

Ultra-structural description of *Haemaphysalis* species on goats in Kerala, south India

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ABSTRACT: Ticks and tick-borne diseases are a major threat to goat farming in hot humid tropical regions of the world. The parasite species and vectors are significantly influenced by topographic and climatic variables. In the context of high prevalence of theileriosis in goats being reported from Kerala, south India, the ixodid tick fauna of the region has to be mapped. Adult ticks collected from 500 naturally infested goats were identified based on gross morphological and scanning electron microscopic studies. Majority of the ticks on goats belonged to *Haemaphysalis* genus (99.1%) followed by *Rhipicephalus*. Scanning electron microscopic features of *Haemaphysalis intermedia* (Warburton and Nuttall, 1909), *H. bispinosa* (Neumann, 1897), *H. kutchensis* (Hoogstraal & Trapido, 196) and *H. megalaimae* (Rajagopalan, 1963) were studied. *H. kutchensis* and *H. megalaimae* are reported for the first time from south India. This study emphasises the need to revisit the tick surveillance on different animal species of the state to provides an insight into the possible outbreaks of fatal tick-borne pathogens in the state.

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KEY WORDS: Ticks, *Haemaphysalis*, *H. kutchensis*, *Rhipicephalus*, ultrastructure, new report

INTRODUCTION

Ticks are haematophagous ectoparasites that cause significant economic losses to small ruminant farming through both their direct biting effects and the transmission of diseases. A substantial proportion (94.36%) of the world's goat population resides in Asia and Africa, highlighting the critical economic role of these small ruminants for impoverished communities in arid, semiarid, hilly, and remote tribal regions (Hegde, 2020). Goat farming in humid tropics faces numerous challenges, with tick and tick-borne diseases warranting particular attention.

Ticks such as *Haemaphysalis bispinosa*, *Rhipicephalus sanguineus* and *R. microplus* have been identified as potential vectors for *Babesia motasi*, *B. ovis* and *Theileria ovis* infections in goats in India, Bangladesh and Pakistan (Ghosh *et al.*, 2007). Previous attempts to identify tick species on domestic animals in Kerala, revealed a high prevalence of *Haemaphysalis* spp on goats based on light microscopic morphological features. However, the species spectrum of ectoparasites continually evolves with changes in time and climate (Prakasan and Ramani, 2007; Balasubramanian and Sahina, 2022; Sahina *et al.*, 2024). In the realm of

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tick taxonomy and speciation, classifying closely related tick species often presents a complex challenge. This complexity is not limited to subspecies but also includes distinct species with remarkably similar morphological features. Such ambiguities are prevalent among various tick species, where differentiation based solely on visual attributes becomes challenging. Moustafa *et al.* (2024) emphasized the importance of integrating molecular, genetic, and morphological approaches to unravel the complexities of tick taxonomy, thereby enhancing our understanding of their ecological roles and disease transmission potential (Rathinam and Sidhik, 2022). The use of scanning electron microscopy (SEM) to investigate the fine structures of *Haemaphysalis* spp on goats in Kerala, which could aid in species distinction, has not been extensively performed.

In light of the increasing prevalence of theileriosis and anaplasmosis reported in small ruminants of south India (Nagaraj *et al.*, 2019), this study aims to investigate the species spectrum of ixodid ticks infesting goats using gross morphological features and to document the ultrastructural morphology of the most prevalent tick species. This study serves as a platform for detailed tick surveillance on goats reared in households of Kerala, which is crucial for assessing the potential risk factors of fatal tick-borne pathogens in both animals and humans.

MATERIALS AND METHODS

Adult ticks (n=1200) were collected from 500 naturally infested goats in small and medium farms of south, central and north districts of Kerala and identified based on standard gross morphological keys (Sen and Fletcher, 1962; Trapido *et al.*, 1964). Adult goats and kids belonging to Malabari and Malabari cross breeds were selected. For SEM studies, the ticks were dehydrated in series of ascending grades of ethyl alcohol (50 to 100%) and dried to critical point drying with CPD (Critical Drying Point) unit. The processed samples were mounted over the stubs with double sided carbon conductivity tape and a thin layer of gold coating was done by an automated sputter-coater for three minutes with vacuum conditions of pressure 1.5×10^{-3}

pa and accelerating voltage of 5-8 kV and scanned under Scanning Electron Microscope (SEM Model: TESCAN VEGA 3) at required magnifications as per the standard procedures at Central Instruments Laboratory, College of Veterinary and Animal Sciences, Mannuthy, Kerala. Ticks were scanned on the dorsal and ventral sides and the external features such as mouth parts, infra internal setae dentition, hypostome, adanal plates, festoons, scutum, porose area, shape of genital sucker, lateral groove and spiracles were observed.

RESULTS

Out of the 1200 adult ticks examined, 1190 (99.1 %) belonged to *Haemaphysalis* genus and ten (0.83%) to *R. haemaphysaloides* based on gross morphological features. The characteristic morphological features of the prevalent tick genus, *Haemaphysalis* spp. under SEM were recorded. The most prevalent species on goats in Kerala as observed in the present study was *H. bispinosa* (n=747), followed by *H. intermedia* (n=435), *H. kutchensis* (n=4) and *H. megalaimae* (n=1). The species of three *Haemaphysalis* specimens could not be ascertained due to mutilation of mouth parts. (Table 1)

Table 1. Tick species distribution

	Tick species	Number (%) n=1200
1	<i>Haemaphysalis bispinosa</i>	747 (62.25 %)
2	<i>H. intermedia</i>	435 (36.25 %)
3	<i>H. kutchensis</i>	4 (0.33%)
4	<i>H. megalaimae</i>	1(0.08 %)
5	<i>Rhipicephalus haemaphysaloides</i>	10 (0.83 %)

Mouth parts: Mouth parts comprising of a hypostome, paired chelicerae, two palps and the lateral prolongation of the second segment of pedipalp were distinctly seen in all the specimens of *Haemaphysalis* spp. as were evident in light microscopy itself. The chelicerae had two digits armed with sharp denticles. The hypostome had

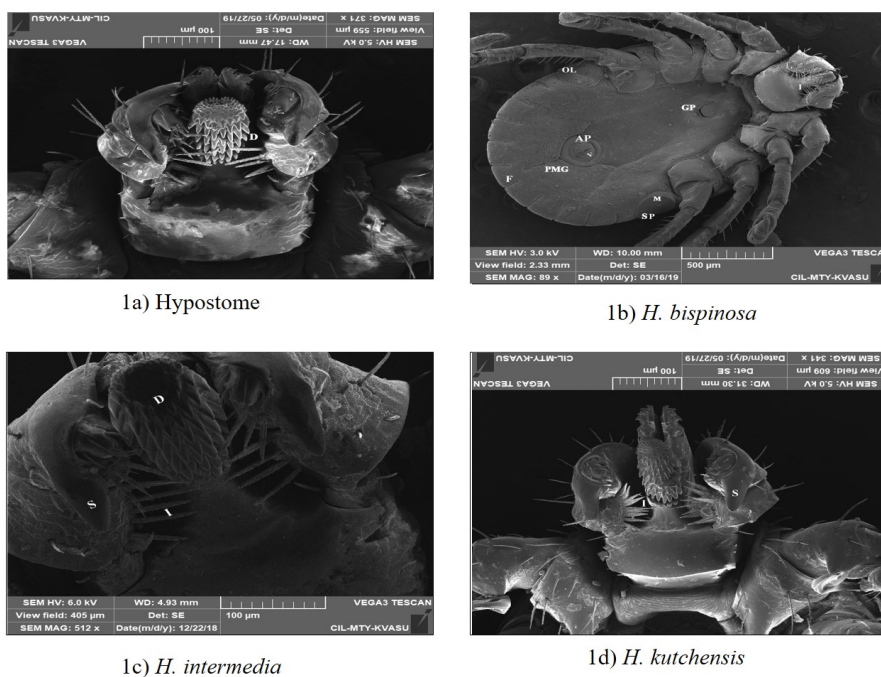


Fig. 1 Mouth parts of *Haemaphysalis* spp. a) Hypostome, b) *H. bispinosa*, c) *H. intermedia*, d) *H. kutchensis*
 AP- anal pore; D- dentition; F- festoon; GP- genital pore; M- macula; I- infrainternal setae; S- spur;
 SP-spiracle; OL- ostial lip; PMG- postero-median groove

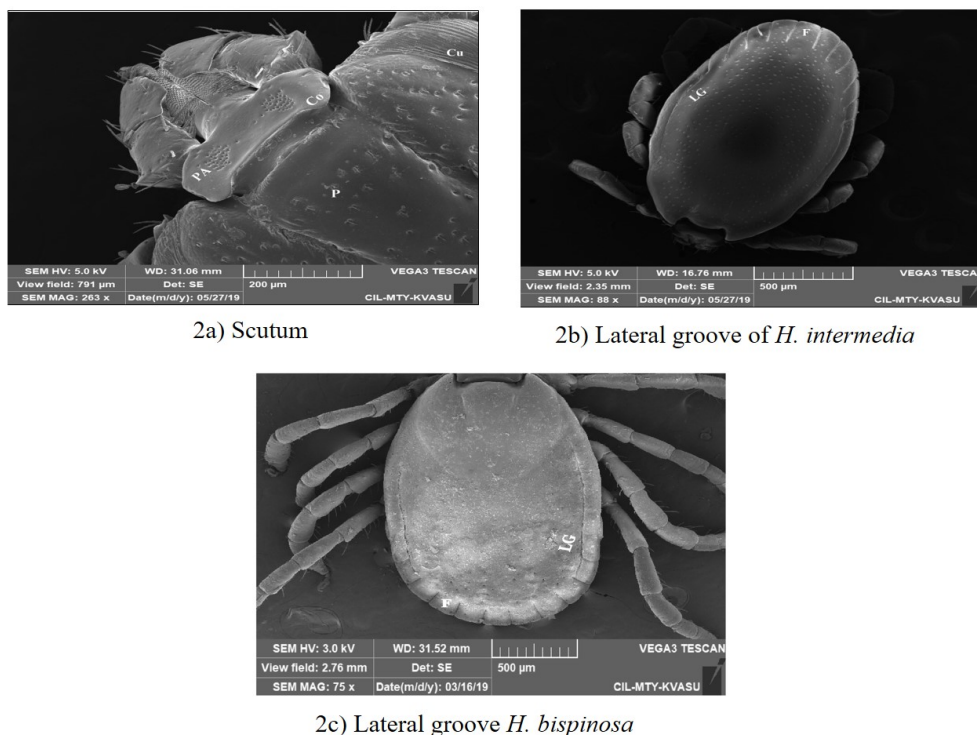


Fig. 2 Dorsal surface of *Haemaphysalis* spp. a) Scutum, b) Lateral groove *H. intermedia*, c) Lateral groove *H. bispinosa*: Co - cornua; Cu - cuticle; LG - lateral groove; PA - porose area; P - punctuations

recurved chitinous plates with minute denticles at the apex. The dentition on the hypostome that play a key role in attachment to the hosts, was 4/4 in all the specimens (Fig. 1a).

The number and appearance of infra internal setae varied from 2 to 10 between species. *Haemaphysalis bispinosa* could be distinguished by the presence of four to five infra internal setae that were non-feathery and distinctly separate (Fig. 1b). The specific feature of the dorsal side of the palp of this species was the long and prominent dorsal retroverted spur (DS) on the palpal article 3 (P3) (Fig. 4). The lateral salience of palp 2 extended well beyond the lateral margin of palpal article 3. The third palpal segment revealed a ventral retroverted spur that was not reaching the basal margin of palp 2 (Fig. 1b). Feathery and closely set six to seven infra internal setae could be distinctly associated with *H. intermedia* (Fig. 1c-I). Besides, in this species, the third segment of pedipalp revealed a ventral spur that was not reaching the basal margin of palp 2 (Fig. 1c-S). The dorsal spur of *H. intermedia* on palpal article 3 was short. Two specimens that possessed eight to ten feathery, closely set, infra internal setae were identified as *H. kutchensis*, while three could be observed in *H. megalaimae*. The rounded projection at the baso-internal angle of palpal segment 3 of *H. kutchensis* stretched beyond the internal margin of segment 2. The ventral spur on the third pedipalp of *H. kutchensis* was exceptionally long reaching the base of the palpal segment 2 (Fig. 1d-S).

Dorsal surface: Cuticle, at higher magnification, showed tiny surface folds giving a finger-print like appearance. On the dorsal surface of basis capitulum, porose areas were visible as a pair of circular depression containing several pores. The lateral cornua were moderately projected in all the specimens. The scutum had tiny pores or sensilla oriformia. The punctuations on the scutum of *H. kutchensis* were more piliferous when compared to other species (Fig. 2a). The lateral grooves enclosed the first festoon as was observed in *H. intermedia* (Fig. 2b), while it reached upto the second festoon in *H. bispinosa* (Fig. 2c).

Ventral surface: Genital aperture of females had a wide open U shape in all the studied species (Fig. 1b), except in *H. kutchensis*, in which it was narrow (Fig. 3a). The anal pore was made up of a central slit and two semicircular valves. A postero-median groove extending between the anal groove and festoons was distinct in all specimens. In *H. kutchensis*, the postero-median groove stretched into the level of festoons (Fig. 3a), while in *H. bispinosa* this groove terminated just above the level of festoons (Fig. 1b). The number of festoons varied from 10 to 12. The spiracles were broadly oval to circular in outline (Fig. 1b), the spiracular plate being elevated from the rest of the integument. There were more than 150 aeropyles of different sizes in each of the spiracle. A well distinct thick cuticular macula with a crescentic ostial lip was discernible in the spiracles (Fig. 3b). The spiracles of *H. kutchensis* were elongate and oval in shape (Fig. 3b).

Haller's organ: The Haller's organ on the tarsus of ticks had a distinct anterior pit with several sensillae and a proximal capsule with a wavy slit. In the anterior pit one sensilla was longer than the rest. Several needle-like bristles were observed around the Haller's organ (Fig. 3c). The terminal portion of leg was armed with a globular adhesive pad like structure adjacent to a pair of curved claws. Ventral and dorsal plates of this pad could be observed. The pad reached almost upto the apical curvature of the claws (Fig. 3d).

DISCUSSION

The distribution of any vector borne pathogen in specific geographic regions is intricately linked to the distribution of its vectors in the particular area. *Haemaphysalis* spp. was the predominant species on goats in Kerala, a hot humid state of south India (Sahina *et al.*, 2024). According to Moustafa *et al.* (2024), the taxonomy and genetic differentiation of *Haemaphysalis*, one of the most widespread genera in Asia, present considerable challenges. Accurate identification of tick species, often through morphological characteristics, is vital for public and veterinary health and for controlling associated tick-borne diseases. One of the key morphologic

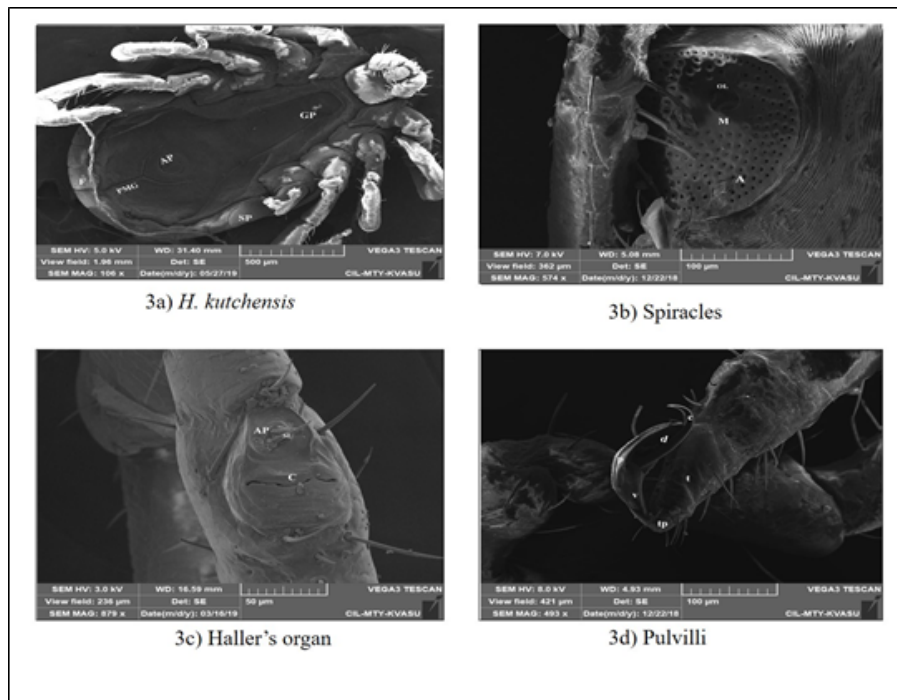


Fig. 3 Spiracles and Haller's organ of *Haemaphysalis* spp. a) *H. kutchensis*, b) Spiracles, c) Haller's organ, d) Pulvilli. A- aeropyle; C- capsule; c- claw; d- dorsal plate; SL- sensilla; t-tarsus; tp- tarsal-pretarsal articulation; v-ventro-lateral plate



Fig. 4 Dorsal view of *H. bispinosa* in light microscopy (Ds - Dorsal spur, P3 - Palpal segment 3)

characteristics used to differentiate ixodid ticks are the mouthparts (Thompson *et al.*, 2020). In this study, mouthparts were examined under scanning electron microscopy to identify the species. The dentition on the hypostome, which plays a crucial role in attachment to hosts, was 4/4 in all four species studied. Jin *et al.* (2016) had reported that male *H. tibetensis* possessed a 5/5 dentition pattern, while females had a 4/4 pattern. The number and appearance of infra internal setae varied from 2 to 10 between *Haemaphysalis* species, serving as a distinctive feature for different species within the genus. These observations concurred with the published keys (Trapido *et al.*, 1964). The length of the spurs on the third pedipalp also varied with species. The ventral spur on the third pedipalp of *H. kutchensis* was exceptionally long reaching the base of the palpal segment 2 as reported by Hoogstraal and Trapido (1963).

The punctuations on the scutum of *H. kutchensis* were found to be more piliferous when compared to other species. In *H. tibetensis*, the punctuations were minute and medium sized, as observed by Jin *et al.* (2016). According to Pegram *et al.* (1987) the punctuations on scutum of *R. sanguineus* were large, deep and piliferous being distributed in four uniform rows.

The lateral grooves enclosed the first festoon in *H. intermedia*, while it reached upto the second festoon in *H. bispinosa*. The reports on the lateral grooves reaching upto the level of coxa III in *H. kutchensis* and in *H. tibetensis* (Hoogstraal and Trapido 1963; Jin *et al.*, 2016) suggested that interspecies variations do exist in this feature as well.

Genital aperture of females had a wide open U shape in all the species studied, except in *H. kutchensis*. This is not a very distinguishing feature as the appearance of aperture in engorged and mated ticks differed from that in unmated ones (Hoogstraal and Trapido, 1963). The number of festoons in *Haemaphysalis* varied from 11 to 12. Caetano *et al.* (2017) had observed 11 festoons in *R. sanguineus*. The spiracles were broadly oval to circular in outline in all species of

Haemaphysalis, while those of *H. kutchensis*, were more elongate. Woolley (1972) noted that the spiracular plates of *Ixodes* and *Haemaphysalis* were rounded in outline, while those of *Rhipicephalus* were subtriangular or comma shaped. There were more than 150 aeropyles of different sizes in each of the spiracles. Spiracular pores are reported to vary with different genera of ticks (Hinton, 1967; Roshdy and Hefnawy, 1973).

A well distinct Haller's organ with a proximal pit containing several sensillae and a capsule was observed on the tarsus in the first pair of appendages. Haller described this for the first time in 1881 as an auditory organ. Studies of Foelix and Axtell (1972) proved that anterior pit functioned as humidity receptor, while the capsule was involved in olfaction. The capsule of the Haller's organ of *Amblyomma* spp was not perforated and the number of slits varied with species (Ghosh *et al.*, 2017). A narrow slit was reported in *A. americanum*, while *A. gervaisi* and *A. helvolum* had six and five slits, respectively. In the present study, the capsule of Haller's organ of *Haemaphysalis* spp. was found to have a single wavy slit.

The dorsal and ventral lobes of the tarsal pad were evident between paired tarsal claws. The claws of *H. tibetensis* were moderate in size and the pad did not extend upto the distal curvature of claws as reported by Jin *et al.* (2016), while it was reaching almost upto the apical curvature of the claws in all the *Haemaphysalis* species in the present study. The position of tarsal pad may, however, vary with the posture of fixing for the SEM study.

The present study puts on record the ultrastructural description of *Haemaphysalis* species on goats in Kerala. Based on morphological features, *H. bispinosa* and *H. intermedia* were the most prevalent species on goats. The study area's local environment may be favorable for the development of *H. bispinosa* (Sahina *et al.*, 2024; Rathinam and Sidhik, 2022). Thus, goats are important reproductive hosts for these ixodid ticks in the study region. The study also recorded *H. kutchensis* and *H. megalaimae* for the first time in the state. The

role of these ticks as potential vectors of pathogens infecting animals and humans needs further investigation in the country. It is interesting to note that *H. spinigera*, the vector of Kyasanur Forest Disease in humans reported from Kerala (Babu *et al.*, 2019), could not be observed on goats of this region. Genetic characterisation has been utilised to differentiate *H. cornupunctata*, *H. kashmirensis* and *H. montgomeryi* infesting goats and sheep in Pakistan (Khan *et al.*, 2023). The description of *Haemaphysalis* species, a potential vector of pathogenic infections would also serve as a platform to redesign the map of vector tick fauna on different species of livestock in this part of the country.

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