2015

Identification Guide to Larval Stages of Ticks of Medical Importance in the USA

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Identification guide to larval stages of ticks of medical importance in the USA

An Honors Thesis submitted in partial fulfillment of the requirements for Honors in Biology

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ABSTRACT

Scanning Electron Micrographs were prepared of four morphologically diagnostic regions (dorsal capitulum, ventral capitulum, scutum, leg coxae) for the larval stage of the 16 species of ixodid (hard) ticks known to parasitize humans in the USA. These species are: *Amblyomma americanum, A. maculatum, A. mixtum, A. tuberculatum, Dermacentor albipictus, D. andersoni, D. occidentalis, D. variabilis, Haemaphysalis leporispalustris, Ixodes angustus, I. cookei, I. pacificus, I. scapularis, I. spinipalpis, I. texanus*, and *Rhipicephalus sanguineus*. Based on the morphological characters observed, a dichotomous identification key to ixodid larvae that parasitize humans in the USA was prepared. Common names, hosts and geographical distributions are included for each tick species.

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April 2015
Biology Department
University Honors Program
Georgia Southern University

Introduction

Wherever present, ticks pose a threat to humans and animals. They are vectors of numerous pathogenic agents that are responsible for disease and fatalities worldwide and are second only to mosquitoes as arthropods of medical-veterinary importance (Oliver 1989). During blood-feeding, ticks can transmit of the causative agents of Rocky Mountain Spotted Fever, Human Monocytic Ehrlichiosis, Human Granulocytic Anaplasmodsis, Lyme disease, Q-fever, Tularemia, Colorado tick fever, and several other vector-borne diseases, in North America alone (Nicholson et al. 2009). Some feeding ticks can also cause paralysis or various forms of toxicosis in their hosts (Durden and Mans, 2015). In addition to affecting human health, ticks and tick-borne diseases can decimate livestock and have a severe monetary impact (Nicholson et al. 2009).

Many tick larvae cannot currently be identified to species so their medical-veterinary importance including their role in pathogen transmission is insufficiently known. However, ticks of all stages (larvae, nymphs, adult males and females) have distinct morphological attributes that can allow them to be accurately identified. A combination of morphological characters of the capitulum (including the hypostome), leg coxae, and scutum (Figures 1, 2) can be used to identify ticks of all stages to species. However, to date, very few identification guides to tick larvae have been available and none have used Scanning Electron Micrographs SEMs), which accurately illustrate key morphological characters, to differentiate species. For the eastern United States, Clifford et al. (1961) produced a key to ixodid (hard tick) larvae and included some line drawings of various species but not all known eastern U.S. species were included in that guide. Similarly, Robbins and Keirans (1992) provided SEMs for the larvae of all 5 U.S. species in the subgenus *Ixodiopsis* (genus *Ixodes*) and Webb et al. (1990) and Kleinjan and Lane (2008) provided diagnostic illustrations and keys to tick larvae belonging to the genus *Ixodes* in California. However, there is no available guide to the larval stages of hard ticks that parasitize humans across the USA and, except for ticks belonging to the subgenus *Ixodiopsis*, none of these larvae have been illustrated using SEMs. Being able to identify tick larvae to species will allow future researchers to determine the medical-veterinary importance of tick larvae of various species including their role in the transmission of pathogens. Knowing exactly which species of ticks can transmit which pathogens in their larval stage would also be highly beneficial for implementing control and treatment programs. Because some tick-borne diseases are only transmitted by certain species, the identification of ticks of all stages is essential to linking tick-borne illnesses to their vectors. This information can also then be used to estimate the geographical range for those particular tick-borne diseases. Being able to match human-biting tick larvae with particular tick-borne diseases will also be important for elucidating the transmission dynamics of recently discovered tick-borne pathogens in the USA including Heartland virus and Bourbon virus (Swei et al. 2013, Kosoy et al. 2015).

As an example, the lone star tick, *Amblyomma americanum*, is known to transmit the causative agents of Human Monocytic Ehrlichiosis, Southern Tick Associated Rash Illness (STARI), and a recently discovered bunyavirus belonging to the genus *Phlebovirus* named Heartland virus (HRTV). As of April 2013, there were two cases of HRTV reported in the United States, both in Missouri (Swei, 2013). Because HRTV is fairly new to the United States, there is little information on how to treat, diagnose, and prevent the disease. If lone star tick larvae can be distinguished from larvae of other species and their populations can be reduced or removed from the area, the transmission of HRTV can be intercepted and prevented from spreading to other parts of the United States.

While some tick species are relatively indiscriminate feeders and will feed on a wide variety of different host species, others are more selective and some are host-specific to a single host species, especially as adult ticks. A North American example of tick-host specificity involves *Amblyomma tuberculatum,* the adults of which exclusively parasitize the gopher tortoise, *Gopherus polyphemus* (Ennen and Qualls, 2011). However, the nymphs and larvae of many tick species are progressively less discriminate in their feeding habits (Durden, 2006). For example, the larvae of *A. tuberculatum* are known to feed on a variety of reptiles, birds and mammals, including humans (Clifford et al., 1961).

Vectors can be difficult to control and the case fatality rates associated with the disease agents they transmit can be remarkably high (Schmidt, 2013). Wherever present, ticks pose a threat to humans and animals. This makes the understanding of tick species identification, hosts and geographical distributions especially important.

The main aim of this project was to prepare an identification guide to the larval stages of medically important ticks of the United States. The guide will be useful to the medical and veterinary community including researchers across North America. This aim was met by:

1. Matching larval ticks with conspecific identified adults.
2. Using a scanning electron microscope to prepare SEM images of morphological characters of larvae.

3. Preparing a dichotomous identification key to larvae for the species included in this project.

4. Recording information about the geographical range, hosts, and medical/veterinary importance for each tick species included in the guide.

Tick species included in this study are those hard tick (family Ixodidae) species recorded to attach to humans in the United States by Merten and Durden (2000) in their survey of human-infesting tick species. The following U.S. tick species of major medical importance and a few additional species of lesser importance are therefore included in this identification guide:

- ***Amblyomma americanum*** (lone star tick)
- ***Amblyomma mixtum*** (cayenne tick)
- ***Amblyomma maculatum*** (Gulf Coast tick)
- ***Amblyomma tuberculatum*** (gopher tortoise tick)
- ***Dermacentor albipictus*** (winter tick)
- ***Dermacentor andersoni*** (Rocky Mountain Wood Tick)
- ***Dermacentor occidentalis*** (West Coast Tick)
- ***Dermacentor variabilis*** (American Dog Tick)
- ***Haemaphysalis leporispalustris*** (Rabbit tick)
- ***Ixodes angustus*** (no common name)
- ***Ixodes cookei*** (no common name)
- ***Ixodes pacificus*** (Western Blacklegged Tick)
- ***Ixodes scapularis*** (Blacklegged Tick)
- ***Ixodes spinipalpis*** (no common name)

-***Ixodes texanus*** (no common name)

- ***Rhipicephalus sanguineus*** (Brown Dog Tick)

Materials and Methods

Tick specimens were selected from collections in the United States National Tick Collection (Georgia Southern University) or the United States Department of Agriculture (Agricultural Research Service – Veterinary Services) Tick Collection (USDA) (Ames, Iowa).

Two principal techniques for identifying larval ticks were employed. Firstly, the identity of some larvae was known because they represented the progeny (from eggs) of identified adult female ticks. Secondly, the identity of some tick larvae used in this project was confirmed by previous molecular matching with DNA from identified adult ticks (Beati & Keirans, 2001). This involved using the Polymerase Chain Reaction (PCR) to amplify and match DNA sequences from selected genes of identified adult ticks and larvae. Some larvae of host-specific ticks were tentatively identified because they were co-collected (on the same host individual) with conspecific nymphs or adults. This latter technique further involved confirmation of larval identities via molecular matching.

Selected, identified tick larvae were prepared for Scanning Electron Microscopy (SEM) firstly by removing debris from individual specimens with fine probes while they were observed under a low-power binocular microscope (MEIJI Model. EMZ-TR). Individual larvae were then positioned on SEM specimen stubs using SEM double-stick tape (Ted Pella Company, Redding, CA) and sputter-coated with a fine coat of gold using a DENTON DESK II sputter coater. Coated specimens were then examined using a JOEL JSM-6610 LV scanning electron microscope (SEM) in the GSU Biological Sciences Building. SEM image files were saved on CDs and then organized into plates and labeled using Adobe Illustrator™ and Microsoft Word. Four images for the larva of each species were prepared and organized into plates. These images show the Dorsal Capitulum, Ventral Capitulum, Scutum, and Leg Coxae, respectively, for each species. Based on morphological characters shown in the SEMs, a dichotomous identification key was prepared to the larvae included in this study. Information on hosts, range, and medical importance was recorded from literature searches and from specimens accessioned into the U.S. National Tick Collection and USDA Tick Collection databases.

Results

SEM plates were prepared to show key morphological characters for the larval stage for all 16 human-biting ticks included in this study (Figs. 5-20). Based on these characters, the following identification key to human-biting hard tick larvae in the USA is presented.

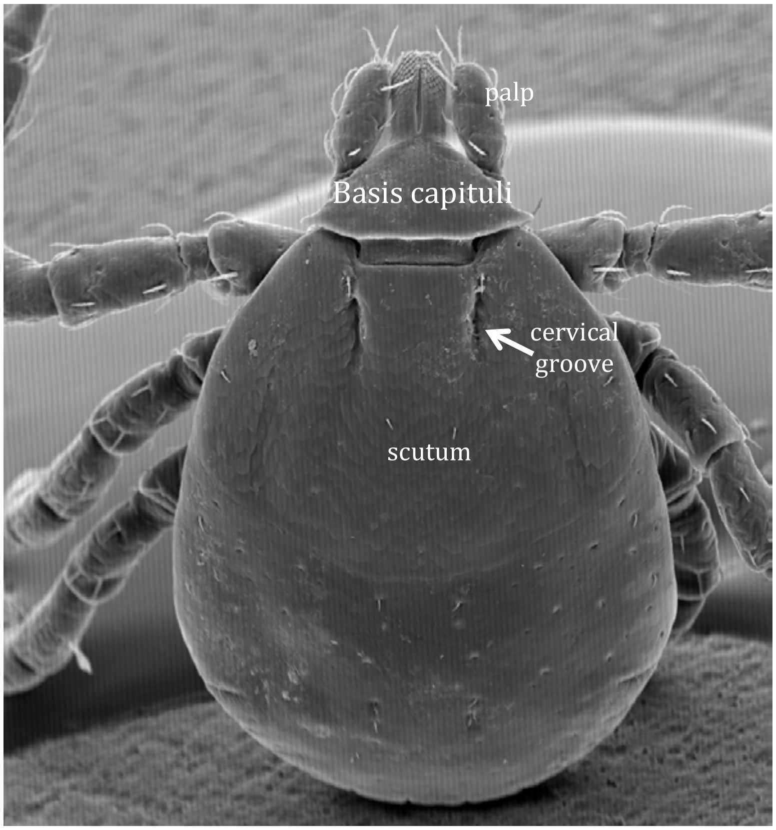

Fig 1. SEM of dorsal view of larval ixodid tick with key morphological characters identified

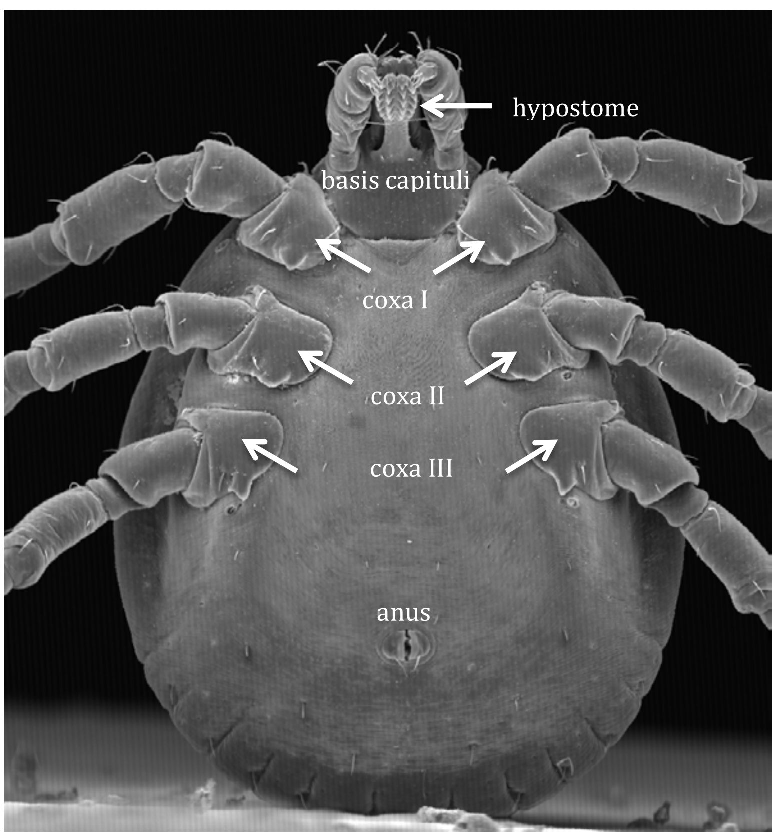

Fig 2. SEM of ventral view of larval ixodid tick SEM with key morphological characters identified

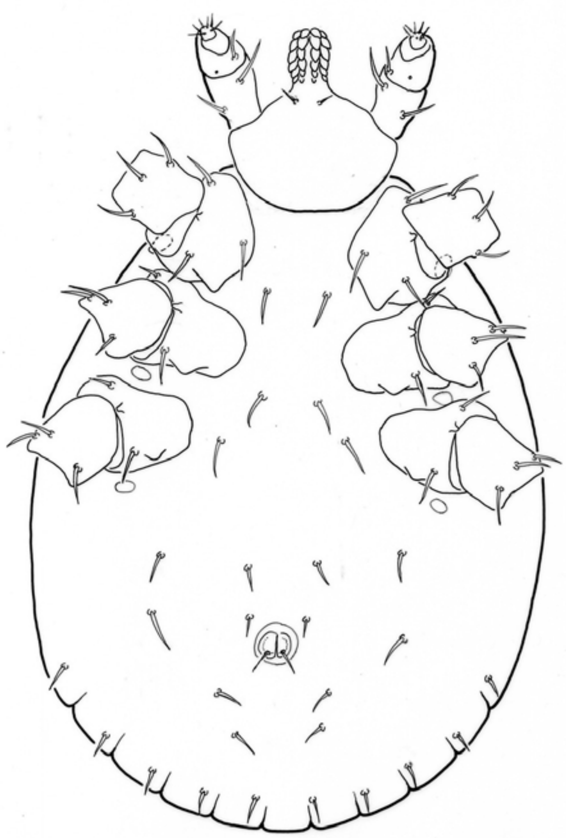

Fig 3. non *Ixodes* tick

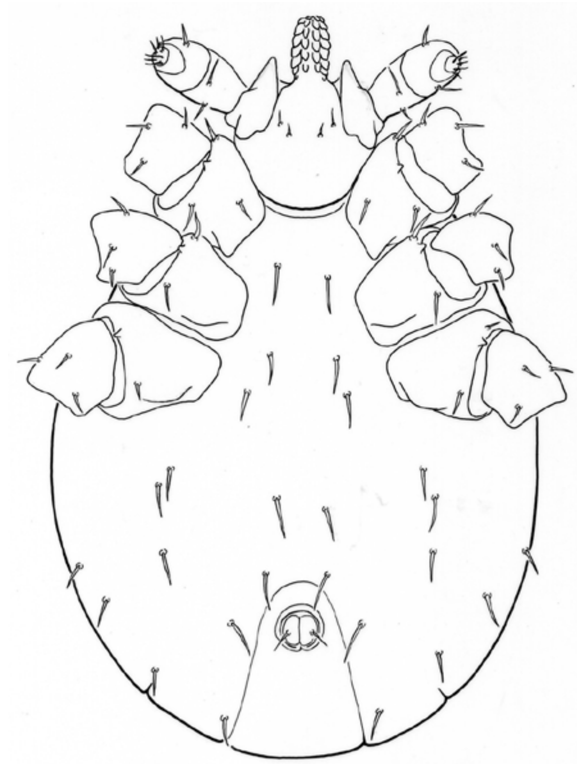

Fig 4. *Ixodes* tick

DICHOTOMOUS IDENTIFICATION KEY TO IXODID (HARD) TICK LARVAE PARASITIZING HUMANS IN THE USA

1A. Anal groove not extending anteriorly around anus (Figure 3)…………………......…7

1B. Anal groove extending anteriorly around anus (Figure 4) (genus *Ixodes*)…...………2

2A. Palpal segment 2 extending anteriorly and posteriorly (Fig. 14B) ….. *Ixodes angustus*

2B. Palpal segment 2 not extending anteriorly and posteriorly …………….…….……3

3A. Palps broad and relatively short (Figs. 15B, 19B) ………………………………..…..4

3B. Palps narrow and long (Figs. 16B, 17B, 18B) ……………………………………….5

4A. Dorsal basis capituli almost triangular (Fig. 15A); small internal spur on coxa I (Fig. 15D) ……………………………………………………………..………. *Ixodes cookei*

4B. Dorsal basis capituli squarish (Fig. 19A); no coxal spurs (Fig. 19D)………………….

…………………………………………………………………..…..……*Ixodes texanus*

5A. Tip of hypostome pointed (Fig. 17B); tiny extensions (auriculae) present on ventral basis capituli (Fig. 17B) ……………………………………………….....*Ixodes scapularis*

5B. Tip of hypostome rounded (Figs. 16B, 18B); large shelf-like extensions (auriculae) present on ventral basis capituli (Figs. 16B, 18B)………………………...……………6

6A. Palps longer (Fig. 18A) ………………………………...………..*Ixodes spinipalpis*

6B. Palps shorter (Fig. 16A)………………………………………….…….*Ixodes pacificus*

7A. Basis capituli almost hexagonal (Fig. 20A) ……………….*Rhipicephalus sanguineus*

7B. Basis capituli almost triangular or squarish (e.g., Figs. 8A, 12A, 13A)………...……8

8A. Palpal segment II greatly expanded laterally so palps appear triangular (Fig. 13AB)……………………………………….…….………*Haemaphysalis leporispalustris*

8B. Palpal segment II not greatly expanded laterally; palps not triangular ………..……..9

9A. Palpal segment II much longer than broad (Figs. 5AB, 6AB, 7AB, 8AB) (genus *Amblyomma)* ……………………………………………………………………………10

9B. Palpal segment II about as long as broad (Figs.9AB, 10AB, 11AB, 12AB) (genus *Dermacentor*)………………………………………………………………………….13

10A. Palps and hypostome short (Fig. 7AB); postero-lateral angles of dorsal basis capituli acute (Fig. 7A)…………………………………………..…….*Amblyomma maculatum*

10B. Palps and hypostome long (Figs. 5AB, 6AB, 8AB); postero-lateral angles of dorsal basis capituli rounded (Figs. 5A, 6A, 8A)……………………………………………….11

11A. External spur on coxa I pointed and distinctly larger than internal spur (Fig. 5D) ……………………………………………..……………… *Amblyomma americanum*

11B. External spur on coxa I rounded and not distinctly larger than internal spur (Figs. 6D, 8D)…………………………………………………………...………………..12

12A. Posterior margin of scutum rounded (Fig. 8C)………..….*Amblyomma tuberculatum*

12B. Posterior margin of scutum slightly concave on either side (Fig. 6C)……………….. ………………………………………………………………...……... *Amblyomma mixtum*

13A. Palps very broad (Fig. 9AB); postero-lateral angles of dorsal basis capituli not extended laterally (Fig. 9A) …………………………..………….*Dermacentor albipictus*

13B. Palps less broad (Figs. 10AB, 11AB, 12AB); postero-lateral angles of dorsal basis capituli extended laterally (Figs. 10A, 11A, 12A)…………………………...………..14

14A. Posterior margin of scutum slightly concave on either side (Fig. 11C)……………… ……………………………………………………….………… *Dermacentor occidentalis*

14B. Posterior margin of scutum rounded (Figs. 10C, 12C)…………….…………………15

15A. Postero-lateral angles of dorsal basis capituli acute (Fig. 10A) ……………………...

……………………………………………………………...……..*Dermacentor andersoni*

15B. Postero-lateral angles of dorsal basis capituli not acute (Fig. 12A) ………………...

………………………………………………………………… *Dermacentor variabilis*

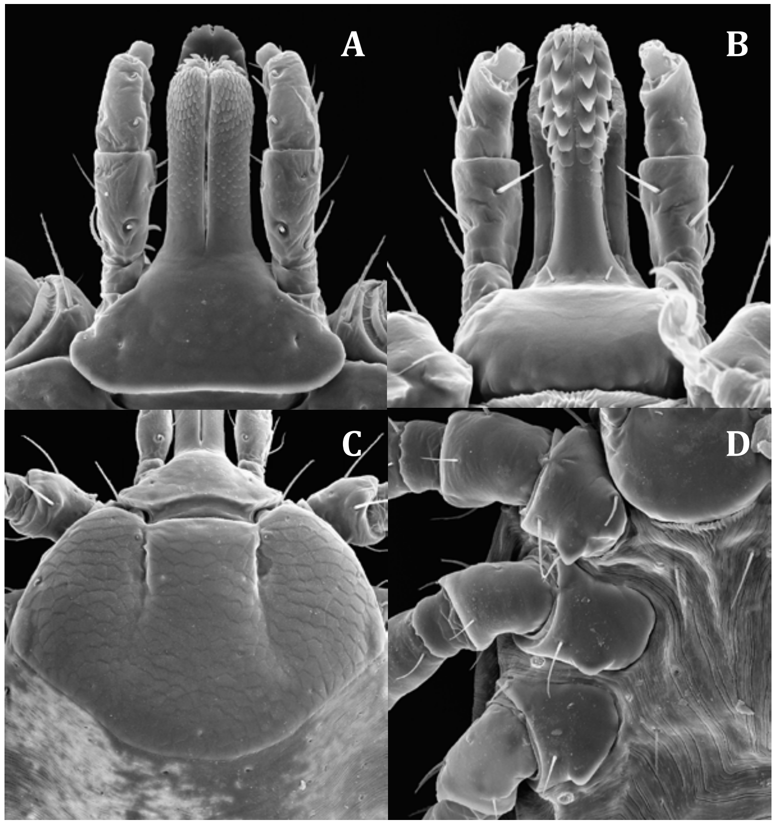

Fig. 5 *Amblyomma americanum*. A, Capitulum, dorsal view. B, Capitulum, ventral view. C, scutum. D, Coxae I-III.

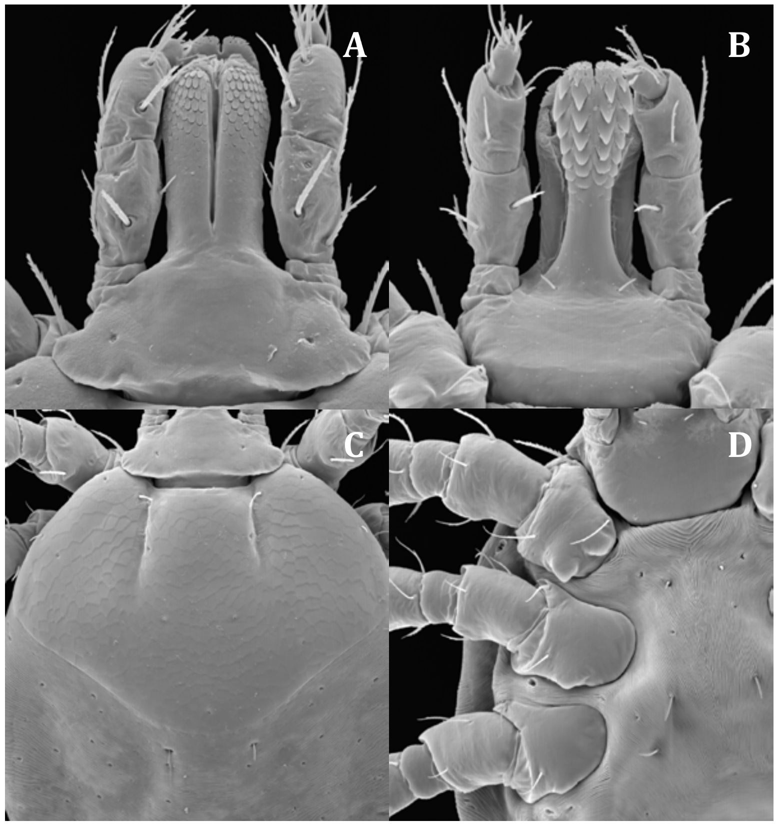

Fig. 6 *Amblyomma mixtum*. A, Capitulum, dorsal view. B, Capitulum, ventral view. C, scutum. D, Coxae I-III.

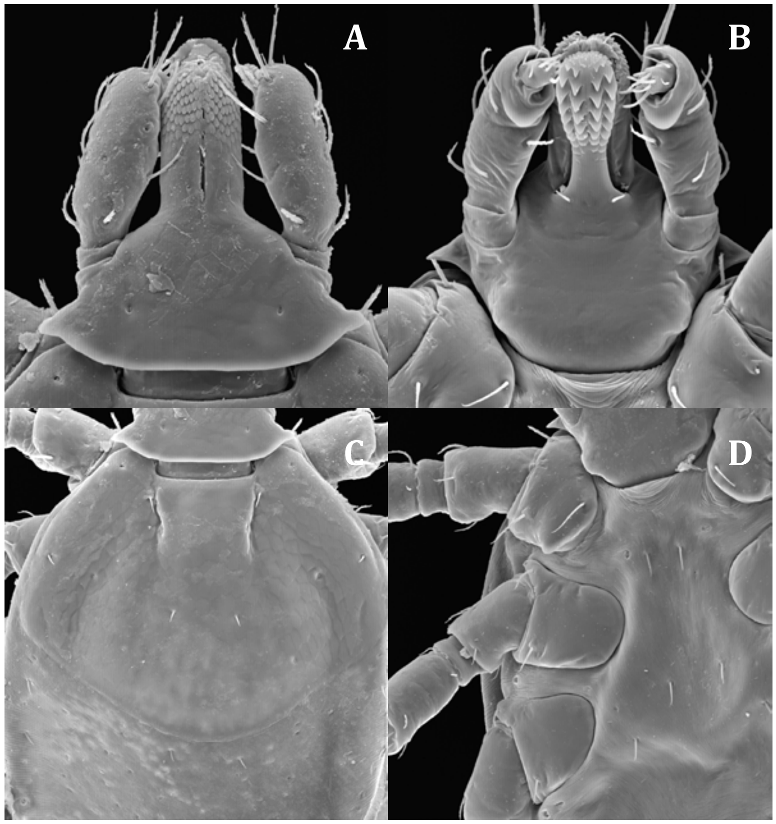

Fig. 7 *Amblyomma maculatum*. A, Capitulum, dorsal view. B, Capitulum, ventral view. C, scutum. D, Coxae I-III.

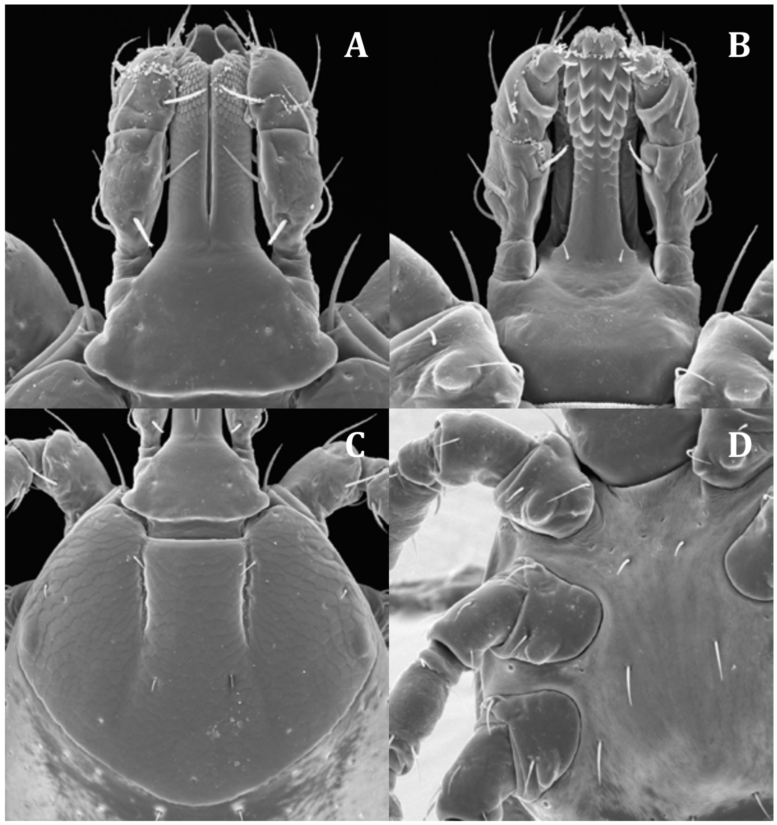

Fig. 8 *Amblyomma tuberculatum*. A, Capitulum, dorsal view. B, Capitulum, ventral view. C, scutum. D, Coxae I-III.

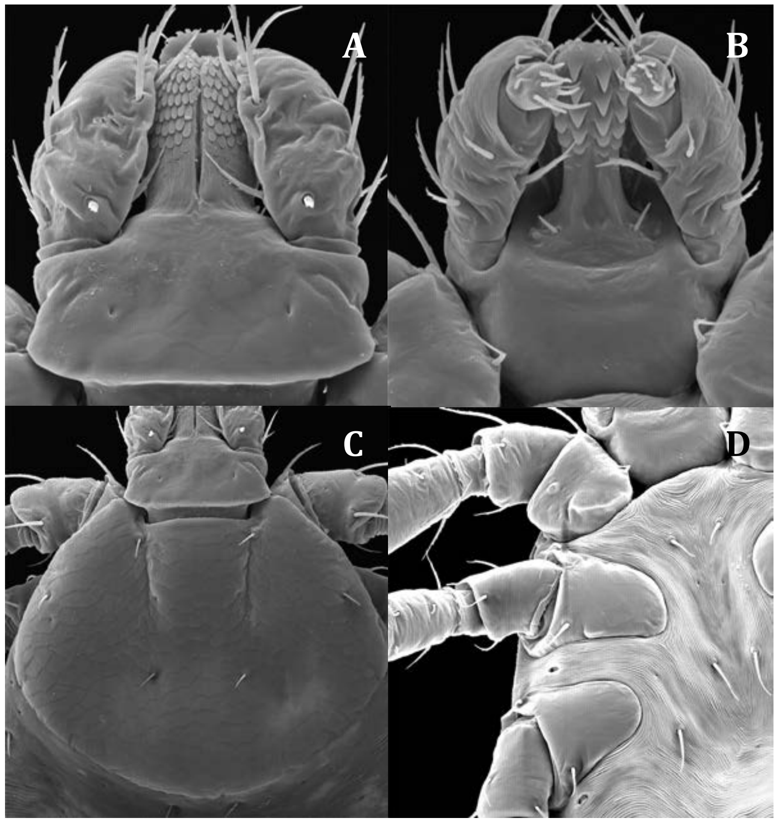

Fig. 9 *Dermacentor albibictus*. A, Capitulum, dorsal view. B, Capitulum, ventral view. C, scutum. D, Coxae I-III.

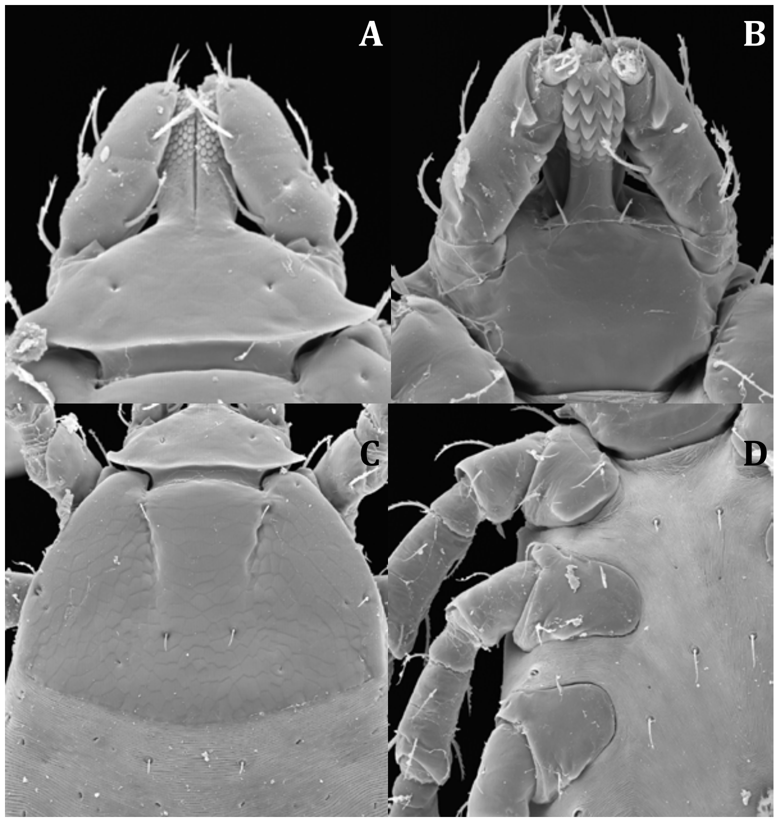

Fig. 10 *Dermacentor andersoni*. A, Capitulum, dorsal view. B, Capitulum, ventral view. C, scutum. D, Coxae I-III.

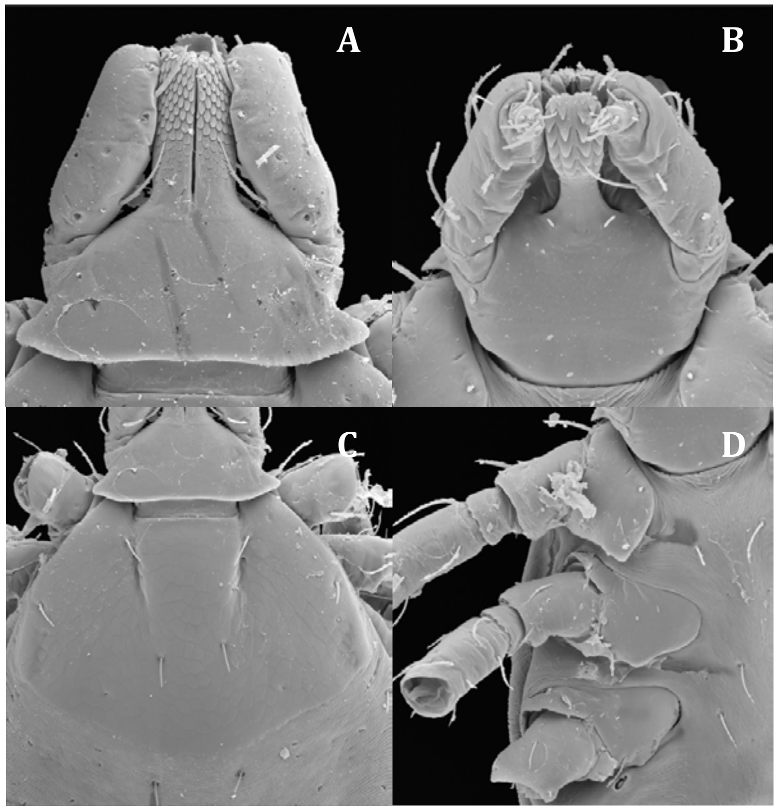

Fig. 11 *Dermacentor occidentalis*. A, Capitulum, dorsal view. B, Capitulum, ventral view. C, scutum. D, Coxae I-III.

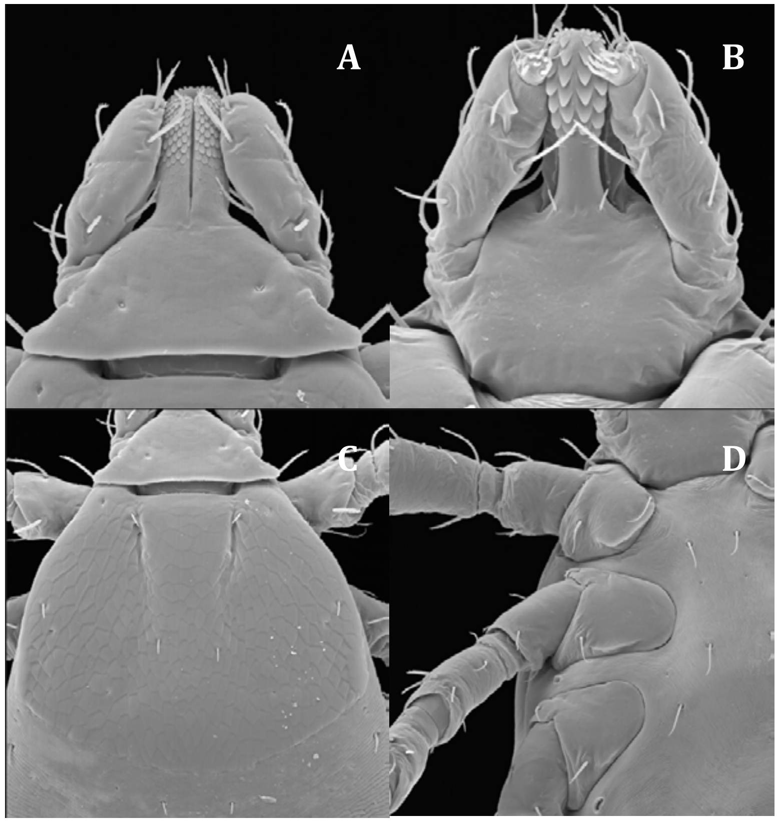

Fig. 12 *Dermacentor variabilis*. A, Capitulum, dorsal view. B, Capitulum, ventral view. C, scutum. D, Coxae I-III.

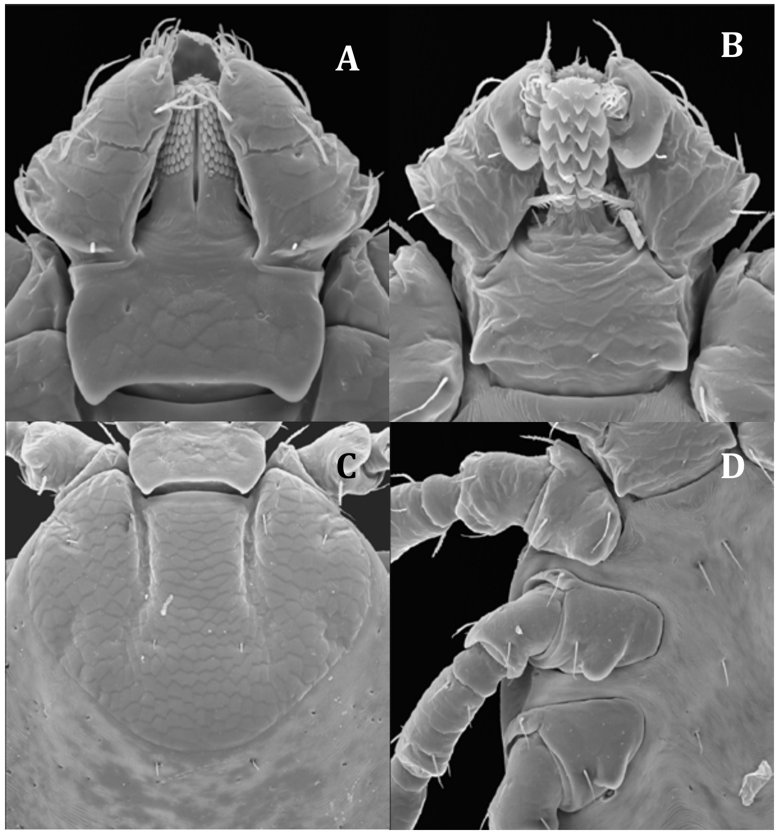

Fig. 13 *Haemaphysalis leporispalustris*. A, Capitulum, dorsal view. B, Capitulum, ventral view. C, scutum. D, Coxae I-III.

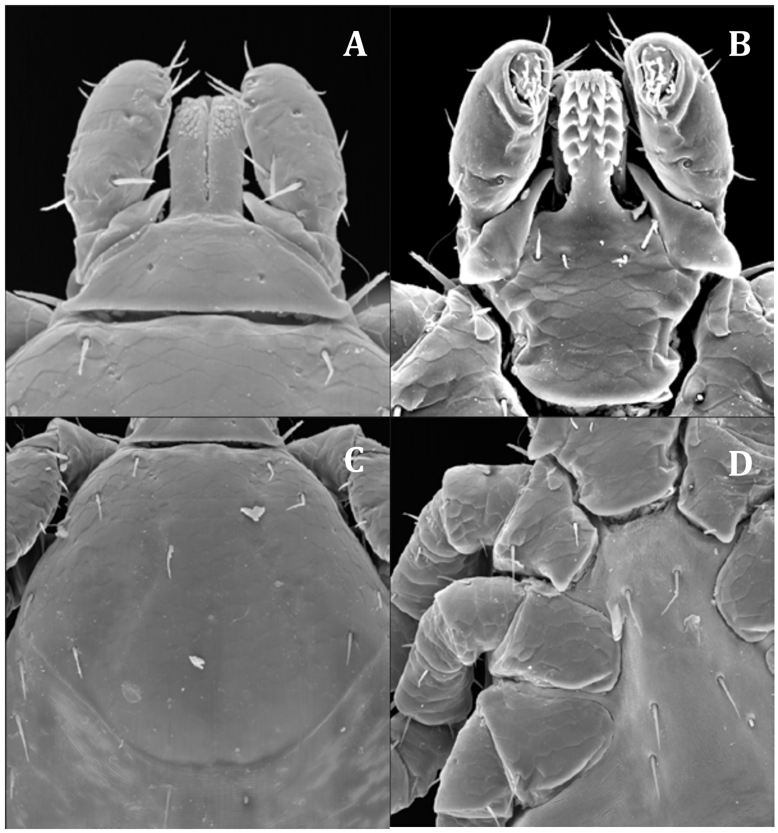

Fig. 14 *Ixodes angustus*. A, Capitulum, dorsal view. B, Capitulum, ventral view. C, scutum. D, Coxae I-III.

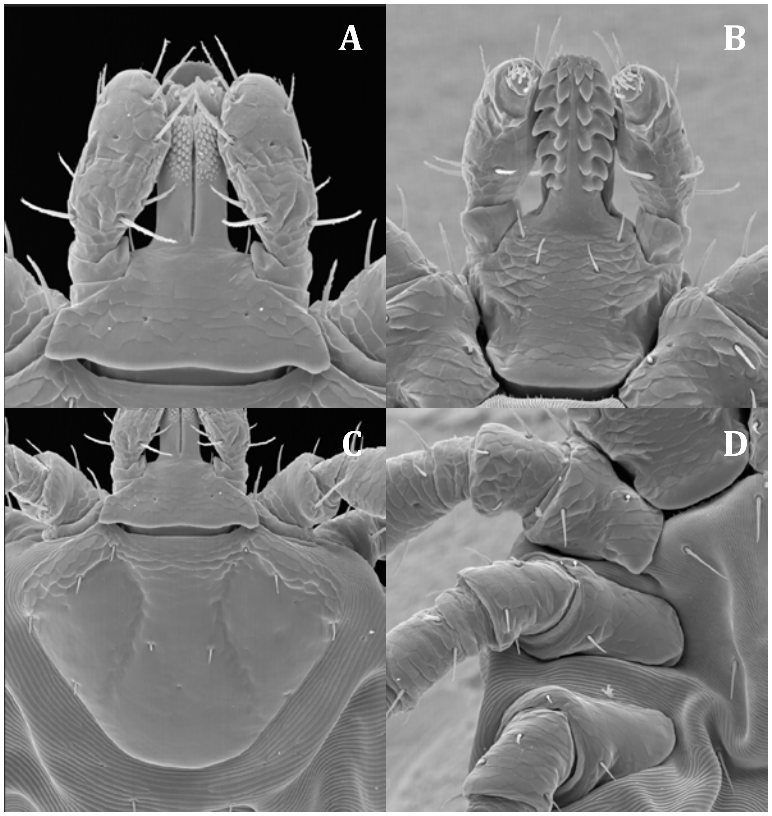

Fig 15 *Ixodes cookei*. A, Capitulum, dorsal view. B, Capitulum, ventral view. C, scutum. D, Coxae I-III.

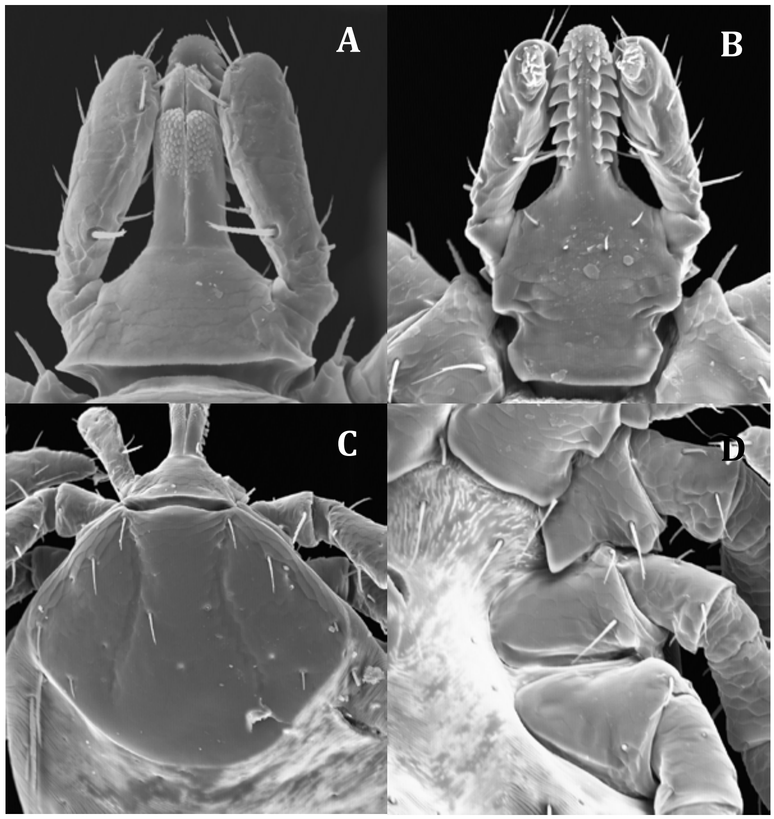

Fig. 16 *Ixodes pacificus*. A, Capitulum, dorsal view. B, Capitulum, ventral view. C, scutum. D, Coxae I-III.

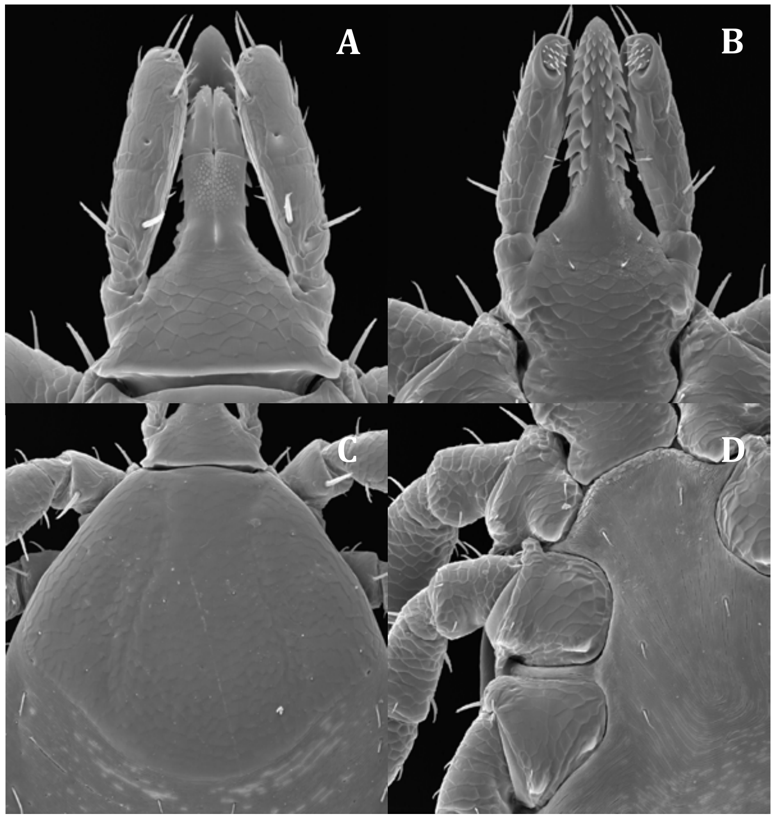

Fig. 17 *Ixodes scapularis*. A, Capitulum, dorsal view. B, Capitulum, ventral view. C, scutum. D, Coxae I-III.

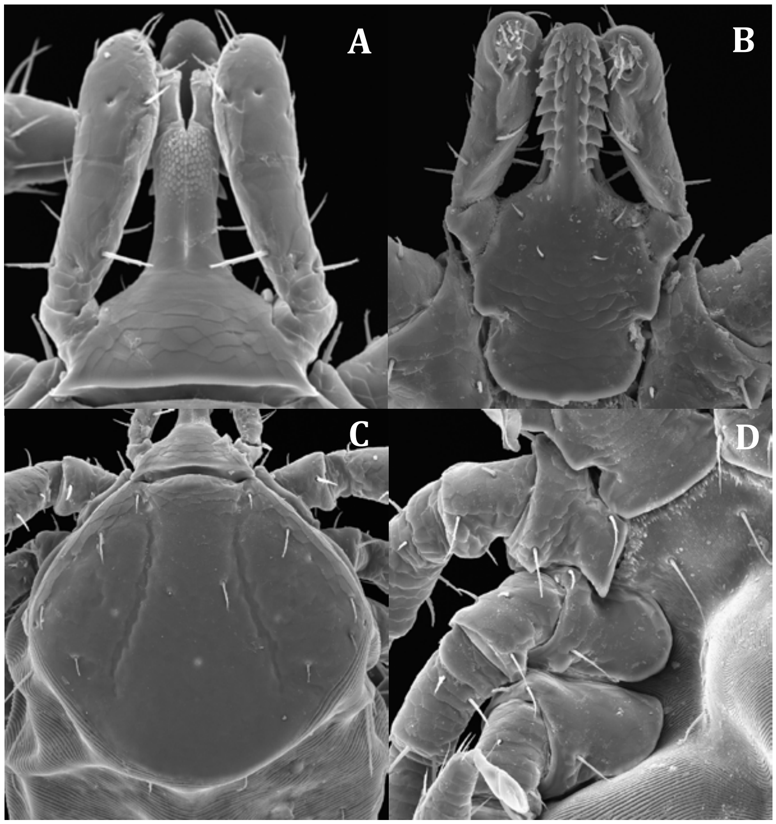

Fig. 18 *Ixodes spinipalpis*. A, Capitulum, dorsal view. B, Capitulum, ventral view. C, scutum. D, Coxae I-III.

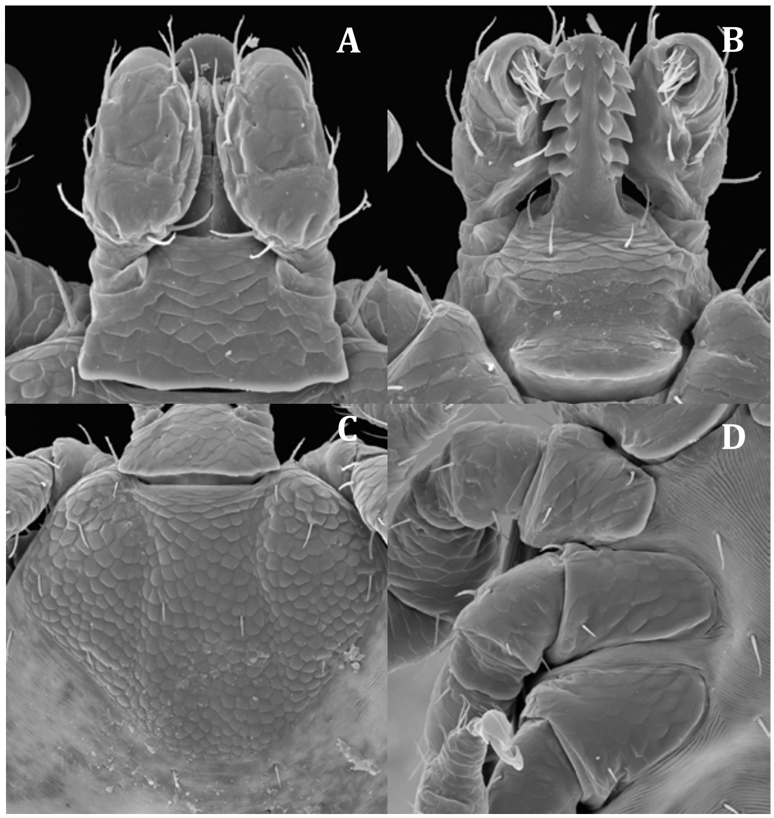

Fig. 19 *Ixodes texanus*. A, Capitulum, dorsal view. B, Capitulum, ventral view. C, scutum. D, Coxae I-III.

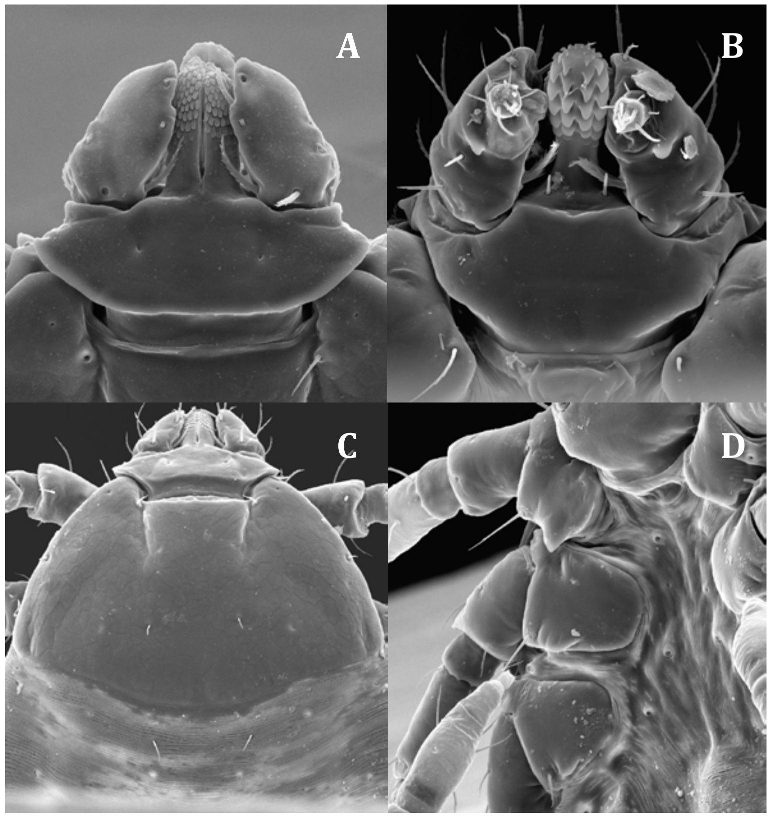

Fig. 20 *Rhipicephalus sanguineus*. A, Capitulum, dorsal view. B, Capitulum, ventral view. C, scutum. D, Coxae I-III.

Discussion

An illustrated identification key to the larval stages of the human-biting hard ticks of the USA is presented for the first time. Although SEMs have been used to illustrate important morphological characters for each tick species, a scanning electron microscope is not needed in order to identify these ticks. The SEMs merely provide high quality images of features that can easily be observed using a low-power binocular microscope. The benefits of being able to identify the larval stage of these ticks has already been emphasizes in the Introduction. The following notes provide information on the medical/veterinary importance, hosts and geographical distribution of the 16 ticks included in this identification guide.

Tick Species	Main Host(s) for larvae	Distribution	Disease(s) Transmitted
Amblyomma americanum (Lone Star Tick) (Fig. 5)	White-tailed deer, humans, raccoons, opossums, birds	Southeastern and Eastern United States	Heartland virus, Human Monocytic Ehrlichiosis, Tularemia, Southern Ttick Associated Rash Illness
Amblyomma mixtum (Cayenne Tick) (Fig. 6)	Birds, small mammals	Texas	Spotted fever
Amblyomma maculatum (Gulf Coast Tick) (Fig. 7)	Birds, rodents	Coastal areas of the United States along the Atlantic coast and the Gulf of Mexico	Spotted fever
Amblyomma tuberculatum (Gopher Tortoise Tick)	Reptiles, birds, mammals	Southeastern United States	None to humans

(Fig. 8)			
Dermacentor albipictus (Winter Tick) (Fig. 9)	Moose, deer, elk, cattle	Throughout the U.S.	None to humans
Dermacentor andersoni (Rocky Mountain Wood Tick) (Fig. 10)	Rodents	Rocky Mountain states	Rocky Mountain spotted fever, tick paralysis, Colorado tick fever
Dermacentor occidentalis (West Coast Tick) (Fig. 11)	Rodents	Western U.S.	Rocky Mountain spotted fever, tick paralysis
Dermacentor variabilis (American Dog Tick) (Fig. 12)	Rodents	East of Rocky Mountains; limited areas of Pacific Coast	Rocky Mountain spotted fever, tularemia, tick paralysis
Haemaphysalis leporispalustris (Rabbit Tick) (Fig. 13)	Rabbits, birds	Eastern United States; also some western states	Rocky Mountain spotted fever, Tularemia
Ixodes angustus (no common name) (Fig. 14)	Rodents, insectivores, rabbits	Higher elevations and latitudes in Western and Eastern U.S.	Lyme disease
Ixodes cookei (no common name) (Fig. 15)	Rodents, raccoons	Eastern United States	Lyme disease, Powassan virus
Ixodes pacificus (Western Blacklegged Tick (Fig. 16)	Lizards,birds, rodents	Pacific coast of the United States	Human Granulocytic Anaplasmosis, Lyme disease, tick paralysis
Ixodes scapularis (Blacklegged Tick) (Fig. 17)	Lizards, birds, mammals	Eastern U.S. States	Human Granulocytic Anaplasmosis, babesiosis, Lyme disease

Ixodes spinipalpis (no common name) (Fig. 18)	Rodents	Rocky Mountain states	Lyme disease, Human Granulocytic Anaplasmosis
Ixodes texanus (no common name) (Fig. 19)	Raccoons, rodents	Eastern U.S. and some western states	None to humans
Rhipicephalus sanguineus (Brown Dog Tick) (Fig. 20)	Dogs	Throughout the United States	Rocky Mountain spotted fever

Acknowledgments

Gratitude is extended to Dr. Lorenza Beati (U.S. National Tick Collection, GSU) and to Dr. H. Joel Hutcheson (USDA, ARS, Ames, Iowa) for providing larval tick specimens used in this study and to Dr. Hao Chen (Department of Biology, GSU) for training and assistance with Scanning Electron Microscopy.

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