Zootaxa 5719 (1): 049–072
https://www.mapress.com/zt/

Article

ISSN 1175-5326 (print edition)
ZOOTAXA
ISSN 1175-5334 (online edition)

https://doi.org/10.11646/zootaxa.5719.1.2
http://zoobank.org/urn:lsid:zoobank.org:pub:83CFF8D5-9CB4-4631-8366-3F981F647E19

Description of all stages of a new tick species from California, *Haemaphysalis vespertina* (Acari: Ixodidae), with redescription of *H. leporispalustris* Packard, 1869 adults and phylogenetic relationships among related U.S. taxa

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Abstract

All active stages of *Haemaphysalis vespertina* **sp. nov. (**Acari: Ixodidae), a tick previously identified as *H. leporispalustris* Packard, 1869, are described from specimens collected on the vegetation and from leporids in California and Oregon. The adults of *H. leporispalustris* Packard, 1969 are redescribed based on type material. Adults of the two species can be distinguished by their overall size, the dorsal shape of palpal segment II, the number and shape of dorsal and ventral setae on palpal segment II, the number of spurs on coxae II, the length of setae on scutum, legs and coxae, and the pattern of

Accepted by O. Seeman: 2 Oct. 2025; published: 11 Nov. 2025

scutal punctations. Phylogenetic analyses support *H. vespertina* as a distinct taxonomic lineage. Additional unresolved lineages within *H. leporispalustris* s.l. were identified, suggesting a need for further taxonomic study of leporid-associated *Haemaphysalis* ticks in North America.

Key words: *Haemaphysalis*, rabbit tick, United States, new species

Introduction

While the genus *Haemaphysalis* Koch 1844 is the 2nd most species-rich genus of all hard ticks (Acari: Ixodidae) (Guglielmone *et al.*, 2020), only five of its species are native to the New World: *Haemaphysalis leporispalustris* Packard, 1869; *H. chordeilis* Packard, 1869; *H. juxtakochi* Cooley, 1946; *H. mariae* Apanaskevich, 2024; and *H. cinnabarina* Koch, 1844, the latter known only from two specimens collected in Brazil more than a century ago (Barros-Battesti *et al.*, 2008). A sixth congener, *H. longicornis* Neumann, 1901, is an invasive species that established populations in the eastern United States sometime prior to 2017 (Beard *et al.,* 2018; Rainey *et al.*, 2018).

Historically, *H. leporispalustris* has been regarded as widely distributed throughout North, Central and South America, from Alaska to Argentina (Guglielmone *et al.*, 2021; Lindquist *et al.*, 2016). Adults are near exclusive parasites of leporids, chiefly *Sylvilagus* Gray, 1867 and *Lepus* L. spp., while immatures can be found on a wider variety of hosts including ground-feeding birds and, rarely, mammals (Cooley, 1946; Merino, 1967; Mertins *et al.*, 1992; Wells *et al.*, 2004). The tick is also often carried by migratory birds reaching the Gulf Coast from Central or South America (Karim *et al.*, 2024; Mukherjee *et al.*, 2014). Though not a frequent human biter (Eisen, 2022; Guglielmone & Robbins, 2018), *H. leporispalustris* has been shown to carry the causative agent of tularemia (Parker *et al.*, 1952; Philip & Parker, 1938) and the "Hlp" strain of *Rickettsia rickettsii* recently found to be pathogenic for humans (Karpathy *et al.*, 2007; Paddock *et al.*, 2014; Parker *et al.*, 1951; Philip *et al.*, 1978). It has also been proposed as an enzootic amplifier of human pathogens such as *R. rickettsii* and *Borrelia burgdorferi* (Lane & Burgdorfer, 1988; Parker *et al.*, 1951). In Central and South America, *H. leporispalustris* is more directly linked to pathogenic *R. rickettsii* strains (Freitas *et al.*, 2009; Fuentes *et al.*, 1985; Hun *et al.*, 2008).

After *H. longicornis* invaded the U.S., to promote rapid identification of this new species among human and animal health practitioners, a pictorial key was developed to differentiate it from native *Haemaphysalis* taxa (Egizi *et al.*, 2019). The key used the number and shape of ventral setae on palpal segment II to distinguish nymphal *H. leporispalustris* from *H. juxtakochi* as proposed by Kohls (1960) and Fairchild *et al.* (1966). However, subsequent observations soon revealed morphological variation in this character among *H. leporispalustris* nymphs, which triggered further investigation.

Colloquially known as the rabbit tick, *H. leporispalustris* was first described as *Ixodes leporis-palustris* based on a single female collected from a marsh rabbit, *Sylvilagus palustris* (Bachman 1837), at the time called *Lepus palustris* (Packard, 1869). The type locality was Fort Macon, North Carolina, the collection date February 1869, and the collector given as Dr. E. Coues. The description is fragmentary and does not include any illustrations. In the same publication, Packard also described *H. chordeilis.* The morphological characters included in the two narratives were, unfortunately, not distinct enough to be considered diagnostic. The rabbit tick was renamed *Haemaphysalis leporis* by Neumann (1897), who provided the first complete description of the female and that of the male, based on samples that had been collected from a variety of locations in Texas, Kansas, California, Mexico, and oddly, Timor. The reasons for the name change were not stated, but Neumann confirmed that he was referring to *H. leporis-palustris.* He vaguely described the nymphal and larval stages. In his opinion, *Gonixodes rostralis* Dugès 1888 and *H. chordeilis* were also synonyms of *H. leporis*. Dugès' illustrations of *G. rostralis* included an eyeless female, which he considered to be a male, that undoubtedly belongs to the genus *Haemaphysalis* because of the morphological features of the capitulum; however, the illustration also showed 17 festoons (Dugès, 1888). The other illustrations of *G. rostralis* (nymph and female) appear to refer to *Ixodes* and/or *Amblyomma* ticks. Banks (1908), redescribed the male and female of *Haemaphysalis leporis-palustris* reverting to the original name. The specimens he examined for his description and illustrations came from Texas, Virginia, Louisiana, Arizona, California, and New York. He also reinstated *H. chordeilis* as a valid species. Hunter & Hooker (1907) and Hooker *et al.* (1912) further described this species based on samples collected in the western part of the U.S. Additional detailed illustrations of all stages are available in Nuttall & Warburton (1915) who examined samples from Canada, Texas and California, but based their illustrations mainly on Texas specimens. The hyphen in *leporis-palustris* was later dropped following the taxonomic code of nomenclature, although the name without the hyphen can be found in the literature as early as in Fairchild (1966).

Our bibliographic search revealed that, while the type locality of *H. leporispalustris* is in North Carolina, the following descriptions of adults and immature stages were based on specimens collected from mixed localities, but mostly from the western half of the U.S. In particular immature stages were illustrated based on samples of unclear geographic origin (Cooley, 1946), collected either in Texas (Nuttall & Warburton, 1915) or California (Furman & Loomis, 1984; Kleinjan & Lane, 2008) and, only in one instance, from the Atlantic states (Clifford *et al.,* 1961). These descriptions might have corresponded to *H. leporispalustris*, to the newly described *H. mariae* (Apanaskevich, 2024), or to a yet to be discovered species within what we can now justifiably call, *H. leporispalustris* sensu lato (s.l.). The extensive variation, at least in size, within this group of ticks was further emphasized by the work of Thomas (1968), a morphometric study of adult and larval specimens across the U.S. Thomas (1968) revealed important variance between U.S. populations, a clear overall decrease in size going from East to West, and an increase in variance in populations containing ticks collected from migrating birds. While he dealt with quantitative features, he did not try to describe any of the qualitative fixed morphological characters coinciding with the morphometric differences.

In order to bring some clarity to the taxonomic status of this group of ticks, we used mitochondrial and nuclear gene sequences to reconstruct the phylogenetic relationships within *H. leporispalustris* s.l. based on specimens collected from across the U.S. The deep split between a clade from California and the remaining *H. leporispalustris* s.l. indicated that the lineage represented a new species, which was confirmed by subsequent morphological reassessment. In this study, we describe all life stages of the new species. We also redescribe the adults of *H. leporispalustris* sensu stricto based on type material, as the original description of Packard (1869) was fragmentary at best. A formal description of *H. leporispalustris* sensu stricto will facilitate future taxonomic study in this group, where growing recognition of cryptic diversity has led to the identification of additional distinct species.

Materials and methods

Sampling for molecular analyses

Specimens sourced from across the US and Canada are listed in Table 1 and their geographical origin illustrated in Figure 1. As the recent description of *H. mariae* (Apanaskevich, 2024) did not include molecular data, we also obtained specimens of *H. mariae* from Texas, USA and included them in the phylogenetic analysis. Sequences of *H. juxtakochi* from Central America (Panama) were used as an outgroup.

DNA extraction

Specimens were extracted following a nondestructive procedure (Beati *et al.*, 2012; Beati & Keirans, 2001) where a small cut is made in the posterolateral abdomen before placing the tick in 180 µl Buffer ATL and 20 µl Proteinase K (Qiagen Inc., Valencia, CA) and incubating overnight at 56°C. After incubation, the exoskeleton is removed and returned to an ethanol-filled cryovial for preservation. The remainder of the extraction follows the manufacturer's protocol for the Qiagen DNeasy Blood & Tissue Kit except for the elution step, where two successive elutions with 25 µl hot (72°C) Buffer AE are performed in the same tube for a final elution volume of 50 µl (30 µl for larvae).

PCR amplification and sequencing

The PCR amplification of 3 mitochondrial (cytochrome c oxidase I, *cox1*; small mitochondrial subunit, *12SrDNA*; large mitochondrial subunit, *16SrDNA*) and one nuclear (Internal Transcribed Spacer 2, *ITS2*) gene sequences was attempted for each specimen. All PCRs were performed with 12.5 µl of AmpliTaq Gold 360 Master Mix (Life Technologies), in a 25µl reaction volume with 1 µl each of 10 µM primers, 0.375µl Bovine Serum Albumin (BSA, 10mg/ml) and 1 µl template DNA. *Cox1* and *ITS2* amplifications mixes were supplemented with 1.6µl MgCl2 (20 mM). Primers LCO1490 and HCO2198 were used to amplify a 680 bp fragment of *cox1* (Folmer *et al.*, 1994), primers 16S+1 and 16S-1 for a 460 bp portion of *16SrDNA* (Black & Piesman, 1994); primers T1B and T2A (Beati & Keirans, 2001) for 360 bp of *12SrDNA*, and F2LITS2 and McLn (Beati *et al.*, 2013; McLain *et al.*, 1995) for 950 bp of *ITS2.* Annealing conditions were: 50°C for 0:30 for *cox1*; at 54°C for 0:30 for 16SrDNA; touchdown over 8

cycles decreasing from 60°C to 50°C, followed by 25 cycles at 50°C for 0:35 for *12SrDNA*; and touchdown over 8 cycles decreasing from 65°C to 54°C followed by 25 additional cycles at 53°C for 0:30 for *ITS2*. All positive PCR products were purified with ExoSAP-IT (USB Corporation, Cleveland, OH) and Sanger sequenced in both directions (Genewiz, South Plainfield, New Jersey). Sequences were quality trimmed and assembled in Geneious 10.2.3 (Kearse *et al.*, 2012) and the consensus sequence for each sample was used in phylogenetic analyses. The list of sequences generated in this study and their GenBank accession numbers are in Table 1.

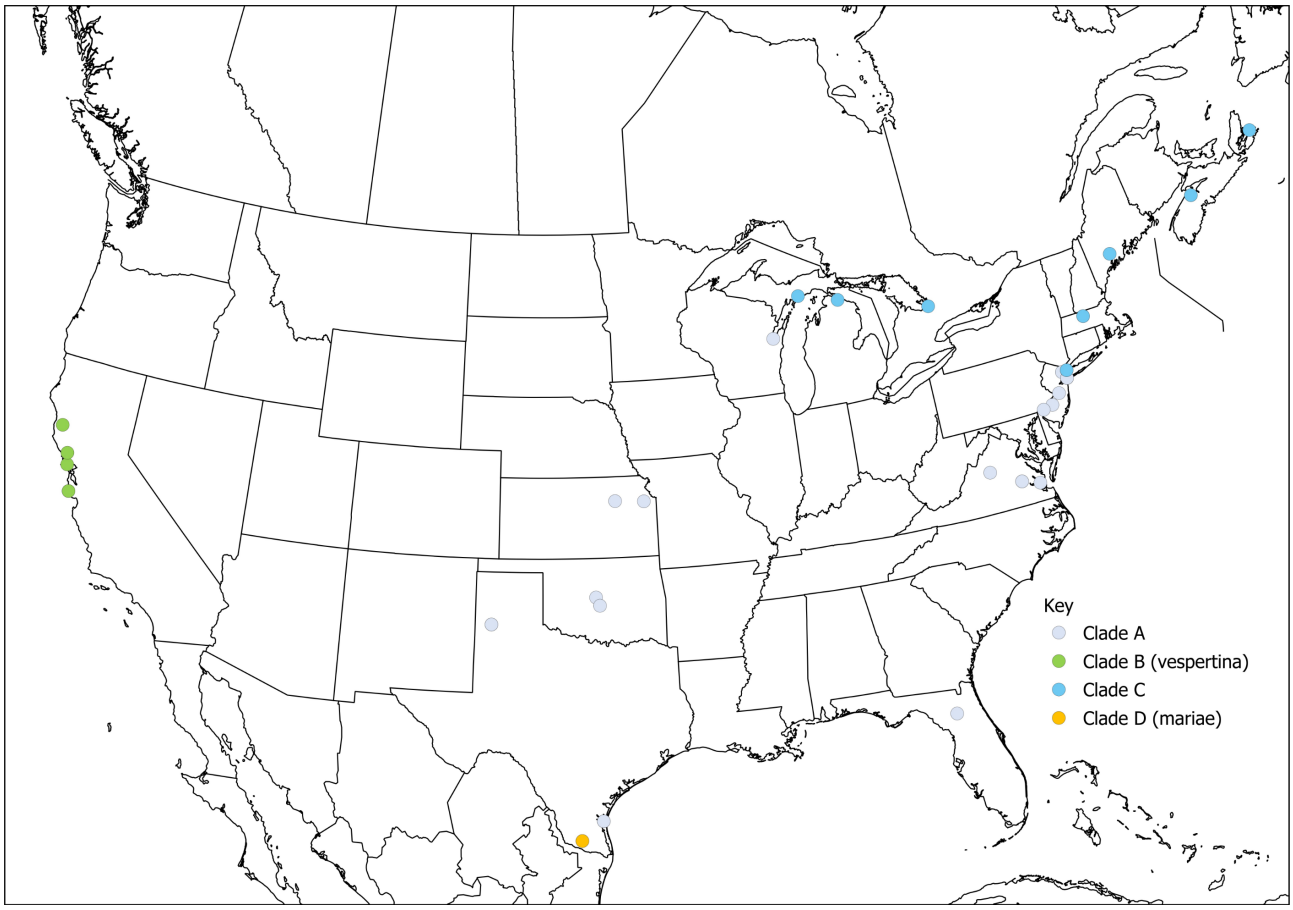


FIGURE 1. Collection locations of *Haemaphysalis leporispalustris* s. l. group specimens analyzed molecularly. Colors represent clades defined by phylogenetic analyses (Figure 2).

Phylogenetic analysis

The sequences were aligned with Mesquite v. 3.61 (Maddison & Maddison, 2018). Phylogenies were generated by Bayesian inference analysis (BA) using MrBayes 3.2.4 (Huelsenbeck & Ronquist, 2001; Ronquist *et al.*, 2011). Two runs with four chains each were run simultaneously for BA analyses (10,000,000 generations). Trees were sampled every 100 iterations. Trees saved before the average standard deviation of split fragments converged to a value < 0.01 were discarded from the final sample. When necessary, the number of generations was increased so that the number of discarded samples would not exceed 25% of the total sampled trees. The 50% majority-rule consensus tree of the remaining trees was inferred, and posterior probabilities (prob) recorded for each branch. PAUP (Swofford, 2000) was used to infer maximum parsimony (MP) trees and calculate MP bootstrap support values. Maximum likelihood (ML) trees with bootstrap support values were constructed with PhyML (Guindon & Gascuel, 2003) and used to evaluate the best fitting nucleotide substitution model (Guindon *et al.*, 2010) for each dataset. Each gene dataset was first analyzed separately. A mitochondrial concatenated matrix (*12SrDNA*, *16SrDNA*, *cox1*) and a concatenated nuclear and mitochondrial matrix (*12SrDNA*, *16SrDNA*, *cox1* and *ITS2*) were also created in Mesquite and analyzed by following the same method used for the separated datasets.

TABLE 1. Collection information for *Haemaphysalis* specimens used in molecular analyses.

Sample name	Species	Stage	Collection Location	Collection Date	Host	USNTC Accession (if	NCBI GenBank Accessions			
							cox1	*16SrDNA*	*12SrDNA*	*ITS2*
Outgroup	*juxtakochi*	Unknown	Summit, Panama	Unknown	Unknown		PV670653	PV671832	PV671757	PV671796
LMTX3	*mariae*	Male	McCook, TX, USA	8/19/2022	*Lepus californicus*		PV670654	PV671833	PV671758	PV671797
LMTX4	*mariae*	Male	McCook, TX, USA	8/19/2022	*Lepus californicus*		PV670655	PV671834	PV671759	PV671798
LMTX1	*leporispalustris*	Nymph	Rangerville, TX, USA	2/16/2023	Environment		PV670656		PV671760	PV671799
LMTX2	*leporispalustris*	larva	Rangerville, TX, USA	1/6/2023	CO2 trap		PV670657	PV671835	PV671761	PV671800
FFTX1	*leporispalustris*	Male	Dimmit Co., TX, USA	3/19/2024	*Sylvilagus floridanus*		PV670658	PV671836	PV671762	PV671801
FFTX2	*leporispalustris*	Female	Dimmit Co., TX, USA	3/19/2024	*Sylvilagus floridanus*		PV670659	PV671837	PV671763	PV671802
Cal_N	*vespertina*	Nymph	Marin Co., CA, USA	7/30/2021	Environment		PV670660	PV671838	PV671764	PV671803
Cal_F	*vespertina*	Female	San Mateo Co., CA, USA	6/15/2021	Environment		PV670661	PV671839	PV671765	PV671804
Cal_M	*vespertina*	Male	San Mateo Co., CA, USA	6/15/2021	Environment		PV670662	PV671840	PV671766	PV671805
Cal_Son	*vespertina*	Nymph	Sonoma Co., CA, USA	5/19/2021	Environment		PV670663	PV671841	PV671767	PV671806
Cal_Mend	*vespertina*	Nymph	Mendocino Co., CA, USA	6/18/2020	Environment		PV670664	PV671842	PV671768	PV671807
NJ_MonL	*leporispalustris*	Larva	Monmouth Co., NJ, USA	2021	Environment		PV670665	PV671843	PV671769	PV671808
NJ_BergA	*leporispalustris*	Female	Bergen Co., NJ, USA	6/28/2018	*Sylvilagus floridanus*		PV670666	PV671844	PV671770	PV671809
NJ_CAM	*leporispalustris*	Nymph	Camden Co., NJ, USA	5/10/2018	Environment		PV670667	PV671845	PV671771	PV671810
NJ_Sal	*leporispalustris*	Nymph	Salem Co., NJ, USA	5/18/2021	Environment		PV670668	PV671846	PV671772	PV671811
WI_F	*leporispalustris*	Female	Brown Co., WI, USA	2018	Host unknown		PV670669	PV671847	PV671773	PV671812
NY563_3	*leporispalustris*	Nymph	Brooklyn, NY, USA	6/14/2015	Environment		PV670670		PV671774	
NY991	*leporispalustris*	Nymph	Bronx, NY, USA	7/12/14	Environment		PV670671	PV671848	PV671775	PV671813
NY134	*leporispalustris*	Nymph	Brooklyn, NY, USA	4/24/2015	Environment		PV670672	PV671849	PV671776	PV671814
NY477_3	*leporispalustris*	Nymph	Bronx, NY, USA	5/23/2015	Environment		PV670673	PV671850	PV671777	PV671815
NY653	*leporispalustris*	Nymph	Staten Island, NY, USA	7/25/2015	Environment		PV670674	PV671851	PV671778	PV671816
VA13	*leporispalustris*	Larva	Varina, VA, USA	2018	Environment		PV670675	PV671852	PV671779	PV671817

.....continued on the next page

TABLE 1. (Continued)

Sample name	Species	Stage	Collection Location	Collection Date	Host	USNTC Accession (if	NCBI GenBank Accessions			
							cox1	*16SrDNA*	*12SrDNA*	*ITS2*
VA9	*leporispalustris*	Larva	Crozet, VA, USA	2018	Environment		PV670676	PV671853	PV671780	PV671818
VA14	*leporispalustris*	Larva	Varina., VA, USA	2018	Environment		PV670677	PV671854	PV671781	PV671819
VA_N	*leporispalustris*	Nymph	York Co., VA, USA	3/18/2016	Environment		PV670678	PV671855	PV671782	PV671820
KS_N1	*leporispalustris*	Unknown	Manhattan, KS, USA	2018	Unknown	USNMENT 1482403	PV670679	PV671856	PV671783	PV671821
KS_N2	*leporispalustris*	Unknown	Manhattan, KS, USA	2018	Unknown	USNMENT 0986102	PV670680	PV671857	PV671784	PV671822
MA_N1	*leporispalustris*	Unknown	Petersham, MA, USA	2013	Unknown	USNMENT 00714383	PV670681	PV671858	PV671785	PV671823
MA_N2	*leporispalustris*	Unknown	Petersham, MA, USA	2013	Unknown	USNMENT 00714383	PV670682	PV671859	PV671786	PV671824
Can_Cope	*leporispalustris*	Nymph	Copeland Forest Ontario, Canada	6/21/2018	Environment		PV670683	PV671860	PV671787	PV671825
Can_F	*leporispalustris*	Female	Coldbrook, Nova Scotia, Canada	5/6/2019	*Canis lupus familiaris*		PV670684	PV671861	PV671788	PV671826
Can_M	*leporispalustris*	Male	Coldbrook, Nova Scotia, Canada	5/6/2019	*Canis lupus familiaris*		PV670685	PV671862	PV671789	
Can_3.1	*leporispalustris*	Nymph	Sydney, Nova Scotia, Canada	7/17/2019	*Canis lupus familiaris*			PV671863		
MaineN	*leporispalustris*	Nymph	Augusta, ME, USA	7/15/2021	Environment		PV670686	PV671864	PV671790	PV671827
MIScN	*leporispalustris*	Nymph	Schoolcraft Co., MI, USA	6/14/2018	Environment		PV670687		PV671791	PV671828
MIChN	*leporispalustris*	Nymph	Cheboygan Co., MI, USA	7/18/2018	Environment		PV670688	PV671865	PV671792	PV671829
OK_L	*leporispalustris*	Larva	Oklahoma City, OK, USA	8/7/2017	Northern Cardinal		PV670689	PV671866	PV671793	
OK_N	*leporispalustris*	Nymph	Norman, OK, USA	6/21/2017	Brown Thrasher		PV670690	PV671867	PV671794	PV671830
FL_N	*leporispalustris*	Nymph	Gainesville, FL, USA	5/17/2021	Environment		PV670691	PV671868	PV671795	PV671831

Morphological analysis

Measurements are all in millimeters, indicated by range, followed by the mean and standard deviation (with three decimals for small phenotypic characters). All adult, nymphal, and part of the larval specimens were cleaned with household detergent in water (1:9), examined and measured under a Nikon SMZ25 stereo microscope (Nikon Instruments, Inc., Melville, NY). Larvae and small characters were measured on Scanning Electron Microscopic (SEM) images obtained with a JEOL JSM6610LV (JEOL USA, Inc, Peabody, MA). Measurements obtained through stereo microscopy were verified and corrected, if needed, by using SEM images of the same ticks. Nomenclature for larval chaetotaxy follows Clifford *et al.* (1961).

Results

Phylogenetic analysis

Information about alignment lengths and basic phylogenetic statistics can be found in Table 2. The Wisconsin *ITS2* sequences of WI-F was, unfortunately, shorter than the other sequences. Therefore, we created two *ITS2* and two mitochondrial + *ITS2* concatenated matrices, with or without the WI-F sequence. Table 2 also provides support for the main identifiable clades (BA posterior probability, MP bootstraps, and ML bootstraps) for comparative purposes. The BA reconstructions obtained by analyzing the concatenated mitochondrial matrix and the concatenated nuclear-mitochondrial matrix are shown in Figs. 2A and 2B, respectively, while the other phylogenetic trees are shown in the Supplemental File.

Within the ingroup, a basal split separated a strongly supported California clade (Clade B, with 1.00 prob, 100% MP and ML bootstrap; Table 2) from a clade including all remaining lineages (Clade A). Clade A was not as consistently supported but was found to be monophyletic in all concatenated data analyses (Figs. 2A and 2B). Clade A was characterized, however, by a basal polytomy and was consistently paraphyletic with variable topology depending on the analyzed genes (Supplemental File). However, within this lineage, two well-supported groups, one including the two *H. mariae* samples (clade D) and one encompassing samples from the northern part of the known distribution of *H. leporispalustris* (Canada, Maine, Michigan, Massachusetts, and New York; clade C) were found in all phylogenies (Figs. 2A–2B and Supplemental File). The remaining *H. leporispalustris* s.l. lineages, ranging from Texas, Oklahoma, Kansas, to Massachusetts, did not cluster in a monophyletic group. In the *ITS2* (Supplemental File) and the nuclear-mitochondrial concatenated reconstructions the female specimen from Wisconsin (F-WI) and a larva from New Jersey (NJ-1) were found to be basal to Clade D although the support for such placement was variable (Table 2).

Descriptions

Family Ixodidae Murray, 1877

Genus *Haemaphysalis* Koch, 1844

Haemaphysalis vespertina Beati, Egizi & Nava, new species (Figs. 3–6)

ZooBank registration: Details of the new species have been submitted to ZooBank (http://zoobank.org/). The Life Science Identifier (LSID) for the new name *Haemaphysalis vespertina* is B61B3440-02C5-4944-9221-7B2E703EA237.

Etymology: The specific epithet is derived from the Latin ‘vesper’ in reference to the evening or the evening star and, by extension, to the West. This species is described from the western coast of North America.

Type-locality: USA: California, San Mateo County, Costanoa (coordinates: 37.154342, -122.341978). Collected from vegetation. Known hosts for all stages are *Lepus californicus* Gray, 1837 and *Sylvilagus* sp. Gray, 1867; avian hosts such as *Melanerpes* sp. Swainson, 1832 and *Toxostoma* sp. Wagler, 1831 have been found infested with immatures.

TABLE 2. Number of sequences, alignment lengths, and basic phylogenetic statistics including support for main identifiable clades (BAPP = bayesian posterior probability, MPB = maximum parsimony bootstraps, MLB = maximum likelihood bootstraps, RC = rescaled consistency index, HI = homoplasy index, and "-" = non supported).

Phylogenetic analyses summary	***12SrDNA*** **(gap = 5th)**	***16SrDNA*** **(gap = 5th)**	***cox1***	**Mitochondrial (concatenated)**	***ITS2***	***ITS2*** **(+WI)**	**All (concatenated)**	**ALL (+WI) (concatenated)**
Number of unique sequences	17	17	23	25	9	9	23	24
Matrix length	315	369	526	1193	935	638	2127	1830
Parsimony informative	30	46	110	193	102	79	302	283
MP number of best trees	140	44	13	136	1	2	20	20
MP score of best tree (score, RC, HI)	76, 0.701, 0.171	95, 0.743,0.158	265, 0.610, 0.279	446, 0.649, 0.256	206, 0.719, 0.131	173, 0.732,0.121	651, 0.698,0.215	642, 0.658, 0.246
PHYML best model	HKY85+I	HKY85+G+I	HKY85+G	HKY85+G+I	GTR+G	GTR+G	GTR+G+I	GTR+G+I
ML score of best tree (-lnL)	770.76	866.12	1844,00	3526,28	2163,26	1612.72	5854.88	5376.94
Clade node support (BAPP, MLB, MPB)								
Ingroup	1.00/98/100	1.00/100/100	1.00/100/100	1.00/100/100	1.00/100/100	1.00/100/100	1.00/100/100	1.00/100/100
H. vespertina	1.00/98/100	1.00/99/100	1.00/100/100	1.00/100/100	1.00/100/100	1.00/100/100	1.00/100/100	1.00/100/100
H. leporispalustris s.l. (clade A)	0.82/-/91	1.00/86/99	1.00/-/100	1.00/84/100	0.90/-/99	-/-/-	1.00/88/100	1.00/93/100
H. mariae	n/a	1.00/100/100	1.00/96/100	1.00/100/100	n/a	n/a	1.00/100/100	1.00/100/100
H. mariae + NjMon	-/-/-	-/-/-	-/-/-	-/-/-	1.00/86/90	n/a	0.98/89/-	0.92/73/-
H. mariae + NJMon +WI	-/-/-	-/-/-	-/-/-	-/-/-	-/-/-	1.00/97/96	n/a	1.00/87/-
North (clade B)	0.98/91/82	1.00/94/100	0.97/81/100	0.98/-/100	1.00/98/100	1.00/93/100	1.00/96/100	1.00/96/100

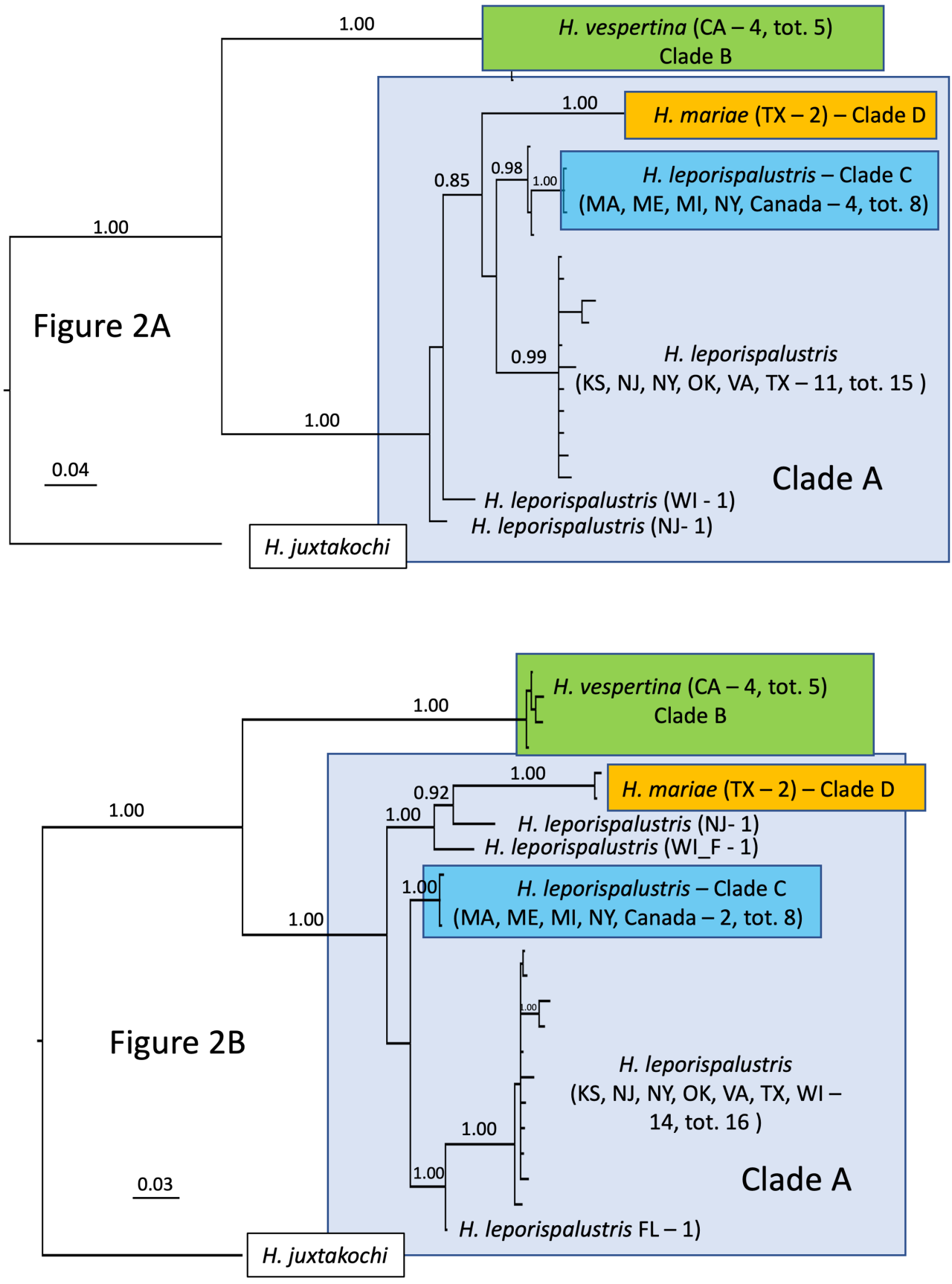


FIGURE 2. Bayesian phylogenetic reconstruction of relationships within the *Haemaphysalis leporispalustris* s.l. group based on the analysis of concatenated mitochondrial (*12SrDNA*, *16SrDNA*, and *cox1*) (Fig 2A) and concatenated nuclear (*ITS2*) and mitochondrial datasets (Fig. 2B).

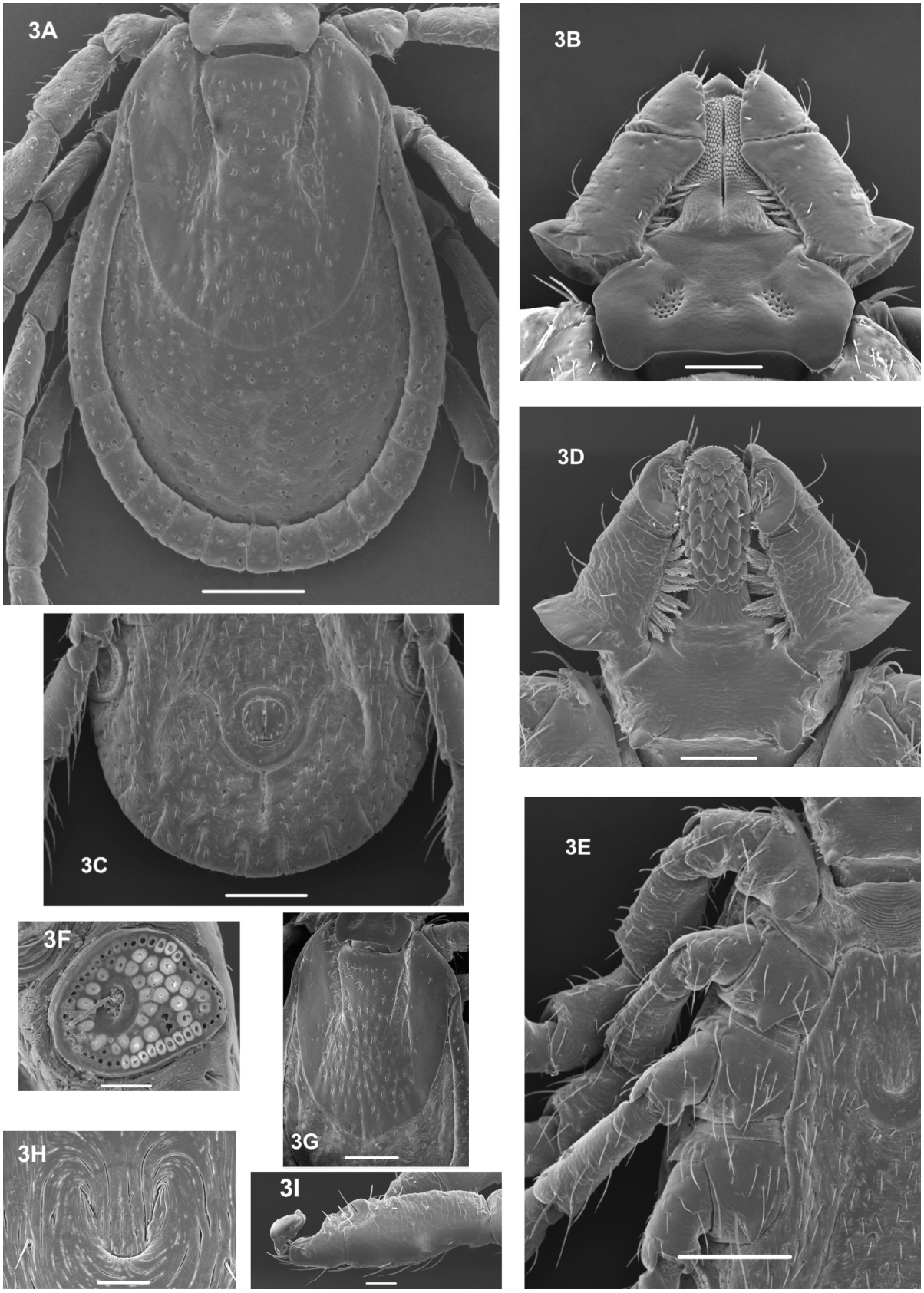


FIGURE 3. Scanning electron microscopic images of the female of *Haemaphysalis vespertina.* sp. n., scales are in parentheses; 3A dorsum (250μm); 3B dorsal capitulum (100μm); 3C ventral posterior idiosoma (200μm); 3D ventral capitulum (100μm); 3E coxa (200μm); 3F spiracular plate (50μm), 3G slightly tilted scutum (200μm); 3H genital aperture (40μm); 3I Haller's organ (50μm).

***Female*—Figs. 3A–3I**

HOLOTYPE: USNMENT1785003 (USA, California, San Mateo County, Costanoa, 37.154342, -122.341978, 9 VII 2024; from vegetation, coll. Tara Roth and Arielle Crews). PARATYPES: USNMENT1510975, 5 females (USA, California, San Mateo County, Costanoa, 37.154342, -122.341978, 9 VII 2024; from vegetation, coll. Tara Roth and Arielle Crews); USNEMNT1510966, 1 female (USA, California, San Mateo County, Costanoa, 37.154342, -122.341978, 15 VI 2021; from vegetation, coll. Angie Nakano). **Other material examined:** USNEMNT1510970, 22 females (USA, California, Mendocino County, Hopland, 38.9729541, -123,1163918, 15 VI 1965, ex. *L. californicus*); USNMENT1510977, 7 females (USA, California, Mendocino County, Hopland, 38.9729541, -123.1163918); USNMENT1510986, 1 female (USA, California, San Benito County, 22 III 1932, ex. *L. californicus*)**;** USNMENT1510974, 6 females (USA, California); USNMENT1510976, 1 female (USA, Oregon, Harney County, Burns, 43.588333, -119.061389, ex. *Sylvilagus* sp.).

Body (Fig. 3A) of unfed specimens dorsally suboval, longer (1.30–1.49; 1.36 ± 0.06) than wide (0.86–0.95; 0.93 ± 0.03); with lateral edges slightly concave at level of coxa II, widest posterior to mid-length. **Scutum** oval, (Fig. 3A, Fig. 3G) longer (0.79–0.84; 0.82 ± 0.02) than wide (0.62–0.68; 0.64 ± 0.02), with posterior margin rounded; cervical grooves very deep and broad, converging posteriorly to almost mid-length of scutum, broadening posteriorly into shagreened triangular shallower area (Fig. 3G) ; scapulae round, with scattered fine punctations, bearing short, fine setae (0.017–0.027; 0.024 ± 0.003), lateral fields and posterior border with few punctations and glabrous; median field with homogeneously distributed, dense, larger, shallow punctations, all bearing fine setae (0.014–0.025; 0.018 ± 0.003), central punctations sometimes confluent producing rugose effect (Fig. 3G). **Alloscutum** (Fig. 3A) with deep, uniformly distributed, very small punctations, all bearing short setae, slightly shorter (0.011–0.022; 0.015 ± 0.003) than scutal setae; marginal groove complete, lining 11 festoons, reaching scutum at level of coxa II; festoons and marginal fold with numerous deep, small, punctations bearing setae. **Venter**: genital aperture at level of coxae II–III (Fig. 3E, Fig. 3H), U-shaped, with almost parallel lateral margins, lined by very narrow sclerotized flaps; anal groove posterior to anus joining genital groove anterolaterally (Fig. 3C); in unfed specimens, bean-shaped areas posterolateral to anus, delimited by posteromedian groove, festoons, posterior part of genital groove, and anal groove; ventral grooves more distinct in unfed specimens; punctations dense, fine, deep, uniformly distributed, bearing fine setae (0.011–0.022; 0.016 ± 0.003); spiracular plates almost round with inconspicuous, blunt, dorsal projection, with 2–4 lines of goblet cells, larger in center, slightly smaller along periphery (Fig. 3F). **Capitulum** (Figs. 3B, Fig. 3D). Dorsal (Fig. 3B): length from palpal apices to tip of cornuae (0.35–0.41; 0.39 ± 0.02); basis capituli broader (0.31–0.35; 0.34 ± 0.01) than long (0.18–0.19; 0.19 ± 0.004), subrectangular, with convex, rounded, lateral edges, posterior margin straight, cornuae wider at insertion than long, rounded; porose areas as narrow flattened ovals (0.050–0.063; 0.055 ± 0.004 long and 0.020–0.035; 0.029 ± 0.04 wide), placed in deep depressions of basis capituli, diverging posteriorly; ventrally (Fig. 3D) basis capituli subrectangular, with lateral edges slightly diverging anteriorly, with short, triangular, rounded, posteriorly directed processes, wider at insertion than long. Palps dorsal (Fig. 3B): palpal segment I inconspicuous; palpal segment II length (0.16–0.17; 0.16 ± 0.02), palpal segment II width at level of lateral projection (0.13–0.15; 0.14 ± 0.01), distance between apices of lateral projections (0.48–0.54; 0.50 ± 0.02), internal edge of palpal segment II almost straight, with 6 flattened, barbed setae; palpal segment III approximately as long (0.09–0.10; 0.10 ± 0.01) as wide (0.10–0.10; 0.10 ± 0.01); lateral length of palpal segments II and III measured from apex of palpal segment III to tip of angle with lateral projection (0.24–0.26; 0.25 ± 0.001). Palps ventral (Fig. 3D): palpal segment I inconspicuous, palpal segment II with no spurs, with approx. 11–12 lanceolate, barbed, flattened median setae, palpal segment III with rounded ventral spur; hypostome clavate, with homogeneous 3:3 dental formula except at crown, approx. 6–7 denticles per file. **Legs.** Coxa I (Fig. 3E) with short, rounded internal spur, wider than long, external spur as triangular ridge, shorter than internal spur, concealed by tuft of long fine, setae; coxa II, III, and IV with single, rounded, triangular spurs, as long as wide at insertion, directed posterolaterally and inserted at mid-width in coxa II and III, directed posteriorly and inserted more medially in coxa IV. Trochanter I with ventral rounded spur; coxae and legs with numerous, very long, fine setae. Haller's organ as in Fig. 3I.

***Male*—Figs. 4A–4H**

ALLOTYPE: USNMENT1785004 (USA, California, San Mateo County, Costanoa, 37.154342, -122.341978, 9 VII 2024; from vegetation, coll. Tara Roth and Arielle Crews). PARATYPES: USNMENT1510975, 4 males (USA, California, San Mateo County, Costanoa, 37.154342, -122.341978, 9 VII 2024; from vegetation, coll. Tara Roth and Arielle Crews); USNEMNT1510966, 2 males (USA, California, San Mateo County, Costanoa, 37.154342, -122.341978, 15 VI 2021; from vegetation, coll. Angie Nakano). **Other material examined:** USNEMNT1510970,

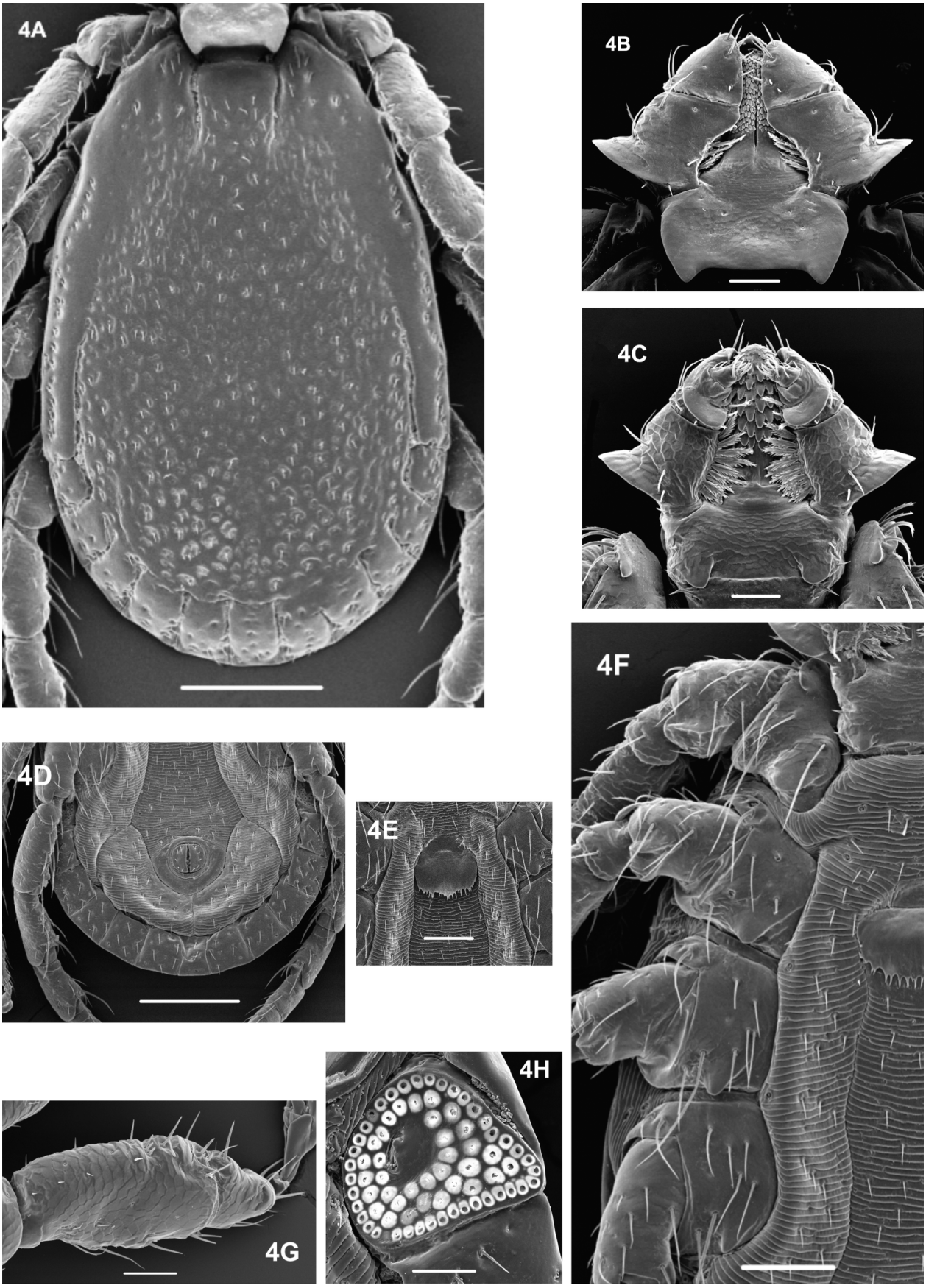


FIGURE 4. Scanning electron microscopic images of the male of *Haemaphysalis vespertina.* sp. n., scales are in parentheses; 4A conscutum (250µm); 4B dorsal capitulum (50µm); 4C ventral capitulum (50µm); 4D ventral posterior idiosoma (250µm); 4E genital apron (100µm); 4F coxa (100µm); 4G Haller's organ (50µm); 4H spiracular plate (50µm).

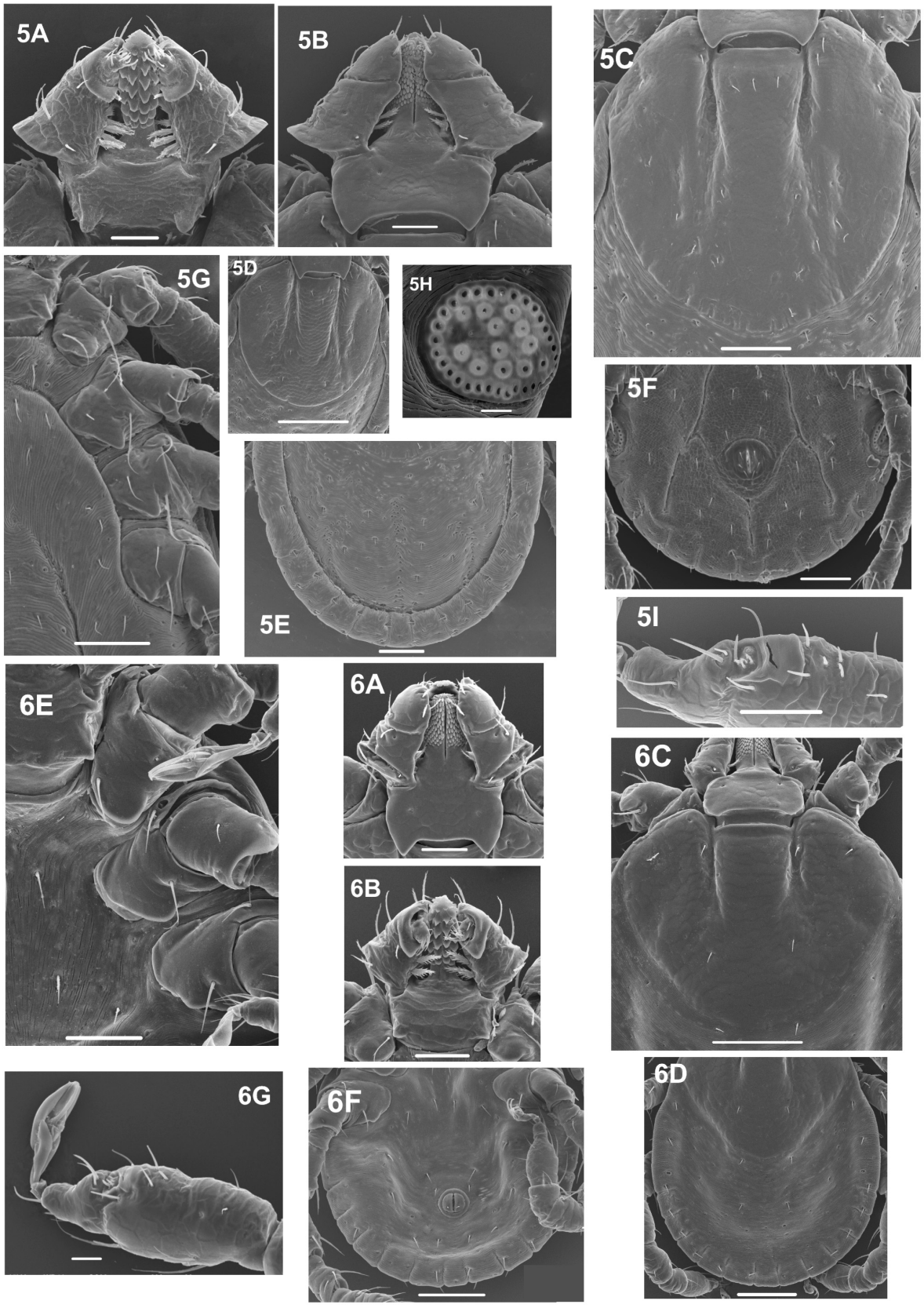
12 males females (USA, California, Mendocino County, Hopland, 38.9729541, -123,1163918, 15 VI 1965, ex. *L. californicus*); USNMENT1510977, 12 males (USA, California, Mendocino County, Hopland, 38.9729541, -123,1163918); USNMENT1510986, 2 males (USA, California, San Benito County, 22 III 1932, ex. *L. californicus*); USNMENT1785016; 23 males (USA, California, Bernardino County, 5 IX 1936, ex. *L. californicus*); USNMENT1510976, 4 males (USA, Oregon, Harney County, Burns, 43.588333, -119.061389, ex. *Sylvilagus* sp.).

Body: conscutum (Fig. 4A) distinctly oval, longer (1.16–1.30; 1.21 ± 0.04) than wide (0.72–0.90; 0.80 ± 0.04); with lateral edges slightly convex, widest posterior to mid-length; cervical grooves very deep, short, almost parallel, reaching level of coxa II; scapulae round with scattered fine punctations, bearing short, fine setae as in female; marginal grooves starting anteriorly at approx. mid length of scutum, deep, reaching and lining first festoon, fragmented along other 9 festoons; festoons with scattered punctations and inconspicuous short, fine setae; median field with homogeneously distributed, dense, large, shallow punctations, all bearing short fine setae (0.012–0.019; 0.015 ± 0.002), central punctations sometimes confluent producing distinct rugose effect; lateral fold anterior to festoons with single, lateral, almost linear line of punctations reaching level of coxa II. **Venter** (Figs. 4D–F): genital aperture at level of coxa II, covered by oval apron as in Fig. 4E; anal groove posterior to anus joining anterolaterally genital groove, bean-shaped areas, posterolateral to anus, delimited by posteromedian groove, festoons, posterior part of genital groove, and anal groove (Fig. 4D); punctations dense, fine, deep, uniformly distributed, bearing fine setae (0.014–0.028; 0.020 ± 0.007); spiracular plates almost round with blunt, dorsal projection, with 2–4 lines of goblet cells, larger in center, slightly smaller along periphery (Fig. 4H). **Capitulum** (Figs. 4B–C). Dorsal (Fig. 4B): length from palpal apices to tip of cornuae (0.25–0.29; 0.27 ± 0.01); basis capituli broader (0.19–0.22; 0.21 ± 0.01) than long (0.10–0.12; 0.11 ± 0.01), subrectangular, with convex, rounded, lateral edges, posterior margin straight, cornuae triangular, wider at insertion than long, rounded; ventrally basis capituli subrectangular, with lateral edges slightly diverging anteriorly, with short rounded, posteriorly directed processes, twice as wide at insertion as long (Fig. 4C). Palps dorsal (Fig. 4B): palpal segment I inconspicuous; palpal segment II length (0.09–0.10; 0.10 ± 0.001), width at level of lateral projection (0.11–0.12; 0.12 ± 0.001), distance between apices of lateral projections (0.33–0.35; 0.34 ± 0.01); lateral length of palpal segments II and III measured from apex of palpal segment III to tip of angle with lateral projection (0.15–0.16; 0.15 ± 0.004); internal margin concave ending anteriorly with inconspicuous medially directed lobe, with 4 flattened, barbed setae; palpal segment III approximately as long (0.07–0.08; 0.07 ± 0.002) as wide (0.08–0.09; 0.08 ± 0.002). Palps ventral (Fig. 4C): palpal segment I inconspicuous, palpal segment II with no spurs, with approx. 9 lanceolate, barbed, flattened median setae; palpal segment III with rounded ventral spur. Hypostome clavate, with homogeneous 3:3 dental formula excepted crown, approx. 6–7 denticles per file. **Legs.** Coxa I with short, rounded internal spur, wider than long, external spur as rounded ridge, shorter than internal spur, concealed by tuft of long, fine setae; coxa II, III, and IV with single, triangular spurs, approx. as long as wide at insertion level, directed posterolaterally (Fig. 4F). Trochanter I with ventral rounded ridge-like spur; coxae and legs with very numerous, long, and fine setae (Fig. 4F). Haller's organ as in Fig. 4G.

***Nymph*—Figs. 5A–5I**

PARATYPES: USNMENT1510966, 13 nymphs (USA, California, San Mateo County, Costanoa, 37.154342, -122.341978, 15 VI 2021; from vegetation, coll. Angie Nakano); USNMENT1510980, 4 nymphs (USA, California, San Mateo County, Costanoa, 37.154342, -122.341978, 15 VI 2021; from vegetation, coll. Angie Nakano). **Other material examined:** USNMENT1510970, 3 nymphs (USA, California, Mendocino County, Hopland, 38.9729541, -123,1163918, 15 VI 1965, ex. *L. californicus*); USNMENT1785016; 5 nymphs (USA, California, Bernardino County, 5 IX 1936, ex. *L. californicus*); USNMENT1510976, 5 nymphs (USA, Oregon, Harney County, Burns, 43.588333, -119.061389, ex. *Sylvilagus* sp.).

Body: Outline overall oval, length from palpal apices to posterior margin (0.08–0.09; 0.08 ± 0.02); width (0.06–0.07; 0.07 ± 0.01), with lateral edges slightly concave at level of coxa III, widest at level of coxa IV. **Scutum** (Figs. 5C–D) oval, longer (0.45–0.47; 0.46 ± 0.01) than wide (0.43–0.46; 0.45 ± 0.01), with posterior margin rounded; cervical grooves very deep, broad, almost parallel anteriorly, broadening posteriorly into triangular shallower area and reaching edge of scutum; scattered, unevenly distributed punctations bearing fine, short setae (0.007–0.020; 0.013 ± 0.003). **Alloscutum** (Fig. 5E) with posteromedian and curved, posterolateral grooves outlined by very fine, deep, glabrous, dense punctations; other areas of idiosoma with sparse, scattered, medium-sized punctations bearing setae (0.009–0.019; 0.013 ± 0.002); 11 festoons, with medially directed 1 or 2 setae each (not on central festoon); marginal groove lining all festoons and reaching scutum at level of coxae III. **Venter** (Figs. 5F–G): anal groove posterior to anus, median postanal groove reaching festoons, joining anterolaterally hint of future genital grooves



FIGURES 5–6. Scanning electron microscopic images of the nymph (Figure 5) and the larva (Figure 6) of *Haemaphysalis vespertina.* sp. n., scales are in parentheses; 5A ventral capitulum (50µm); 5B dorsal capitulum (50µm); 5C scutum (100µm); 5D slightly tilted scutum (200µm); 5E posterior alloscutum (100µm); 5F ventral posterior idiosoma (100µm); 5G coxa (100µm); 5I Haller's organ (50µm); 6A dorsal capitulum (50µm); 6B ventral capitulum (50µm); 6C scutum (100µm); 6D alloscutum (100µm); 6E coxa (50µm); 6F posterior ventral idiosoma (100µm); 6G Haller's organ (20µm).

that extend posteriorly to festoon level (Fig. 5F); scattered, sparse, small punctations bearing fine setae (0.020–0.038; 0.026 ± 0.004); spiracular plates sub-oval with 1–3 lines of goblet cells, larger in center, smaller in peripheral line (Fig. 5H). **Capitulum** (Figs. 5A–5B). Dorsal: length from palpal apices to tip of cornuae 0.25; 0.24 ± 0.01); basis capituli broader (0.18–0.18; 0.18 ± 0.001) than long (0.09–0.1; 0.09 ± 0.002), subrectangular, with convex, rounded, lateral edges, posterior margin convex, cornuae wider at insertion than long, triangular, and pointed (Fig. 5B); ventral basis capituli subrectangular with lateral edges diverging anteriorly, with distinct, rounded, posterolaterally directed processes (Fig. 5A). Palps dorsal (Fig. 5B): palpal segment I inconspicuous; palpal segment II length (0.08–0.09; 0.08 ± 0.003), palpal segment II width at level of lateral projection (0.09–0.10; 0.10 ± 0.003), distance between apices of lateral projections (0.28–0.30; 0.29 ± 0.006), internal edge of palpal segment II almost straight, with 2 flattened, barbed setae; palpal segment III approximately as long (0.06–0.07; 0.07 ± 0.003) as wide (0.06–0.07; 0.06 ± 0.003); lateral length of palpal segments II and III measured from apex of palpal segment III to tip of angle with lateral projection (0.12–0.13; 0.13 ± 0.003). Palps ventral (Fig. 5A): palpal segment I inconspicuous, palpal segment II with no spurs, with 5 lanceolate, barbed, flattened median setae, palpal segment III with rounded ventral spur. Hypostome clavate, with homogeneous 2:2 dental formula excepted crown, approx. 7 denticles per file. **Legs.** Coxa I (Fig. 5G) with rounded internal spur, as wide as long, external spur as inconspicuous ridge, concealed by long, fine seta; coxa II, III, and IV with single, rounded, triangular spurs, directed posterolaterally and inserted at mid-width in coxa II and III, directed posteriorly in coxa IV. Trochanter I with ventral triangular spur; coxae and legs with numerous, 2–3 very long, fine setae. Haller's organ as in Fig. 5I.

***Larva*—Figs. 6A–6G**

PARATYPES: USNMENT1510966, 2 larvae (USA, California, San Mateo County, Costanoa, 37.154342, -122.341978, 15 VI 2021; from vegetation, coll. Angie Nakano); USNMENT1510991, 1 larva (USA, California, San Mateo County, Costanoa, 37.154342, -122.341978, 15 VI 2021; from vegetation, coll. Angie Nakano). **Other material examined:** USNMENT1510964, 1 larva (USA, California, Los Angeles County, 13 XI 1987, ex. *Toxostoma redivivum*, sent by K.C. Emerson); USNMENT1510958, 5 larvae (USA, California, San Diego County, Imperial Beach, 21 XII 1968, ex. *Toxostoma bendirei*); USNMENT1510962, 8 larvae (USA, California, Sonoma County, Jack London State Historic Park, 38.350556, -122.543056, 24 IX 2021, ex. vegetation, coll. Megan Saunders); USNMENT 1510970, 50 larvae (USA, California, Mendocino County, Hopland, 38.9729541, -123,1163918, 15 VI 1965, ex. *Lepus californicus*); USNMENT1785017, 1 larva (USA, California, Yalo County, Winters, 38.525, -121.970833, XII 1965, ex. *Melanerpes formicivorus*).

Body*:* (Fig. 6C–D): dorsally subcircular, lateral margins slightly concave at level of leg 2, length from tip of scapulae to posterior edge (0.45–0.50; 0.47 ± 0.02), widest (0.40–0.46; 0.43 ± 0.02) near midlength, median area of idiosoma posterior to scutum convex; 11 festoons. **Scutum** (Fig: 6C): length (0.24–0.25; 0.25 ± 0.002), breadth (0.30–0.32; (0.31 ± 0.06), outline broadly cordiform; scapulae rounded; cervical grooves almost parallel, distinct, deep, wide, with margins slightly diverging posteriorly, reaching 1/3 of scutal length; cervical fields wide, very shallow, reaching edge of scutum, delimiting slightly convex posterior central field and convex anterolateral scutal areas; 3 pairs of scutal setae (0.009–0.014; 0.012 ± 0.002) and 4 pairs of small wax glands. **Alloscutum** (Fig. 6D**):** large wax glands (or sensilla sagittiformia) present; 8 pairs of fine dorsomarginal setae (0.014–0.022; 0.017 ± 0.002), two anterior to large wax glands; 2 pairs of central dorsal setae (0.009–0.014; 0.011 ± 0.002); supplementary setae absent. **Venter** (Fig. 6E–F) with 3 pairs of large wax glands posterior to each coxa; 3 pairs of sternal setae (0.016–0.024; 0.020 ± 0.002), two aligned with coxae III and one with coxae II; 2 pairs of preanal setae (0.010–0.020; 0.014 ± 0.004); 4 pairs of premarginal setae (0.011–0.016; 0.013 ± 0.002), 4 pairs of marginal ventral (0.013–0.018; 0.015 ± 0.002), and 1 pair of minuscule anal setae. **Capitulum** (Fig. 6A–B): dorsal length (Fig. 6A) from palpal apices to tip of cornua (0.13–0.15; 0.14 ± 0.07), width between tips of lateral extensions of palpal segments II (0.15–1.60; 0.16 ± 0.03). Basis capituli length from papal insertion to tip of cornua (0.06–0.07; 0.07 ± 0.002), width (0.11–0.12; 0.12 ± 0.01), with dorsal posterior edge concave, lateral margins rounded, with small notch under insertion of palps, cornua short, wider at insertion than long, bluntly pointed and posteriorly directed. Palpal segment I inconspicuous, palpal segment II almost as long (0.05–0.05; 0.05 ± 0.006) long as broad (0.05–0.05; 0.05 ± 0.006) (width measured at level of lateral extensions), palpal segment III (0.03–0.03; 0.03 ± 0.001) long by (0.04–0.04; 0.04 ± 0.001) broad. Palpal segments II and III not fused, delimited by distinct dorsal suture; palpal segment II dorsally with deep groove going from posteromedian edge to tip of lateral protrusion and with 1 medially directed flattened barbed seta. Ventrally (Fig. 6B), basis capituli broadly rectangular, with posterolaterally directed, bluntly pointed, triangular auriculae. Hypostome spatulate, toothed portion covering approx. 3⁄4 of hypostomal

length; dental formula below crown 2:2 on 4–5 rows, denticles of approx. similar size; one pair of post-hypostomal short and fine setae. Palpal segment II with 2 long, flattened, barbed setae inserted on median edge and covering in part hypostome and post-hypostomal setae; palpal segment III with rounded subtriangular ventral spur; palpal segment IV extruding, with approx. 7 apical setae. Palpal setation as in Fig. 6A–B. **Legs**: Coxa I with internal broad, rounded spur; coxa II with one, short, wide, rounded spur; coxa III with a ridge-like spur; coxal setae approx. twice as long as idiosomal setae (0.03–0.05; 0.04 ± 0.005) (Fig. 6E). Haller's organ as in Fig. 6G.

Redescription of *H. leporispalustris* adults

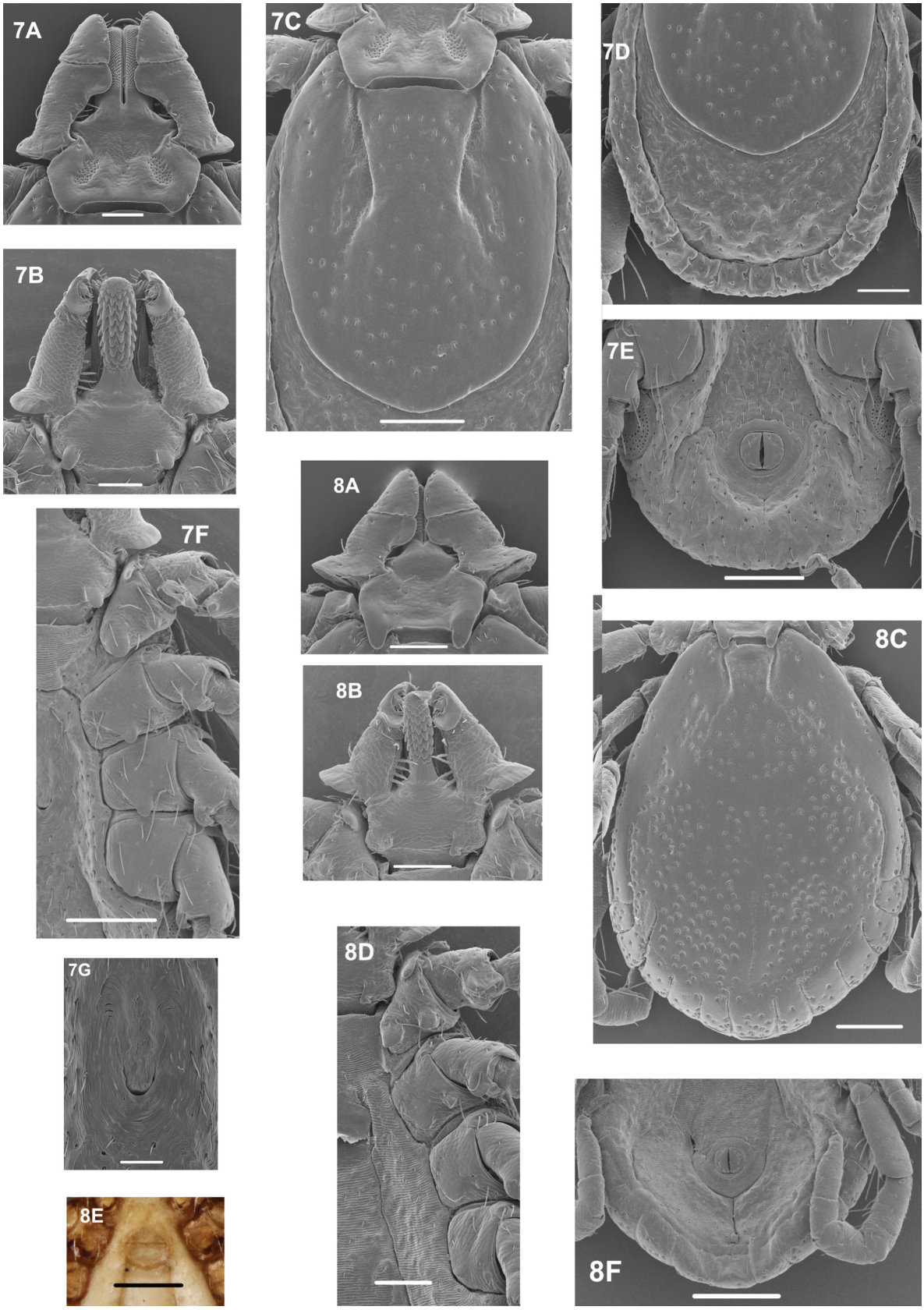
Family Ixodidae Murray, 1877

Genus *Haemaphysalis* Koch, 1844

Haemaphysalis leporispalustris (Packard, 1869)

Female—**Figs. 7A–7G**; based on 14 specimens, some partially engorged, from Packard's type series deposited at the Harvard Museum of Comparative Zoology (MCZ IZ 47339)

Body of unfed specimens dorsally suboval, longer (1.27–1.65; 1.54 ± 0.10) than wide (0.87–1.14; 1.00 ± 0.07); widest posterior to mid-length. **Scutum** (Fig. 7C) oval, longer (0.86–0.96; 0.91 ± 0.03) than wide (0.63–0.806; 0.74 ± 0.05), with posterior margin rounded; cervical grooves deep and broad, converging posteriorly to almost mid-length of scutum, then diverging; scapulae with scattered fine, punctations, bearing very short, fine setae, lateral fields and posterior border with few inconspicuous punctations and glabrous; median field with homogeneously distributed, scattered, small, shallow punctations all bearing very short, fine setae (0.001–0.003; 0.002 ± 0.001) (Fig. 7C). **Alloscutum** (Fig. 7D) with deep, uniformly distributed, small punctations all bearing short setae (0.002–0.004; 0.003±0.001); marginal groove complete, lining 11 festoons, reaching scutum at level of coxa II; festoons and marginal folds with numerous deep, small, punctations bearing short fine setae. **Venter** (Figs. 7E–G): genital aperture at level of coxae III, U-shaped, with almost parallel lateral margins (Fig. 7G); anal groove posterior to anus joining anterolaterally genital groove; bean-shaped areas posterolateral to anus, delimited by posteromedian groove, festoons, posterior part of genital groove, and anal groove; ventral grooves more distinct in unfed specimens; punctations dense, fine, deep, uniformly distributed, bearing fine setae (0.001–0.003; 0.002 ± 0.001) (Fig. 7E); spiracular plates almost round with inconspicuous, blunt, dorsal projection, with 3–5 rows of small goblet cells, slightly smaller along periphery. **Capitulum** (Figs 7A–B). Dorsal (Fig. 7A): length from palpal apices to tip of cornuae (0.49–0.58; 0.53 ± 0.03); basis capituli broader (0.39–0.44; 0.41 ± 0.01) than long (0.15–0.19; 0.17 ± 0.01), subrectangular, with convex, rounded, lateral edges, posterior margin straight, cornuae wider at insertion than long, rounded; porose areas narrowly flattened and oval (0.07–0.11; 0.09 ± 0.01) long and (0.04–0.09; 0.06 ± 0.01) wide, placed in deep depressions of basis capituli, diverging posteriorly, inter-porose area concave; ventrally (Fig. 7B), basis capituli subrectangular, with lateral edges slightly diverging anteriorly, with short rounded, posteriorly directed processes, almost as wide at insertion than at apex. Palps dorsal (Fig. 7A): palpal segment I inconspicuous; palpal segment II length (0.21–0.28; 0.24 ± 0.01), palpal segment II width at level of lateral projection (0.13–0.18; 0.15 ± 0.01), distance between apices of lateral projections (0.54–0.65; 0.60 ± 0.03); internal edge of palpala segment II markedly concave ending in conspicuous medially directed anterior lobe, with approx. 3 fine, barbed setae; palpal segment III approximately as long (0.13–0.18; 0.14 ± 0.01) as wide (0.09–0.12; 0.11 ± 0.01); lateral length of palpal segments II and III measured from apex of palpal segment III to tip of angle with lateral projection (0.29–0.35; 0.33 ± 0.02). Palps ventral (Fig. 7B): palpal segment I inconspicuous, palpal segment II with no spurs, with approx. 8 fine, barbed, flattened, median setae (damaged in Fig. 7B, but basal insertion holes are clearly visible, and confirmed by the examination of other type specimens); palpal segment III with rounded ventral spur; hypostome clavate, with homogeneous 3:3 dental formula excepted crown, approx. 9 denticles per file. **Legs.** Coxa I (Fig. 7F) with short, wide, rounded internal spur, wider than long; distinct, smaller, rounded, external spur; coxa II with internal, round, posteriorly directed spur and external very small, rounded spur; coxa III–IV with single, triangular spur, as long as wide at insertion, directed posteriorly, inserted at mid-width in coxa III, inserted more medially in coxa IV. Trochanter I with ventral rounded spur; coxae and legs with scattered, long, fine setae.



FIGURES 7–8. Scanning electron microscopic images of the female (Figure 7) and the male (Figure 8) of *Haemaphysalis leporispalustris s.s.* 7A; scales are in parentheses; dorsal capitulum (100µm); 7B ventral capitulum (100µm); 7C scutum (200µm); 7D alloscutum (150µm); 7E ventral posterior idiosoma (200µm); 7F coxa (200µm); 7G genital aperture (50µm); 8A dorsal capitulum (100µm); 8B; ventral capitulum (100µm); 8C conscutum (200µm); 8D coxa (100µm); 8E genital apron (macroscopic image; 200µm); 8F ventral posterior idiosoma (200µm).

***Male*—Figs. 8A–8G**; based on 2 specimens, some partially engorged, from Packard's type series deposited at the Harvard Museum of Comparative Zoology (MCZ IZ 47339); therefore, standard deviation values are missing when only 2 measurements were available.

Scutum (Fig. 8C) oval, longer (1.33–2.12; 1.73) than wide (0.95–1.43; 1.19); with lateral edges convex, widest posterior to mid-length; cervical grooves very deep, short, almost parallel, reaching level of coxa II; scapulae round with scattered fine punctations, bearing short, fine setae as in female; marginal grooves starting anteriorly at approx. mid length of scutum, deep, reaching and lining first festoon, absent along other 9 festoons; festoons with scattered punctations and inconspicuous short, fine setae; median field with unevenly distributed, medium sized shallow punctations, all bearing very short, fine setae (0.004–0.011; 0.008 ± 0.003), glabrous crescent outlining pseudoscutum and glabrous median longitudinal line reaching central festoon, interrupting punctation pattern, lateral fold anterior to festoons with single, lateral, almost linear line of punctations reaching almost level of coxa II (Fig. 8C). **Venter**: genital aperture at level of coxa II, covered by oval apron as in Fig. 8E; anal groove posterior to anus joining genital groove anterolaterally, bean-shaped areas posterolateral to anus, delimited by posteromedian groove, festoons, posterior part of genital groove, and anal groove; punctations dense, fine, shallow, and uniformly distributed, bearing fine, very short setae (0.006–0.009; 0.008 ± 0.001) (Fig. 8F); spiracular plates almost round with blunt, inconspicuous, dorsal projection, with 4–5 lines of small goblet cells, smaller along periphery. **Capitulum** (Figs. 8A–B). Dorsal: length from palpal apices to tip of cornuae (0.33–0.35; 0.34); basis capitula broader (0.23–0.24; 0.235) than long (0.13–0.14; 0.135), subrectangular, with convex, rounded, lateral edges, posterior margin straight, cornuae at least as long as wide, bluntly rounded (Fig. 8A); ventrally basis capituli subrectangular, with lateral edges slightly diverging anteriorly, with short rounded, posteriorly directed processes as wide at insertion as long (Fig. 8B). Palps dorsal: palpal segment I inconspicuous; palpal segment II length (0.13–0.14; 0.13 ± 0.005), width at level of lateral projection (0.10–0.13; 0.11 ± 0.01), distance between apices of lateral projections (0.39–0.41; 0.40); lateral length of palpal segments II and III combined measured from apex of palpal segment III to tip of angle with lateral projection (0.18–0.19; 0.18 ± 0.006); internal margin concave ending in conspicuous medially directed lobe, with 2–3 fine, barbed setae (visible in specimen not used for SEM); palpal segment III approximately as long (0.06–0.1; 0.08 ± 0.02) as wide (0.07–0.08; 0.08 ± 0.002) (Fig. 8A). Palps ventral: palpal segment I inconspicuous, palpal segment II with no spurs, with approx. 5, lanceolate, barbed, fine, median setae; palpal segment III with rounded ventral spur. Hypostome clavate, with homogeneous 3:3 dental formula excepted crown, approx. 8 denticles per file (Fig. 8B). **Legs.** Coxa I (Fig. 8D) with short, rounded internal spur, wider than long, external spur shorter and round; coxa II with round almost ridge-like internal spur and pointed short external spur; coxa III with round, very short, internal spur, coxa IV with inconspicuous barely noticeable internal ridge. Trochanter I with ventral rounded ridge-like spur; coxae and legs with scattered, long, and fine setae (Fig. 8D).

Diagnostic characters

The diagnostic characters unique for the female of *H. vespertina* are a combination of the following characters: palpal segment II with pointed lateral projection and 6 dorsal, flattened, barbed setae inserted on an almost straight internal margin, with approximately 11–12 ventral, lanceolate, long, barbed medially inserted setae, palpal segment III with a rounded ventral spur; ventral process of basis capituli short, subtriangular with rounded anterior apex. Hypostome clavate with homogeneous 3:3 dental formula except crown; scutum with lateral fields and posterior border almost devoid of punctations and glabrous, median field with homogeneously distributed very large, shallow, somewhat confluent punctations; coxa I with internal short and rounded spur and external spur as a triangular ridge, coxae II–IV with a single triangular spur, coxae and other leg segments with numerous, long, and fine setae; genital aperture round with small lateral chitinous flaps, and spiracular plate with 3–4 rows of goblet cells, the central ones being much larger than those of the marginal row.

Males of *H. vespertina* can be diagnosed by the following combination of characters: basis capituli with rounded, triangular, and short cornuae; palpal segment II with pointed lateral projection and with 4 dorsal barbed setae inserted medially into concave internal margin and with approximately 9 ventral, barbed, long and lanceolate setae; ventral process of basis capituli short and triangular, hypostome clavate with homogeneous 3:3 dental formula except crown; scutum with homogeneously distributed, very large, shallow, and somewhat confluent punctations; coxa I with 2 spurs, the internal short and rounded spur, the external ridge-like, coxae II–IV with a single triangular spurs; coxae and other segments of legs with very numerous, long, and fine setae; spiracular plate as in female.

The diagnostic morphological characters for the female of *H. leporispalustris* are as follows: palpal segment II with pointed lateral projection, with 3 dorsal, fine, barbed setae inserted along the concave internal margin, posterior to the conspicuous anterior lobe, and with approximately 8 ventral fine barbed setae; palpal segment III with a rounded ventral spur; ventral process of basis capituli short and rounded; hypostome clavate with homogeneous 3:3 dental formula except crown; lateral fields and posterior margin of scutum almost devoid of punctations and glabrous, median field of scutum with homogeneously distributed medium-sized, non-confluent, shallow punctations; coxa I with two spurs, coxa II with two short, rounded, spurs, internal longer than external; coxae III–IV with a single triangular spurs; coxae with scattered, long, fine setae; spiracular plate with 3–5 lines of goblet cells, almost identical in size.

Males of *H. leporispalustris* can be diagnosed by a combination of the following characters: long, rounded cornuae; palpal segment II with pointed lateral projection and with 2–4 dorsal, barbed, fine setae inserted medially posterior to a conspicuous anterior median lobe and with approximately 5 ventral, barbed fine setae; ventral process of basis capituli short and rounded; hypostome clavate with homogeneous 3:3 dental formula except crown; scutum with unevenly distributed medium sized, shallow punctations, with a crescent-shaped glabrous area outlining the pseudoscutum and a glabrous median longitudinal line reaching the central festoon and separating the lateral punctation pattern; coxa I–II with two spurs, external shorter than internal, coxa III with a single triangular spur, coxa IV with an inconspicuous sclerotized ridge; coxae and other segments of legs with scattered, long, fine setae; spiracular plate as in female.

Species relationships

Females of *H. vespertina* can easily be distinguished from *H. leporisplaustris* and *H. mariae* by the presence on the ventral internal margin of palpal segment II of approximately 11–12 lanceolate and long barbed median setae (7–8 and 15–16 thinner, barbed setae in *H. leporispalustris* and *H. mariae,* respectively), palpal segment II dorsally with 6 barbed setae (3 fine setae in *H. leporispalustris* and *H. mariae*) and with the internal margin almost straight (concave and with an anterior lobe in *H. leporispalustris* and *H. mariae*), coxae II with one spur (2 in *H. leporispalustris* and 1 in *H. mariae*), setae on coxae and legs more numerous than in *H. leporispalustris* and *H. mariae,* and punctations in the central field of the scutum larger than in *H. leporispalustris* and *H. mariae.* In addition, *H. leporispalustris* and *H. mariae* differ in the length of the internal spur on coxa I, which is much longer in *H. mariae.*

Males of *H. vespertina* can be differentiated from those *H. leporisplaustris* and *H. mariae* by the presence on the ventral internal margin of palpal segment II of approximately 9 lanceolate and long barbed median setae (5 and 14–15 thin barbed setae in *H. leporispalustris* and *H. mariae,* respectively), palpal segment II dorsally with 4 barbed setae (2–3 and 5–6 fine setae in *H. leporispalustris* and *H. mariae,* respectively), setae of scutum longer than in *H. leporispalustris* and *H. mariae*, coxae II with 1 spur (2 in *H. leporispalustris* and 1 in *H. mariae*) and setae on coxae more numerous than in *H. leporispalustris* and *H. mariae.* In addition, *H. leporispalustris* and *H. mariae* differ by the length of the internal spur on coxa I, which is longer in *H. mariae* and by the length of the cornuae, which are larger in *H. leporispalustris* than in *H. mariae.*

Haemaphysalis juxtakochi is the other closely related species of the genus *Haemaphysalis* sporadically collected in the U.S. (Keirans & Restifo, 1993). This tick can clearly be differentiated from *H. vespertina, H. leporispalustris* and *H. mariae* adults by the presence of a hypostome with a dental formula of 4:4, segment III of palps with a longer and retrograde ventral spur, and ventral processes of the basis capituli absent (Cooley, 1946).

Discussion

Analyses of all datasets, whether of individual genes or concatenated sequence fragments, concurred in finding the western *H. vespertina* to be a strongly supported monophyletic group, sister to clade A (=*H. leporispalustris* s.l.), which contains lineages from the rest of the U.S (Fig. 2). This corroborates the morphological findings, and shows that *H. vespertina* is well defined by both phenotypic and molecular fixed characters. California, a known hotspot of diversity and endemism in the U.S. (Davis *et al.*, 2008), has already proven to be an important area of tick endemism (Backus *et al.*, 2022; Furman & Loomis, 1984; Lado *et al.*, 2021). The extent of *H. vespertina*'s geographical

distribution remains to be determined. In our study, only specimens from California were examined genetically; however, specimens from Oregon were morphologically identical to *H. vespertina.* Therefore, its distribution certainly extends beyond California in the Western US. Samples from Montana and Idaho (Apanaskevich 2024), share some aspects with *H. vespertina*, but they appear to be distinctly larger. Their morphology should be examined in more detail for a meaningful taxonomic assessment, and DNA sequences should be generated for further comparisons. In California, there are previous records of *H. leporispalustris* (= *H. vespertina*) from several lagomorphs: *Sylvilagus audubonii* (Baird)*, Sylvilagus bachmani* (Waterhouse)*,* and *L. californicus* (Furman & Loomis, 1984; Merino, 1967, Schmitz *et al.*, 2014). It is important to mention that the Costanoa adult ticks we examined were all collected by flagging the vegetation, a method that usually fails to yield significant numbers of adult *H. leporispalustris* in the eastern U.S.

Within *H. leporispalustris* s.l. (clade A, Fig. 2A–B) we observed considerable genetic divergence as noted in a prior study (Thompson *et al.*, 2020). Clades C and D are strongly supported within clade A. The morphological evidence provided for considering *H. mariae* (clade D) to be a distinct species is quite compelling (Apanaskevich, 2024). Clade C appears to encompass mostly ticks collected north of New Jersey, from New York state to Maine and Canada, while clade D is mostly found in the south-central part of the U.S. The molecular findings, however, do not support these two lineages within *H. leporispalustris* s.l. as being fully distinct species. Indeed, the phylogenetic species concept would require Clade A to be fully resolved, and not polytomic, with mutually excluding monophyletic lineages in its midst. The remaining lineages in clade A have a wide geographic distribution from Texas to Florida and New York state. The taxonomic status of all lineages within Clade A will need to be further assessed. It appears that, as was the case for *I. mojavensis-I.minor* (Backus *et al.*, 2022) and *A. maculatum* morphotype II and III (Lado *et al.*, 2018), speciation events within *H. leporispalustris* s.l. are very recent or, possibly, still ongoing. In this study, *ITS2* and total evidence analyses provided slightly more information than the three mitochondrial gene sequences combined (Supplemental File). Nevertheless, additional markers, such as microsatellite or SNP loci, could suitably resolve some of the main questions raised by our phylogenetic results as was the case with *A. maculatum* s.l. (Lado *et al.*, 2018; Dorsey *et al.*, 2025.)

The presence of monophyletic clades C and D within a cluster of unranked lineages may be evidence of past segregation, either due to survival in ecologically disjoint geographical refugia or temporary association to specific lagomorph taxa, themselves isolated from each other by environmental conditions. Introduction of different, but reproductively compatible, tick genotypes through bird migration, but maybe also following the introduction of cottontail rabbits in many areas of the eastern states (for hunting), could have caused partial blurring of the initial divergence signal in clade A.

Given the diversity in geographic origin of the lineages aligned along the basal polytomy of clade A, it would be premature to redescribe immature specimens for the eastern part of the U.S. as they could belong to different species. Tick colonies should be established from North Carolina specimens matching the redescription of the adult type material, in order to be able to describe the *H. leporispalustris* s.s. immatures with confidence. Only then, could other immature morphotypes found in the eastern and north-eastern areas of the U.S. be further characterized. Nevertheless, given the geographic origin of the examined specimens, description of *H. leporispalustris* in Clifford *et al.* (1961) may well correspond to the "real" *H. leporispalustris* larva.

The position of WI-F (from Wisconsin), and NJ-Mon (from New Jersey), basal to *H. mariae*, in the *ITS2* containing phylogenetic reconstructions, also requires further investigation. The inclusion of these two lineages in Clade D is, at best, fragile but, for instance, it would be important to know if these two ticks are morphologically similar to *H. mariae*, a tick described so far from Texas, Colorado, and Oklahoma, or to other *H. leporispalustris* s.l. groups. Although mitochondrial genes sequences are marginally informative, GenBank BLAST comparison of the 12SrDNA and 16SrDNA gene sequences reveal that the closest relative of both WI-F and NJ-Mon, is a sample collected in Georgia (Norris *et al.*, 1999). *Haemaphysalis leporispalustris* s.l. immature ticks are known to parasitize birds, although we do not know if *H. mariae* larvae and nymphs also parasitize avian hosts. Migratory birds carry exotic ticks to the U.S. on a regular basis and, sometimes, contribute to establish temporary or permanent imported tick populations far away from their area of origin (Mukherjee *et al.*, 2014). The fact that the two ticks have been collected in such different areas (Wisconsin and New Jersey) and that their seemingly closest relative was collected in Georgia, would probably indicate that the evolutionary geographical origin of Clade D (*H. mariae*) may be found in Central or South America at the confluence of the two most trafficked bird flyways that bring birds to the Great Lakes or the eastern coast, respectively. While our markers might not be sufficiently informative to resolve the

polytomy at the base of Clade A, a larger sampling of *H. leporispalustris*, including specimens from South America, could certainly prove helpful in finding missing basal lineages and in providing better clade ranking.

In this study we have described all stages of a new species, *H. vespertina*, from the western U.S. and have redescribed the adult stage of *H. leporispalustris*, a necessary step in order to further untangle the taxonomic complexity of this virtually ignored taxonomic group. Additional field surveys are needed to fully describe and compare the ecological features associated with the different phylogenetic lineages.

Acknowledgements

We thank Adam Baldinger of the Harvard Museum of Comparative Zoology for allowing us to examine the type series of *H. leporispalustris*. The National Ecological Observatory Network is a program sponsored by the U.S. National Science Foundation and operated under cooperative agreement by Battelle. This material uses specimens and/or samples collected as part of the NEON Program.

We thank the Monmouth County Board of County Commissioners for their ongoing support of the Tick-borne Disease Program, and the following individuals for contributing specimens: Katie Clow, Francisco C. Ferreira, Julia González González, Robert A. Jordan, Terry Riotto, James L. Occi, Matthew Bickerton, Joanna Barron, Sam Wisely, Kristen Wilson, Manuel Zavala, Christopher Kilonzo, Viva Kobbekaduwa, Jean Tsao, and the Virginia Department of Health Vector-borne Disease Team: David Gaines, Kyle Girone, Elena Diskin, and Joshua Bernick.

Publication Disclaimer

The findings and conclusions in this article are those of the authors and do not necessarily represent the views or opinions of the California Department of Public Health or the California Health and Human Services Agency.

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Supplementary Materials. The following supporting information can be downloaded at the DOI landing page of this paper.

Supplemental Files. Bayesian phylogenetic trees of individual loci: S1, *12SrDNA*; S2, *16SrDNA*; S3, *cox1*; S4, *ITS2* with WI-F; S5, *ITS2* without WI-F; and S6, concatenated nuclear-mitochondrial (without WI-F).