	4	13	4	40	0	1	4	66	7	9.4
A7	4	143	22	1	0	0	0	170	7	24.3
A8	1	179	19	4	0	0	0	203	7	29.0
A9	0	3	214	8	29	0	0	254	7	36.3
A10	0							0	1	0.0
A11	0	8	63	5	4	0	0	80	7	11.4
A12	0	0						0	2	0.0
A13	0	4	250	6	0	0	0	260	7	37.1
A14	0	18	154	20	5	27	0	224	7	32.0
A15				2	0	0	0	2	4	0.5
A16			1356	382	54	99	0	1891	5	378.2
FA5			3			6	0	9	3	3.0
FA6			6			2	0	8	3	2.7
FA7						1	0	1	2	0.5
FA8			36				0	36	2	18.0
FA9			0	51	40		0	91	4	22.8
FA11				5	8		0	13	3	4.3
FA13					57	3	0	60	2	30.0
FA14			32	105			0	137	3	45.7

<sup>1</sup>AD1-D15: Transects in the Natural Protected Area of Doñana; FD: Buffer areas in Doñana; A1-A16: Transects in the Natural Protected Area of Los Alcornocales; FA: Buffer areas in Alcornocales

**Supplementary table 13.** Maximum Identities of the sequences obtained in this study comparing with public sequences of GenBank.

Organisms	Target genes	Species	No. of sequences	GenBank acc. No.	Identities			
					bp	%	GenBank Acc. No.	QC
Ticks	16S rRNA	<i>Haemaphysalis punctata</i>	1	PZ060376	387/388	99.7	MF002566*	100
			3	PZ060377	404/404	100	MG021187*	100
		<i>Hyalomma lusitanicum</i>	26	PZ060378	402/402	100	ON366982	100
			1		402/403	99.8	ON366982*	100
				PZ060379	392/392	100	LC508317	97
			1	PZ060380	401/403	99.5	ON366982*	100
			1	PZ060381	401/403	99.5	ON366982*	100
		<i>Hyalomma marginatum</i>	1	PZ060382	403/404	99.8	MW366630*	100
			2	PZ060383	403/403	100	MW366630*	100
		<i>Ixodes acuminatus</i>	1	PZ060384	407/409	99.5	PQ144653	100
		<i>Ixodes ricinus/inopinatus</i>	1	PZ060385	406/407	100	GU074598*	100
			1	PZ060386	390/390	100	OP375394*	100
		<i>Rhipicephalus annulatus</i>	4	PZ060387	402/403	99.8	L34311*	100
		<i>Rhipicephalus bursa</i>	2	PZ060388	401/401	100	OR773535*	100
<i>Rhipicephalus pusillus</i>	1	PZ060389	405/408	99.3	OL454855*	100		
<i>Rhipicephalus sanguineus sensu stricto</i>	2	PZ060390	384/384	100	OR946410	100		
	2	PZ060391	412/412	100	JX997393*	100		
<b>Borrelia</b>	<i>flaB</i>	<i>Borrelia lusitaniae</i>	6	PZ112783	453/453 391/391	100	CP132468	100
<b>Rickettsia</b>	<i>ompA</i>	<i>Candidatus Rickettsia rioja</i>	39	PZ112789	488/488	100	GQ404429*	100
			2	PZ112790	486/488	99.6	GQ404429*	100
			2	PZ112791	487/488	99.8	GQ404429*	100
		<i>Rickettsia aeschlimannii</i>	2	PZ112792	588/588	100	HQ335157*	100
		<i>Rickettsia barbariae</i>	4	PZ112793	583/583 488/488	100	MK028340*	100
			<i>Rickettsia massiliae</i>	16	PZ112794	590/590 490/490	100	CP000683*
		2		PZ112795	448/448	100	CP003319*	100
		<i>Rickettsia monacensis</i>	5	PZ112796	598/603 590/590	100	LN794217* PP176516	100 98

Organisms	Target genes	Species	No. of sequences	GenBank acc. No.	Identities				
					bp	%	GenBank Acc. No.	QC	
		<i>Rickettsia raoultii</i>	1	PZ112797	587/587	100	AH015609	100	
		<i>Rickettsia slovaca</i>	14	PZ112798	588/588 491/491	100	CP002428	100	
	<i>ompB</i>	<i>R. barbariae</i>	3	PZ112799	382/382	100	MK028339*		
		<i>R. massiliae</i>	2	PZ112800	483/483	100	CP000683*	100	
			3	PZ112801	483/483	100	CP003319*	100	
	<i>Sca4</i>	<i>R. barbariae</i>	2	PZ112802	803/803	100	PP954858*	100	
<b><i>Ehrlichia</i></b>	<i>groESL</i>		5	PZ112784	1308/1310	99.9	KJ930191	98	
			2		PZ112785	1089/1089 705/705	100	OQ185227 KJ930193*	82 100
			4	PZ059407	659/659	100	AF414399*	100	
	16S rRNA		4						
	<i>gltA</i>			4	PZ112786	920/920	100	KX987353*	100
				2	PZ112787	848/920	92.2	AF311965	100
1				PZ112788	646/647	99.9	OQ185264*	100	

\* among others