

海南省血红扇头蜱不同发育阶段形态电镜观察

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摘要: **目的** 了解海南地区优势种血红扇头蜱(*Rhipicephalus sanguineus*)不同发育阶段的外部形态特征及超微结构,为海南省血红扇头蜱谱系的识别及防控提供理论基础。**方法** 借助扫描电子显微镜观察海南省优势种血红扇头蜱幼蜱、若蜱和成蜱不同发育阶段的外部形态。**结果** 幼蜱须肢各节之间分界不明显,刚毛数少且呈锯齿状,口下板齿式为2|2,有3对肢节,肛门瓣上有1对肛毛,无肛后沟、气门板、多孔区和生殖孔;若蜱须肢各节开始有明显分界,口下板齿式为2|2,有4对肢节,有肛后沟,肛门瓣上有3对肛毛,无生殖孔和双孔区;雄成蜱须肢覆盖螯肢鞘,刚毛整体为圆锥形,口下板齿式为齿式3|3,有4对肢节,有肛后沟和肛侧板,肛门瓣上有7根肛毛,生殖孔呈卵圆形;雌成蜱口下板齿式为3|3,有4对肢节,有1对孔区,共有3根刚毛,有肛后沟,肛门瓣上有4对肛毛和2个气孔样小孔,生殖孔呈宽U型。此外,雄成蜱盾板几乎占据整个背面,幼蜱、若蜱和成蜱的假头基均呈六角形,并且第1对足上均发现哈氏器的存在。**结论** 对血红扇头蜱不同发育阶段进行扫描电子显微镜观察,根据其形态结构特点初步认为海南省血红扇头蜱为血红扇头蜱热带谱系(*Rhipicephalus linnaei*),为后续血红扇头蜱的鉴别和地方蜱传疾病综合防控提供一定的参考意义。

关键词: 血红扇头蜱;形态学观察;超微结构;扫描电子显微镜;海南

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Scanning electron microscopic observation of morphology of *Rhipicephalus sanguineus* at different developmental stages in Hainan ProvinceLIANG Dejuan^{1,2}, AN Liping^{1,2}, LI Yao^{1,2}, ZHAO Peizhen^{1,2}, GUAN Qingfeng^{1,2}, ZHAO Jianguo^{1,2}

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Abstract: **Objective** To understand the morphological characteristics and ultrastructure of the dominant species of *Rhipicephalus sanguineus* in Hainan at different developmental stages, and provide theoretical basis for the identification of the lineage and control of *Rhipicephalus sanguineus*. **Methods** The external morphology of different developmental stages of the dominant species of *Rhipicephalus sanguineus*, including larva, nymph and adult tick in Hainan were observed by scanning electron microscope. **Results** The division between each segment of larva pedipalps was not obvious, and setae was serrated; dental formula type 2 | 2; 3 pairs of podomere; a pair of setae on the anal valve; none of anal groove, spiracular plate, porous area and genital aperture. There was a clear boundary at the beginning of each segment of nymph pedipalps; dental formula type 2 | 2; 4 pairs of podomere; 3 pairs of setae on the anal valve; anal groove; none of porous area and genital aperture. The male adult tick's trichotheca are covered by the pedipalps, and the whole bristles are conical; dental formula type 3 | 3; 4 pairs of podomere; anal groove and paraprotects; 7 setae on the anal valve; genital aperture was oval. The female of adult tick can be distinguished by dental formula 3 | 3; pairs of podomere; porous areas with 3 short setae; anal groove; 4 pairs of setae and 2 pores on the anal valve; genital pore was broadly U-shaped. In addition, the male adult's scutum occupies almost the entire dorsal surface, the basis capituli of larva, nymph and adult tick all were hexagonal, and the existence of Haller's organ was found on the first pair of legs. **Conclusions** Scanning electron microscopy observation of the different developmental stages of *R. sanguineus* revealed clear morphological features, preliminarily suggesting that *R. sanguineus* in Hainan Province may belong to the tropical lineage, which provide a certain experimental basis for the identification of the tick and the comprehensive prevention and control of local tick-borne diseases.

Keywords: *Rhipicephalus sanguineus*; morphological observation; ultrastructure; scanning electron microscopy; Hainan

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蜱是一种重要的吸血性寄生虫,广泛寄生于家畜、野生动物和人类等脊椎动物体表,可传播细菌(螺旋体、无浆体等)、病毒(蜱传脑炎病毒)及寄生虫(巴贝虫、泰勒虫)等多种病原体,是人畜共患病传播的主要媒介^[1]。人畜共患媒介传播疾病是世界上重要的公共卫生问题。据报道,蜱虫是美国每年报告的近95%的媒介传播疾病中的罪魁祸首^[2-3],并且每年因蜱虫和蜱传疾病造成的经济损失达139亿~197亿美元^[4],在医学、兽医学和经济方面产生巨大的影响。

血红扇头蜱(*Rhipicephalus sanguineus*)是我国常见的蜱种之一,隶属于蛛形纲(Arachnida),蜱螨目(Acarina),硬蜱科(Ixodidae),扇头蜱属(*Rhipicephalus*),全年存在于热带和亚热带地区^[5],而温带地区,春末秋初最为活跃^[6]。血红扇头蜱属于不完全变态发育,其发育阶段可分为卵、幼蜱、若蜱和成蜱4个时期,除卵外,其他3个时期都需要吸血蜕皮后,进入下一个生长阶段。雌成蜱平均一生能产1500~4000粒卵^[7-8],其产卵的数量与体重和产卵期的长短有关^[7],在有利的条件下,如温度、相对湿度和宿主,它们每年可以完成3~4代,繁殖快,如巴西中西部的记录^[9-10]。血红扇头蜱是一种三宿主蜱,家犬是主要宿主^[11],但也寄生在猫、啮齿动物、鸟类和人类等动物身上^[12-15]。血红扇头蜱是海南省的优势种,常年普遍存在,通过叮咬宿主可传播发热伴血小板减少综合征病毒(severe fever with thrombocytopenia syndrome bunyavirus, SFTSV)、犬巴贝斯虫(*Babesia canis*)、犬肝簇虫(*Hepatozoon canis*)、扁平无浆体(*Anaplasma platys*)、嗜吞噬细胞无浆体(*Anaplasma phagocytophilum*)和柯克斯氏体(*Coxiellaceae bacterium*)等病原体^[16-18],从而导致宿主贫血、消瘦、皮肤损伤、炎症及生产性能下降,严重时会导致宿主瘫痪甚至死亡等^[19]。然而,与大多数蜱类不同,血红扇头蜱在室内就能完成所有生命周期,对于房主和害虫防治公司来说是一个极大的挑战。

除了是一种高产的体外寄生虫外,血红扇头蜱还与兽医和公共卫生密切相关。全球气候变暖、蜱虫宿主的分布和生态、人为引起的变化将有助于蜱虫和蜱传病原体的地理扩张,影响世界各地的人群,已经发展成为一个重要的全球性健康问题。但由于原始描述资料不全,模式标本丢失,导致血红扇头蜱的许多形态学描述都基于别的蜱种,在某些情况下,可能会显示出生物不相容和显著的遗传分歧,使得血红扇头蜱的分类地位一直存在质疑和争论^[20]。目前有研究数据表明,血红扇头蜱可分为温带、热带和东南欧3个谱系^[21-23]。热带谱系血红扇头蜱已被重新命名为*Rhipicephalus linnaei*^[24-26],而且血红扇头蜱谱系可能与

特定地点相关的环境变量(如气候、生境或寄主特异性)有关。因此,本实验以海南省血红扇头蜱为研究对象,借助扫描电子显微镜对血红扇头蜱不同发育阶段的形态结构进行观察,为海南省血红扇头蜱后期的分类及防控提供一定的理论依据。

1 材料与方法

1.1 蜱虫采集及饲养 从海南省儋州市和庆镇狗场里采集蜱,带回海南大学病媒生物学实验室,于(27±1)℃、相对湿度90%±5%条件下的人工气候箱中进行饲养^[27],在新西兰兔上进行寄生传代,直至实验需要。

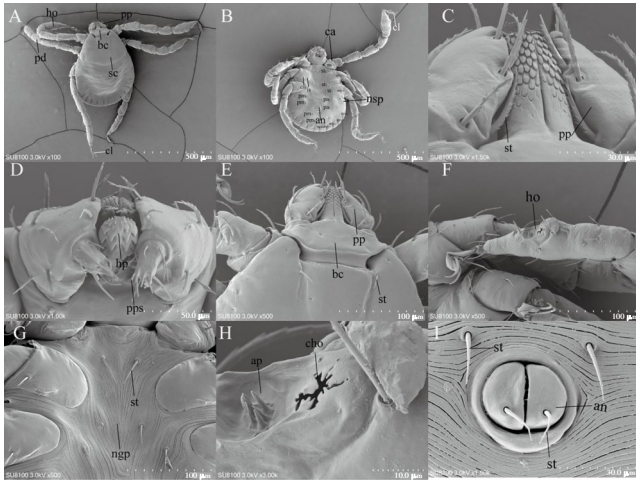
1.2 试剂与仪器 扫描电子显微镜(hitachi,SU8100)、临界点干燥仪(Quorum,K850),离子溅射仪(hitachi,MC1000),人工气候培养箱(广州泰宏君,250H),小号勾线画笔,PBS溶液,2.5%戊二醛固定液(上海源叶生物科技有限公司),乙酸异戊酯(上海麦克林生化科技有限公司),无水乙醇。

1.3 样品制备 取不同发育阶段的血红扇头蜱(幼蜱、若蜱、雄成蜱和雌成蜱),用70~80℃热水烫杀,于pH为7.4的0.1 mol/L磷酸盐缓冲液清洗3次,每次10 min,然后用2.5%戊二醛固定液于4℃固定24 h(避光),之后依次用浓度50%、70%、80%、90%、95%、100%酒精脱水,每次15 min;然后用乙酸异戊酯置换15 min;将样品放入临界点干燥仪内进行干燥。干燥处理结束后,将蜱背面、腹面和侧面粘贴于黑色双面导电胶上,进行喷金处理,在扫描电镜下观察并拍照。

2 结果

2.1 幼蜱 躯体:幼蜱轮廓近似椭圆形,在第3对足附着点处最宽,边缘稍弯曲,颈沟浅,背面和腹部有皱纹样褶皱,缘垛清晰可见(图1A)。盾板呈亚圆形,存在极少量的几乎看不见刻点和气孔样小孔,刚毛数量少,零散覆盖于整个躯体(图1A~图1B)。18对腹刚毛:3对胸骨刚毛(St)、4对前边缘对(Pm)、2对肛前刚毛(Pa)、8对边缘腹侧(Mv)和1对肛毛(图1B);肛门位于腹部的1/3处,由两个叶状的肛瓣构成,肛瓣上有1对肛毛,无气孔样小孔,无肛后沟(图1B、图1I);无生殖孔(图1G),无气门板。假头:由须肢,口下板,假头基和螯肢组成的,假头基呈六角形,宽大于长(图1E);螯肢的两侧为须肢,须肢呈圆柱形,各节分界不明显,刚毛呈锯齿状(图1C~图1E)。口下板短,钝的,长度是口下板的顶端到口下板刚毛处,齿式为2|2,每纵列5~6枚小齿(图1D)。足:有3对肢节,每1对足有六节,即基节、转节、股节、胫节、后跗节和跗节,每一节表面都附着几对刚毛(图1A~图1B);哈氏器仅存在于第一对足跗节的背面,前囊内有刚毛,后囊刚毛不明显,哈氏器囊孔形状呈裂缝状(图1F、图1H);基

节 I~III各有1个单一的、短的、圆形的、外距,大小从基节 I 到基节 III 逐渐减小(图 1B、图 1G)。



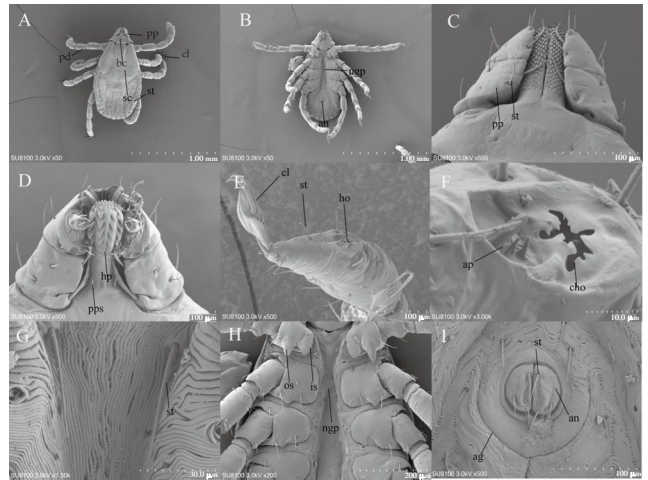
注:A. 躯体背面;B. 躯体腹面;C、E. 假头背面;D. 假头腹面;F. 肢节;G. 腹面局部;H. 哈氏器;I. 肛门区;ho. 哈氏器;pd. 肢节;pp. 须肢;bc. 假头基;sc. 盾板;cl. 爪;nsp. 无气孔板;ngp. 无生殖孔;an. 肛门;st. 刚毛;ph. 口下板;pps. 口下板后毛;cho. 后囊;ap. 前窝;ca. 基节;Pm. 前边缘刚毛;Pa. 肛前刚毛;Mv. 边缘腹侧刚毛。Note: A. Body dorsal; B. Body abdomen; C, E. Capitulum dorsal; D. Capitulum abdomen; F. Podomere; G. Local abdomen; H. Haller's organ; I. Anal area; ho. Haller's organ; pd. Podomere; pp. Palpus; bc. Basis capitulum; sc. Scutum; cl. Claw; nsp. No stigmal plate; ngp. No genital aperture; an. Anus; st. Seta; ph. Hypostome; pps. Posthypostomal seta; cho. Capsule of Haller's organ; ap. Anterior pit; ca. Coxae; Pm. Pre-marginal seta; pa. Pre-anal seta; mv. Marginal ventral seta.

图1 血红扇头婢幼婢形态结构扫描电镜观察

Fig. 1 Morphology and structure of larva of *R. sanguineus* by scanning electron microscopy

2.2 若婢 躯体:若婢轮廓似椭圆形,在第4对足附着点处最宽,前端窄后端宽,缘垛清晰可见(图2A);盾板呈亚圆形,长宽大小近似相等,侧边缘较直,后缘宽且弯曲,颈沟短,前深后浅,盾板皮纹似鳞片状,有少量浅刻点,几乎不可见,短刚毛很少(图2A);躯体全身被长短不一的刚毛覆盖(图2A、图2B),无生殖孔(图2B、图2H),肛门位于腹后部1/3处,由两个叶状的肛瓣构成,肛瓣下方无气孔样,肛瓣上有3对肛毛,有肛后沟(图2B、图2I),腹部体表皮纹纹路清晰,以弧状的形式存在(图2G)。假头:假头基位于躯体的前端,略似六角形,无多孔区(图2A、图2C);螯肢1对,螯肢两侧为须肢,须肢各节分布明显且按顺序逐渐变窄,刚毛锯齿逐渐退化(图2C、图2D);口下板长,钝的,齿式为2|2,每纵列有6~7枚小齿(图2D)。腿:有4对肢节,足上6节,第1对足跗节背缘近端部有哈氏器,哈氏器囊孔呈裂缝不规则状(图2A~图2B、图2E~图2F);足基节 I 距裂明显,有两个类似于三角形的

内外距,外距略长于内距,基节 II~IV 各有一个单一的、短的、突起的外距,外距长度从基节 II~IV 逐渐减小(图2B、图2H)。



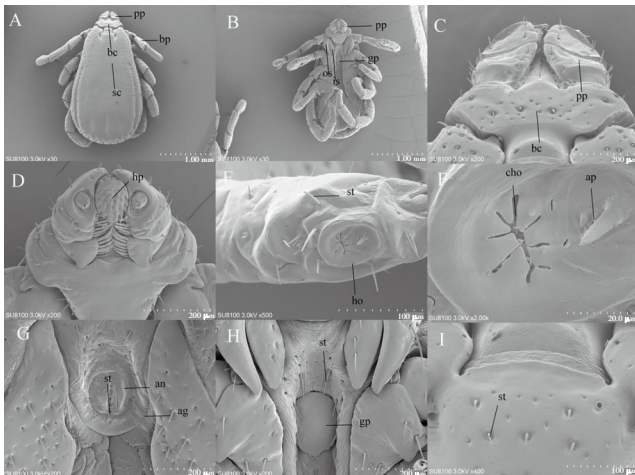
注:A. 躯体背面;B. 躯体腹面;C. 假头背面;D. 假头腹面;E. 肢节;F. 哈氏器;G、H. 腹面局部;I. 肛门区;ho. 哈氏器;pd. 肢节;pp. 须肢;bc. 假头基;sc. 盾板;cl. 爪;sp. 气孔板;ngp. 无生殖孔;an. 肛门;st. 刚毛;ph. 口下板;pps. 口下板后毛;cho. 后囊;ap. 前窝;ca. 基节;os. 外距;is. 内距;ag. 肛沟。Note: A. Body dorsal; B. Body abdomen; C. Capitulum dorsal; D. Capitulum abdomen; E. Podomere; F. Haller's organ; G, H. Local abdomen; I. Anal area; ho. Haller's organ; pd. Podomere; pp. Palpus; bc. Basis capitulum; sc. Scutum; cl. Claw; sp. Stigmal plate; ngp. No genital aperture; an. Anus; st. Seta; ph. Hypostome; pps. Posthypostomal seta; cho. Capsule of Haller's organ; ap. Anterior pit; ca. Coxae; os. Outer spur; is. Inner spur; ag. Anal groove.

图2 血红扇头婢若婢形态结构扫描电镜观察

Fig. 2 Morphology and structure of nymph of *R. sanguineus* by scanning electron microscopy

2.3 雄成婢 躯体:雄成婢轮廓近似椭圆形,在第4对足附着点处最宽,前窄后宽,缘垛清晰可见(图3A)。盾板为深红褐色,长大于宽,侧缘较直,后缘弯曲,几乎占据整个背部,呈卵圆形,表面光滑,有很多大小不一的刻点和短刚毛,分布不均匀,广泛密集地分布在前端和后端,边缘凹槽深且从第2对足处延伸至躯体后边缘,颈沟短且深,呈逗号状(图3A);全身被长短不一的刚毛覆盖(图3A~图3B),生殖孔位于第2对足之间,呈卵圆形,气门板一对,位于足IV基节的后外侧(图3B、图3H);肛门位于腹部1/3处,由两个叶状的肛瓣构成,肛瓣上有7根肛毛,有肛后沟,肛侧板长,几乎为宽的3倍长,后缘较平直,体后缘未观察到尾突(图3B、图3G)。假头:假头基位于躯体的前端,略似六角形,长明显短于宽,无多孔区(图3C)。须肢前端圆钝,由4节组成,第1、2节腹面内缘刚毛较粗,排列紧密,第3节背面呈梯形,较宽,能覆盖螯肢鞘,第4节短小,嵌出于第3节端部腹面小凹陷内(图

3C)。口下板短,钝的,齿式3|3,每纵列有4~5枚小齿(图3D)。腿:有4对肢节,足上6节,足I跗节背缘近端部具哈氏器,哈氏器囊孔呈裂缝不规则状,末端有爪和爪垫(图3B、图3E~图3F);基节I有2个类似于三角形的内外距,外距比内距窄,两者平行,基节II~IV各有一个短的外距,基节大小从I~IV逐渐变大(图3B、图3H)。



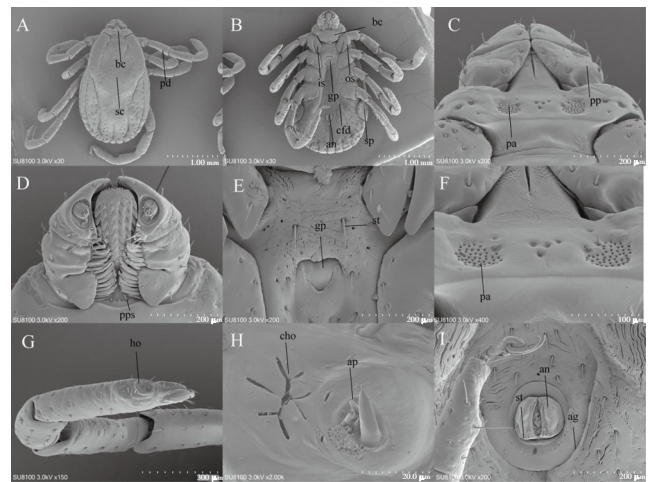
注:A. 躯体背面;B. 躯体腹面;C. 假头背面;D. 假头腹面;E、F. 哈氏器;G. 肛门区;H. 生殖区;I. 盾板区;ho. 哈氏器;pd. 肢节;pp. 须肢;bc. 假头基;sc. 盾板;cl. 爪;sp. 气孔板;an. 肛门;st. 刚毛;ph. 口下板;pps. 口下板后毛;cho. 后囊;ap. 前窝;ca. 基节;os. 外距;is. 内距;ag. 肛沟;gp. 生殖孔。Note: A. Body dorsal; B. Body abdomen; C. Capitulum dorsal; D. Capitulum abdomen; E, F. Haller's organ; G. Anal area; H. Germarium area; I. Scutum area; ho. Haller's organ; pd. Podomere; pp. Palpus; bc. Basis capitulum; sc. Scutum; cl. Claw; sp. Stigmal plate; an. Anus; st. Seta; ph. Hypostome; pps. Posthypostomal seta; cho. Capsule of Haller's organ; ap. Anterior pit; ca. Coxae; os. Outer spur; is. Inner spur; ag. Anal groove; gp. Genital aperture.

图3 血红扇头蜱雄成蜱形态结构扫描电镜观察

Fig. 3 Morphology and structure of male adult of *R. sanguineus* by scanning electron microscopy

2.4 雌成蜱 躯体:雌成蜱轮廓近似椭圆形,在第4对足附着点处最宽,前端窄,后端宽,缘垛清晰可见(图4A);盾板为红褐色,长略大于宽,后缘弯曲,处于躯体1/3的位置,呈亚圆形,表面粗刻点少而零散,细刻点较多,密集分布在整个盾板上;颈沟宽且浅,侧沟约达盾板的后侧缘,较少的短刚毛分布于盾板的前端(图4A);躯体全身被长短不一的刚毛覆盖(图4A~图4B),有发育成熟的生殖孔,呈宽U型,侧缘向前发散,位于第2对足之间(图4B、图4E);气门板1对,位于足IV基节的后外侧,气门板宽阔且有1个小孔(图4B);肛门位于腹后部,由两个叶状的肛瓣构成,肛瓣下方有两个气孔样,肛瓣上有4对肛毛,有肛后沟(图4B、图4I);腹部体表皮纹纹路清晰,以弧状的形式存在

(图4E)。假头:假头基位于躯体的前端,略似六角形,长明显短于宽,背面有1对多孔区,具有感觉及分泌液体帮助产卵的功能(图4C、图4F);螯肢1对,螯肢的两侧为须肢,须肢前端圆钝,由4节组成,第1、2节腹面内缘刚毛较粗,排列紧密,第3节背面呈梯形,较宽,能覆盖螯肢鞘,第4节短小,嵌出于第3节端部腹面小凹陷内(图4C);口下板短且钝,齿式3|3,每纵列有6~7枚小齿(图4D)。腿:有4对足,足上6节,足I跗节背缘近端部具哈氏器,哈氏器囊孔呈裂缝不规则状,末端有爪和爪垫(图4G~图4H);足基节I距裂深,有两个类似于三角形的内外距,外距比内距窄,两者平行,基节II~IV各有1个短的外距,基节大小从I~IV逐渐变大(图4B)。



注:A. 躯体背面;B. 躯体腹面;C. 假头背面;D. 假头腹面;E. 生殖区;F. 假头基;G. 肢节;H. 哈氏器;I. 肛门区;ho. 哈氏器;pd. 肢节;pp. 须肢;bc. 假头基;sc. 盾板;cl. 爪;sp. 气孔板;an. 肛门;st. 刚毛;ph. 口下板;pps. 口下板后毛;cho. 后囊;ap. 前窝;os. 外距;is. 内距;ag. 肛沟;gp. 生殖孔;pa. 孔区。Note: A. Body dorsal; B. Body abdomen; C. Capitulum dorsal; D. Capitulum abdomen; E. Germarium area; F. Basis capituli; G. Podomere; H. Haller's organ; I. Anal area; ho. Haller's organ; pd. Podomere; pp. Palpus; bc. Basis capitulum; sc. Scutum; cl. Claw; sp. Stigmal plate; an. Anus; st. Seta; ph. Hypostome; pps. Posthypostomal seta; cho. Capsule of Haller's organ; ap. Anterior pit; ca. Coxae; os. Outer spur; is. Inner spur; ag. Anal groove; gp. Genital aperture; pa. Porose area.

图4 血红扇头蜱雌成蜱形态结构扫描电镜观察

Fig. 4 Morphology and structure of female adult of *R. sanguineus* by scanning electron microscopy

3 讨论

血红扇头蜱是人畜共患病原体(如立克次体)的主要传播媒介,有研究表明,暴露在高温下的血红扇头蜱更容易叮咬兔子和人类^[27]。随着全球化和气候变暖,血红扇头蜱可能会影响世界各地的人群,从而影响某些蜱传染病的流行病学^[28]。由于血红扇头蜱模式标本的丢失^[29],因此缺乏该物种真正的分类学定义。

为了避免在蜱传疾病流行病学中对血红扇头蜱的错误识别,本研究借助扫描电子显微镜来观察血红扇头蜱不同发育阶段的形态,以获得了更为详细的形态结构信息。结果发现不同发育阶段的蜱在形态上存在一定的差异性。幼蜱、若蜱和雌成蜱盾板均占据躯体背部的1/3,而雄成蜱盾板几乎占据整个躯体背部;幼蜱盾板刻点少,几乎看不见,刚毛数量少且分叉呈锯齿状,3对肢节,口下板齿式为2|2,无气门板、肛后沟、多孔区和生殖孔,肛门瓣上有一对肛毛;若蜱盾板皮纹似鳞片状,有少量浅点,短刚毛很少,无气门斑、双孔区和生殖孔,有4对肢节,肛门瓣上有3对肛毛和肛后沟,口下板齿式为2|2;雄成蜱盾板上刻点数量适中,大小不等,刚毛整体为圆锥状,表面光滑,边缘凹槽明显,具有4对肢节、气门板和气门斑,无双孔区,生殖孔为卵圆形,肛门瓣上有7根肛毛,口下板齿式为3|3;雌成蜱盾板存在许多刻点,刚毛整体为圆锥状,具有4对肢节、气门板、气门斑、双孔区和肛后沟,生殖孔呈宽U型,肛门瓣上有4对肛毛和气孔样小孔,口下板齿式为3|3。此外,幼蜱、若蜱和成蜱的假头基均呈六角形,第1对足上均发现哈氏器的存在。以上结果说明了幼蜱发育成成蜱时,呈锯齿状刚毛逐渐蜕变成成熟的圆锥形状,体表的刚毛逐渐增多,须肢上的刚毛变多且向口下板内弯。除幼蜱外,其他时期的蜱均出现第4对足、气门板和肛后沟,气门板与呼吸有关,但若蜱的气门板无气门斑,因此推测,幼蜱和若蜱的主要呼吸方式是体表^[30]。生殖孔仅在成蜱中被发现,用于交配,而多孔区仅存在于雌成蜱中,具有感觉及分泌体液帮助产卵的功能。哈氏器是蜱独有的感觉器官,位于第1对足的附节上,是化学感觉的主要感觉器官,参与检测信息素,聚集或者交配行为以及宿主生命所需的环境线索^[31]。本实验仅靠形态观察无法辨别幼蜱和若蜱的性别,蜱类性别是由染色体数量决定的^[32]。

在过去的几十年遗传研究和交叉交配试验中所获得的数据显示,不同地区血红扇头蜱种群之间存在复杂的关系,形态描述上存在高度变异性,如雌性生殖孔的形状、雄性的肛侧毛和气门板以及盾板刻点等^[20],已发现的高度变异性可能是发生错误识别的主要原因之一^[25]。因此,正确识别节肢动物(包括蜱虫)至关重要,因为这是成功控制体外寄生虫的第一步。目前研究发现,海南省血红扇头蜱形态结构与SLAPETA等^[25-26]描述的血红扇头蜱热带谱系形态结构极为相似,因此初步推测海南省血红扇头蜱可能为血红扇头蜱热带谱系,后期可进一步利用分子生物学的方

法对其加以验证。这一发现丰富了该地区对血红扇头蜱的认识,并且对确定血红扇头蜱种群内物种的多样性和分类学地位至关重要,为后期蜱虫的识别和防控奠定了理论基础。

伦理审查与知情同意 本研究不涉及伦理批准和患者知情同意

利益冲突声明 所有作者声明不存在利益冲突

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