



universität
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MASTERARBEIT

Titel der Masterarbeit

„Structure and possible functions of the spiracle glands in
Ixodes ricinus L.“

verfasst von

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angestrebter akademischer Grad

Master of Science (MSc)

Wien, 2013

Studienkennzahl lt. Studienblatt:

A 066 831

Studienrichtung lt. Studienblatt:

Masterstudium Zoologie

Betreut von:

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Summary

The spiracles, openings of the tracheal system, in nymphal and adult ticks are complex cuticular structures. In the family Ixodidae, the spiracles contain numerous dermal glands, the spiracle glands. The function of spiracle glands is uncertain but it is suggested that their secretion reduces water loss through the spiracles.

In the present study the structure of the spiracle glands and the appearance of the secretory product within their secretory reservoirs are examined by transmission electron microscopy in unfed adult *Ixodes ricinus*. The genus *Ixodes* is basal within Ixodidae. It is to be expected that the examination of *I. ricinus* will show the original structure of the glands within this tick family. Previous descriptions of the glands from other tick genera show morphological differences. The results are discussed primarily regarding their assumed function in the water balance of ticks. Some aspects of *Ixodes laguri* are also included because nidicolous tick species are thought to have lower resistance to desiccation. The tick with the highest water loss rate is the one-host tick *Rhipicephalus (Boophilus) annulatus*. Spiracle descriptions from the literature of one-host species are therefore also added. Assuming that the spiracle glands in all ixodid tick species are associated with pores (aeropyles) in the spiracle plates, the pore numbers provide information on the maximum possible number of spiracle glands. This approach indeed indicates a possible function of spiracle glands in the water balance of ixodid ticks.

Key words: ticks, dermal glands, spiracle, basal plate glands, sub-atrial glands, calyx, apocrine secretion, water balance, pheromone, *Haemaphysalis concinna*

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1 Introduction

Ticks are notorious blood feeders transmitting a broad spectrum of pathogens in man and other vertebrates (Estrada-Peña and Jongejan, 1999). Due to their life style with alternating on- and off-host periods they are also called gorging-fasting animals. Familiar as blood feeding organisms able to take up huge amounts of blood they are passing the gorging period characterized by rapid metabolism and development. After digestion (and moult into the next life stage) ticks enter the inconspicuous fasting state. From now on they do not feed and rely exclusively on storage substances. To conserve energy the metabolism is reduced and water becomes a limiting factor for survival. Inactivity is typical for this period and ticks usually only move between suitable positions for entering a new host and microhabitats with sufficiently high air humidity. This period lasts for more than 90% of the life time (up to several years) in most tick species, not ending till they get contact to a suitable host again, or die (Needham and Teel, 1991).

Systematically ticks (Ixodida) belong to the large group of mites (Acari) within the Arachnida. Ticks are the largest mites (unfed adults range between 2 and more than 20 mm) and comprise the families Nuttalliellidae, Argasidae and Ixodidae with nearly 900 species worldwide. The Ixodidae include the largest number of species and according to Russian scientists they are subdivided into the two subfamilies Ixodinae (all species of *Ixodes*) and Amblyomminae (all other genera of the Ixodidae). Western authors divide the Ixodidae in the five subfamilies Ixodinae, Bothriocrotoninae, Amblyomminae, Haemaphysalinae and Rhipicephalinae. (Appendix, Table 1). The Ixodinae are also referred to as Prostriata and include all species of the genus *Ixodes*. The remaining subfamilies are commonly termed Metastriata and unite all other recent eleven genera of the Ixodidae. Supporters of

the latter system recognize Ixodinae and Amblyomminae instead of Prostriata and Metastriata but hold on the separation into five subfamilies (Barker and Murrell, 2008, Guglielmone et al., 2010; Keirans and Durden, 2005; Mans et al., 2012; Oliver, 1989; Sonenshine, 2005). I use Ixodinae and Amblyomminae in the same sense.

There are also different terms for the two major branches of mites, Parasitiformes and Acariformes (Oliver, 1989) and Anactinotrichida and Actinotrichida (Alberti and Coons, 1999). I follow the nomenclature of Alberti and Coons (1999).

Within mites Ixodida belong together with Opilioacarida, Holothyrida and Gamasida to the smaller group of Anactinotrichida. The Anactinotrichida are traditionally separated from Actinotrichida by the lack of birefringent setae in polarized light. Another feature common to Anactinotrichida are dermal (integumental, cuticular) glands with a typical cuticular apparatus, called calyx and usually pores (Alberti and Coons, 1999).

Five types (Figure 1) of dermal glands are distinguished in ticks: type 1 glands, type 2 glands, spiracle (spiracular) glands, foveal glands and porose areas (Coons and Alberti, 1999). The number of gland types differs among the three different tick families and between Ixodinae and Amblyomminae (Figure 1). The variation in gland type numbers is most likely caused by differences in the general life style of these groups.

The greatest variability of dermal glands is documented in Ixodidae. With the exception of *Ixodes kopsteini* (lacking porose areas) three gland types are present in its basal subfamily Ixodinae. All five integumental gland types are found in Amblyomminae, containing the most derived genera of ixodid ticks (Coons and Alberti, 1999; Sonenshine, 1991).

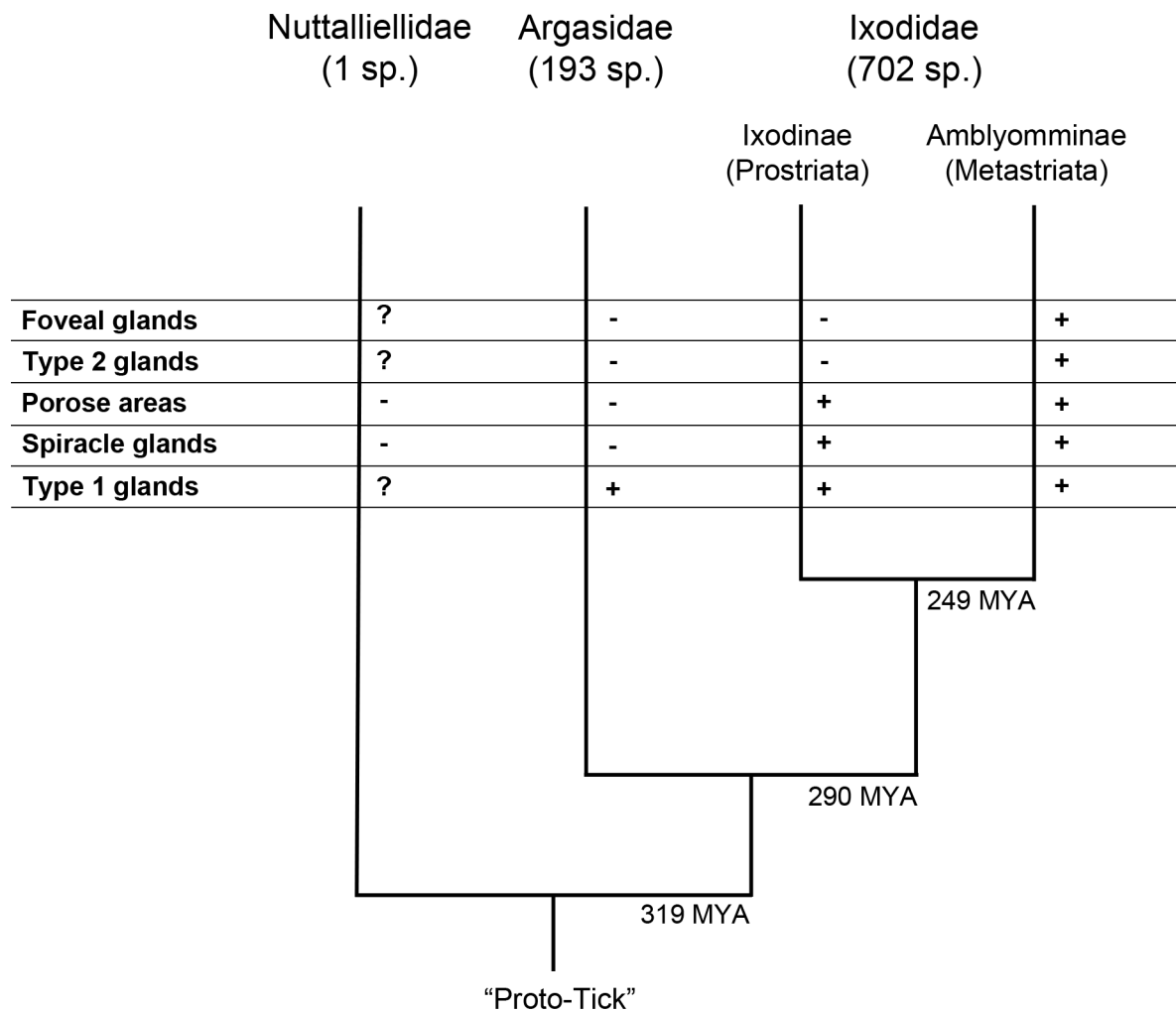


Figure 1: The distribution of dermal gland types among the major clades of Ixodida

Simplified dendrogram of the Ixodida (based on Mans et al., 2012) combined with the various distributions of dermal gland types among the three tick families and between Ixodinae (Prostriata) and Amblyomminae (Metastriata).

Diverging branches within the Argasidae and Ixodinae (Prostriata) of Mans et al., 2012 are not included because there are no indications that they differ in their gland type numbers. Only a single species of the Ixodinae, *Ixodes kopsteini*, does not fit into this scheme by lacking porose areas (Klompen et al., 1996). The numbers on the right side of the divergence points are their estimated dates (MYA, million years ago) as given by Mans et al., 2012.

The five different gland types (in bold) are named after Coons and Alberti, 1999. A plus (+) marks the presence and a minus (-) the absence of this gland type. The question marks symbolize that no information for Nuttalliellidae (*Nuttalliella namaqua*) is found on these gland types in literature. References for the absence or presence of a certain gland type are: Latif et al., 2012 and Roshdy et al., 1983 for the Nuttalliellidae; Coons and Alberti, 1999, Klompen et al., 1996 and Sonenshine, 1991 for the Argasidae and Ixodidae. Several synonyms for the different gland types exist and are summarized in Table 2, appendix of this work. The numbers of species within the tick families are based on the list from Guglielmone et al., 2010.

Spiracle glands are restricted to Ixodidae. These glands are assumed to produce hygroscopic substances decreasing water loss via the spiracles (Pugh, 1997). A secretory function of the spiracle glands is not reported by all authors. The glands are also interpreted as sensory structures (Nordenskiöld, 1906; Roshdy and Hefnawy, 1973) or are assumed to fulfil glandular and perceptive functions as “Krobylophoren” (Schulze, 1942).

A comparison of descriptions from spiracle glands in different species of Amblyomminae revealed morphological disparities (Baker, 1997; Sixl and Sixl-Voigt, 1974; Walker et al., 1996). Therefore the present study focuses on the morphology of spiracle glands in *Ixodes ricinus* a species of the basal subfamily Ixodinae (Sonenshine, 1991) which most likely exhibits the original characteristics of these glands. The main part of the study is based on examinations of unfed adult *Ixodes ricinus* but few aspects of fed female specimens from the burrow-inhabiting species *I. laguri* are also included. Since nidicolous tick species typically show more limited tolerance to desiccation (Sonenshine, 2005) *I. laguri* is investigated in view of a potential reduction (structural or numerical) of spiracle glands and feeding as possible trigger for their secretion. Also included are some specimens of nymphal *I. ricinus* and a male *Haemaphysalis concinna* to describe differences in the morphology of spiracles between nymphal and adult stages and between spiracles of Ixodinae and Amblyomminae. Finally the morphological findings are compared with descriptions of spiracle- and other dermal glands of various tick species and possible functions of the spiracle glands are discussed.

2 Materials and methods

Adult *Ixodes ricinus* L. used for transmission electron microscopy (TEM) were from a deciduous forest on the southern border of Vienna. The unfed specimens were collected during March 2012 by dragging a white cotton cloth through vegetation. After separating males and females, the species was determined under a stereo microscope. Till dissection the ticks were kept in glass jars. Perforated lids and regular moistening of paper towels within the containers ensured air supply and high air humidity (Suppan et al., 2013).

All specimens were processed under a fume hood within one week after collection. The ticks were immersed and dissected in cold fixative (modified Karnovsky: 3% glutaraldehyde and 2% paraformaldehyde in 0.1 M phosphate buffer at a pH of 7.2). The parts of the specimens with the spiracles were fixed for 12 h at 4°C followed by rinsing in phosphate buffer solution (PBS) and postfixation on ice with 2% osmium tetroxide in PBS for 2 h. After dehydration in a graded series of ethanol they were embedded in Epon 812 resin with acetone as intermedium. Sectioning was done with a Reichert Ultracut E. Semithin sections made for orientation within the specimens were stained with toluidine blue and examined with a Nikon Y-THS light microscope (LM) equipped with an OPTOCAM-I. Ultrathin sections were mounted on Formvar-coated slot grids and stained with uranyl acetate followed by lead citrate. The sections were viewed in a Zeiss Libra 120 at 120 kV (Suppan et al., 2013).

Specimens of *Ixodes laguri* were kindly provided by Carina Siutz, a colleague from the Department of Behavioural Biology (University of Vienna) working on Common hamsters, *Cricetus cricetus* L. Hamsters captured with life traps (Siutz and Millesi, 2012) were inspected for ticks. All manipulations were approved by the Austrian Ministry, the City of Vienna (MA22-1216/2009), and the Ethical Committee for Animal

Welfare (GZ 68.210/12-BrGT/2003 and 66.006/0007/II/10b/2009). Some ticks of various life- and feeding stages were collected with forceps from their hosts and transferred into sealable boxes with sufficient humidity and air supply. Subsequent examination under a stereo microscope showed that these specimens were immature stages and females of *Ixodes laguri* Olenov, 1929. For TEM living females were treated like *I. ricinus* but were occasionally stored longer than one week after collection under the same condition as described for specimens of *I. ricinus*.

For scanning electron microscopy (SEM), additional adults and nymphal stages of *I. ricinus* were collected (same method as described above). In the course of these collections also specimens of *Haemaphysalis concinna* Koch, 1844 (one larva, one nymphal stage and a male) were found on the cotton cloth. These specimens were stored in 70% ethanol till they were either critical point dried (Leica EM CPD 300) or dried chemically with hexamethyldisilazane. Razor blade sections through spiracle plates of *I. ricinus* were also dried chemically. All samples were glued to aluminium stubs using carbon plates, coated with gold in an Agar B734 sputter coater, and examined in a Philips REM XL 20 at 10 or 15 kV.

Pores (aeropyles) and pore row numbers of spiracle plates were counted on the scanning electron micrographs in this study. They are only used for description of the examined specimens but are not statistically valid. A comparison from the pore numbers of nymphs, males and females in different species (Baker, 1997) suggests that individual differences between specimens of a certain life stage are low within the same species.

3 Results

3.1 Dermal gland openings

Three different dermal gland openings are found in *Ixodes ricinus*: Type 1 glands, the spiracle (spiracular) plates and the porose areas. The latter are only present in females. Type 1 glands are scattered over the whole body of adult *I. ricinus*. They are numerous on the ventral plates of males and they are in great number on the dorsolateral portions of the palps in females. Single openings of this gland type are found scattered on the legs of both sexes. The openings of type 1 glands contain two crescent-shaped parts orientated in the long axis of a slightly oval depression. The latter can be shallow as on the palps of females or sunken into the cuticle like on the ventral plates of males (Figure 2a). Type 1 gland openings are clearly visible in sclerotized body parts but not in the folded cuticle of the alloscutum in females. The gland openings appear closed or only slightly open (Figure 2a).

The porose areas in female specimens of *I. ricinus* (Figure 2b) consist of two well separated pear-shaped fields on the dorsal side of the *basis capituli* of the gnathosoma. A single field contains numerous pores which are round to oval in shape and often filled with undefined material and bacteria. Clean pores reveal deep depressions getting slightly narrower to the ground.

Examination of a larval and nymphal specimen of *Haemaphysalis concinna* also revealed openings of the two other gland types, the type 2 glands and the foveal glands (Figure 2c and 2d). The external openings of type 2 glands are characterized by a slightly elevated and thick wall surrounding an oval pit (Figure 2c). They also seem to contain crescent-shaped parts. Material protruding from the ground of the pit often covers further details of the gland openings.

Examination of the nymphal stage of *Haemaphysalis concinna* allows the identification of two *foveae dorsales* (Figure 2d) containing the surface openings of foveal glands.

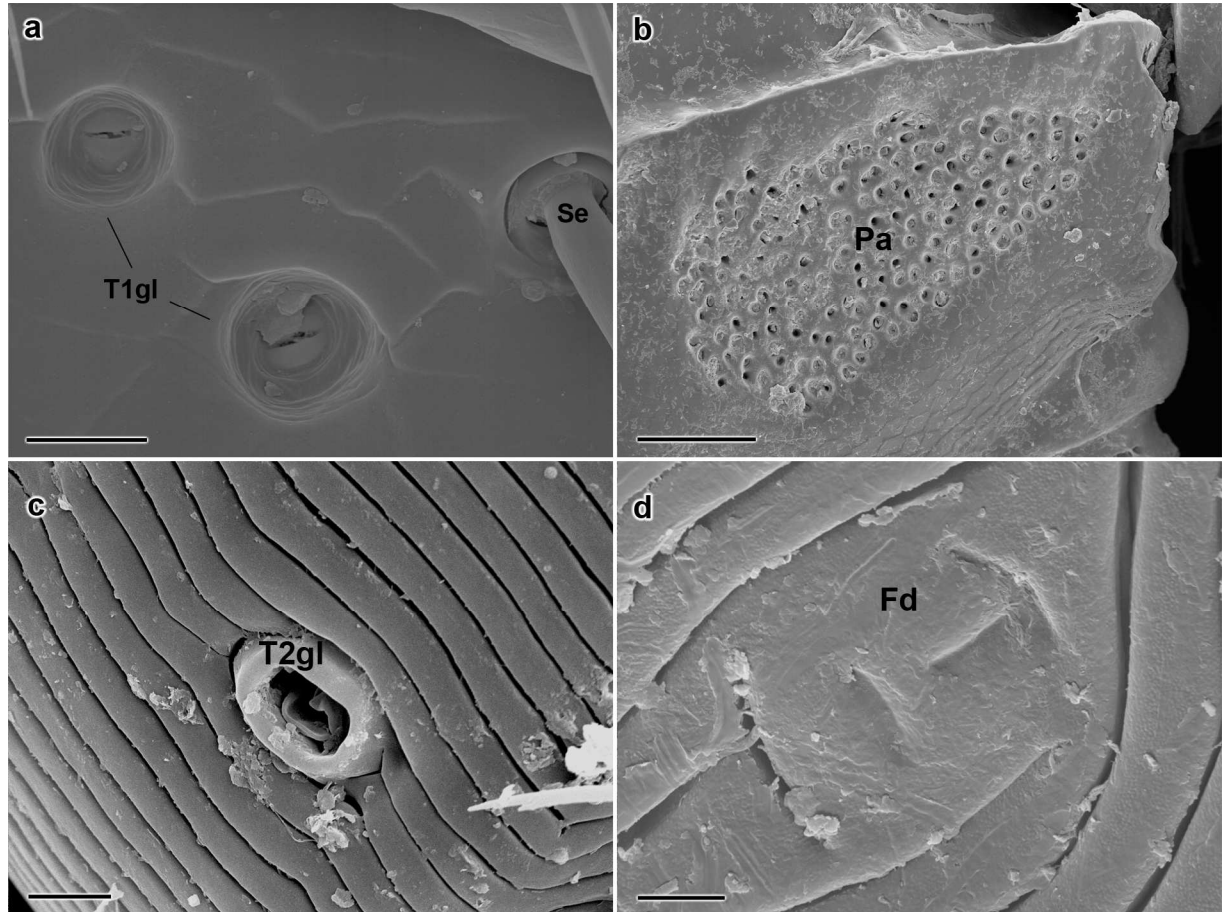


Figure 2: External openings of different dermal glands on the body surface of ixodid ticks. Certain gland types can be identified by their structure and position on the tick's body (SEM images).

a) *Ixodes ricinus*, male: Two type 1 gland openings each in a small depression on the ventral side (left lateral plate). Scale bar: 10 µm. b) *Ixodes ricinus*, female: Surface openings of porose areas on the basis capitulum (left dorsal side). Scale bar: 50 µm. c) *Haemaphysalis concinna*, larva: Opening of a type 2 gland on a festoon (on the ventral side). Scale bar: 5 µm. d) *Haemaphysalis concinna*, nymph: One of the small plates (*Foveae dorsales*) with few slit-like openings which appear sealed. Scale bar: 5 µm.

Fd, *Fovea dorsales*; Pa, porose areas; Se, seta; T1gl, type 1 glands; T2gl, type 2 gland

The *foveae dorsales* are two small cuticular fields which interrupt the cuticular folding on the dorsal surface behind the scutum and are situated at a height close to the posterior end of the third coxae. Each of the two fields contains few elongated

depressions. Four depressions can be distinguished on the right field and only two on the left field. The two fields also differ in the arrangement and orientation of the elongated depressions. In the examined nymphal stage the *foveae dorsales* seem to lack real openings.

3.2 Spiracle morphology

A single pair of spiracle plates is present in nymphal and adult stages of *I. ricinus* (Figure 3a). Each plate is located laterally, on the ventral body side behind each coxa of the fourth legs. The slightly elevated spiracle plates are circular in nymphs and females (Figures 4a and 4b) and ovoid in males (Figure 3b).

The margin of nymphal spiracle plates is serrated giving the plate a “saw blade” appearance (Figure 4a). These serrations are also present on the plates of adults but are not as obvious as in nymphal stages.

In adults the spiracle plates bear several rows of pores (aeropyles) and the macula, a pore-less area with the “ostium” (Figures 3b and 4b). The latter can include a narrow cleft or it appears sealed. In males and females the eccentric macula is depressed whereas in nymphal stages this feature is not as pronounced as in adults. The “ostium” is missing in the macula of nymphal stages. Instead a single small deepening is present (Figure 4a).

Depending on the extension of the surface area between the macula and the margin of the spiracle plate the number of pores (aeropyles) within an individual plate varies. In nymphal and adult *Ixodes ricinus* the pores appear as distinct rows but not all of them are continuous over the entire plate. In the female spiracle plate (Figure 4b) are about 208 pores (120 without the outermost peripheral row of distinctly smaller pores) arranged in four to six rows. The spiracle plate of the male (Figure 3b)

contains between three and eight rows of about 159 pores (88 excluding the peripheral row). The smaller plate of the nymph (Figure 4a) bears 120 pores (57 without the peripheral row) in four to five rows. In all life stages the outermost (peripheral) row consists of pores with a smaller diameter compared to the neighbouring row (Figures 3b, 4a and 4b).

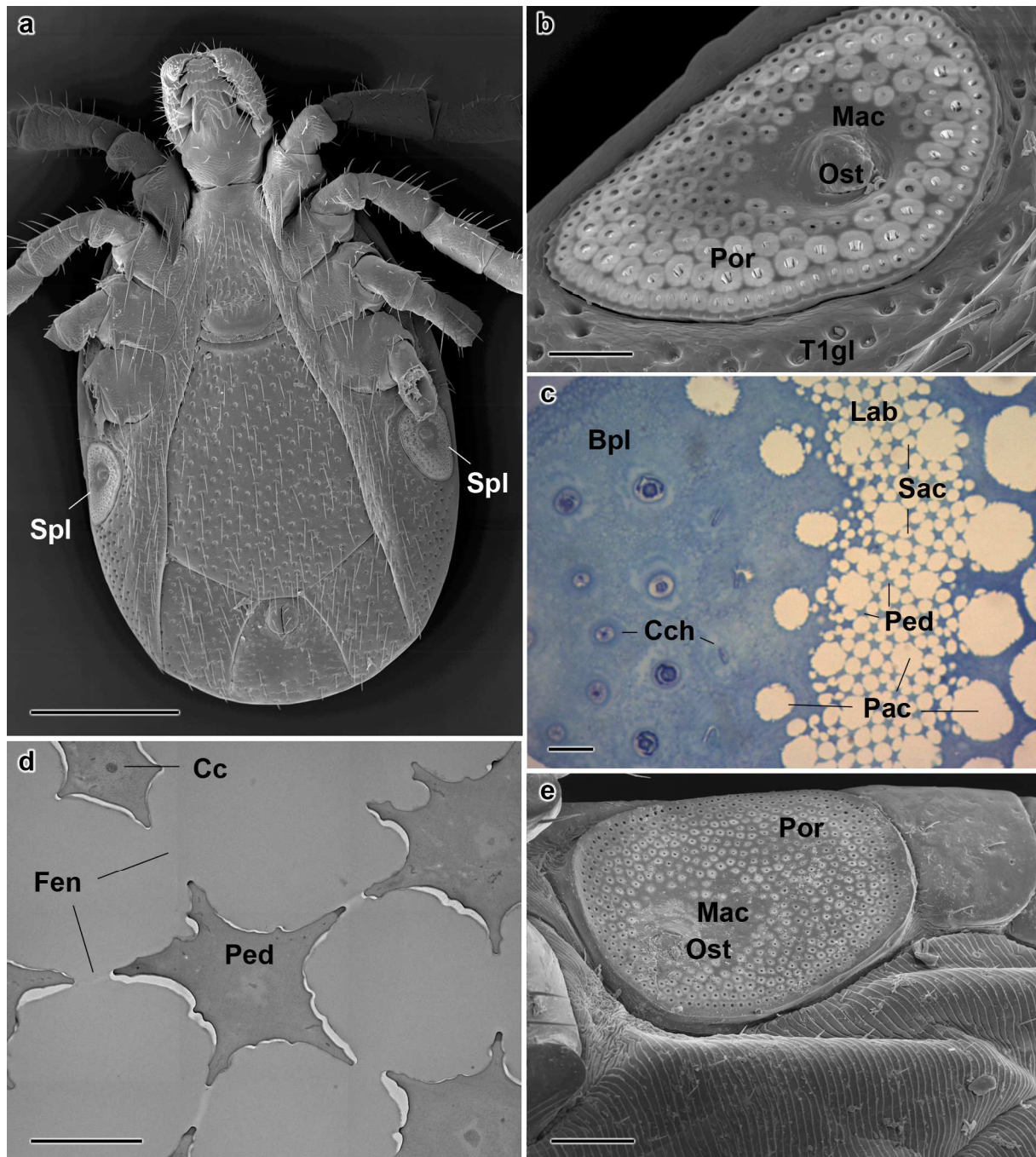


Figure 3: a) *Ixodes ricinus*, male: Position of the paired spiracle plates (Legs III and IV are partially removed). SEM image, Scale bar: 500 μ m. b) *Ixodes ricinus*, male: Oval shaped spiracle plate with the pore-less macula and the rows of pores for gas-exchange. The cuticle around the spiracle plate bears

openings of type 1 glands. SEM image, Scale bar: 50 μm . c) *Ixodes ricinus*, male: Cross section through the posterior part of a spiracle. The cuticular channels in the baseplate contain extensions from the spiracle glands. The larger primary atrial chambers are surrounded by smaller secondary atrial chambers. The arrangement of primary atrial chambers and cuticular channels in the baseplate reflects the rows of pores in the spiracle plate. LM image (toluidine blue), Scale bar: 10 μm . d) *Ixodes ricinus*, female: Cross section of pedicels from the labyrinth. The narrow fenestrations between them allow air flow. TEM image, Scale bar: 2 μm . e) *Haemaphysalis concinna*, male: Spiracle plate bearing larger numbers of pores than *I. ricinus*. SEM image, Scale bar: 100 μm .

Bpl, baseplate; Cch, cuticular channel; Cc, central channel of pedicels; Fen, fenestrations; Lab, labyrinth; Mac, macula; Ost, "ostium"; Pac, primary atrial chambers; Ped, pedicel(s); Por, pores; Sac, secondary atrial chambers; Spl, spiracle plate; T1gl, type 1 glands

The spiracle plate of a fed female specimen from *Ixodes laguri* is similar to that described in female *I. ricinus*. It is only smaller in size and bears three to six pore rows. The number of pores counted is 171 (87 without those in the peripheral row). The bodies of the females investigated are covered for the most part by a dense tight fitting pelage (Figure 4e).

The male *Haemaphysalis concinna* prepared for comparison with specimens of *Ixodes ricinus* has a rectangular rather than ovoid spiracle outline. In contrast to the specimens of *I. ricinus* and *I. laguri* the spiracle margin lacks serrations. The spiracle plate (Figure 3e) is larger than that of the male *I. ricinus* and contains a greater number of pores (about 514, excluding the outermost row about 400). Distinct pore rows are difficult to distinguish. The macula is situated close to the anterior edge of the spiracle plate resulting in two pore rows in the anterior part. The greatest number of pore rows is about 14 in the posterior part of the spiracle plate. The pore size in the outermost and the neighbouring rows does not differ markedly as in *Ixodes* sp. (Figure 3e).

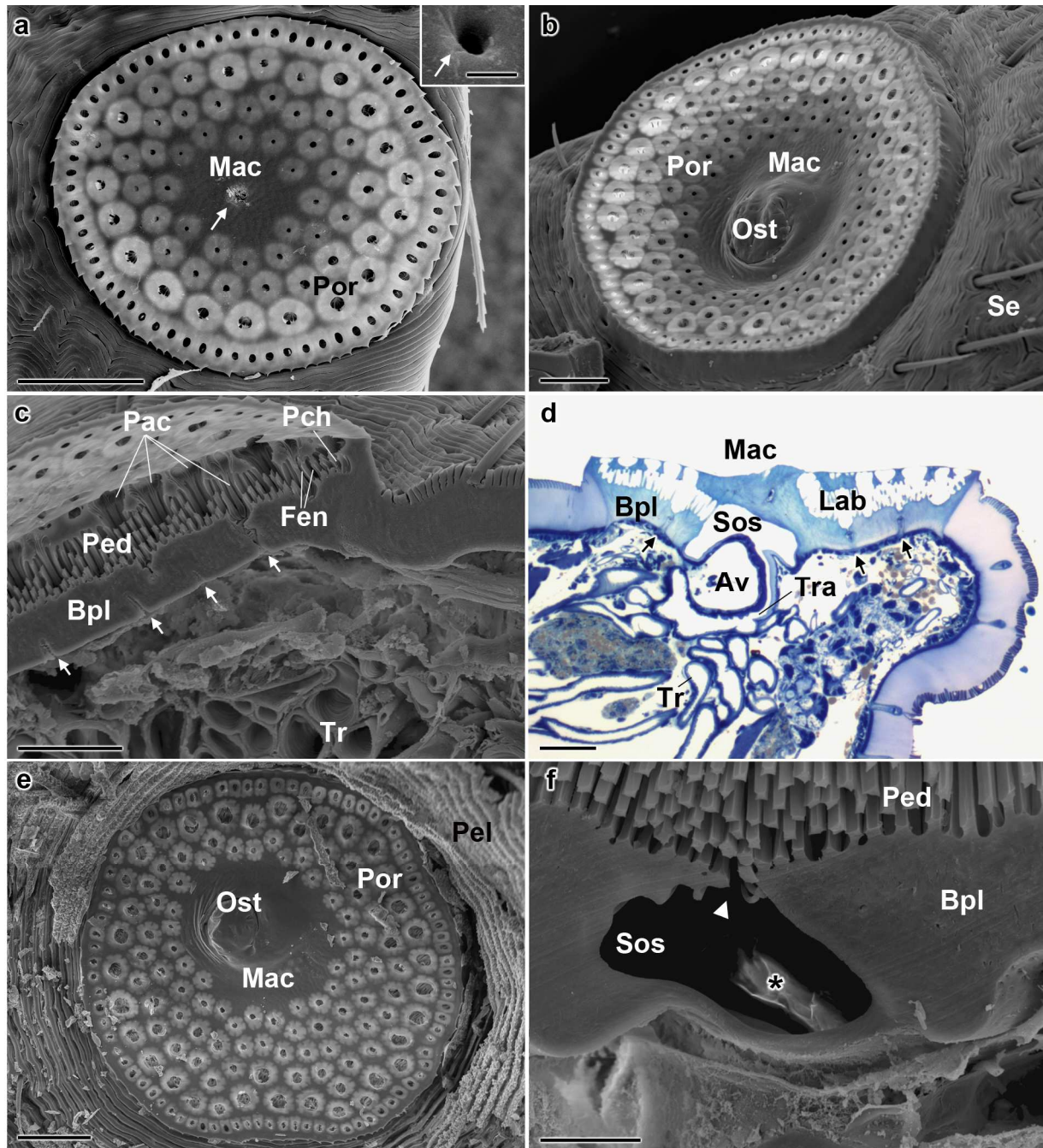


Figure 4: a) *Ixodes ricinus*, nymph: Nymphal spiracle with a single pore (arrow) instead of the “ostium” within the macula. SEM image, Scale bar: 50 μ m. Insert: Pore. SEM image, Scale bar: 5 μ m. b) *Ixodes ricinus*, female: Spiracle plate with circular outline. SEM image, Scale bar: 50 μ m. c) *Ixodes ricinus*, female: Razor blade section through the spiracle showing primary atrial and peripheral chambers in the labyrinth. Fenestrations are between the pedicels. The arrows mark the cuticular channels of the spiracle glands. SEM image, Scale bar: 50 μ m. d) *Ixodes ricinus*, female: Overview of the spiracle with the main compartments labyrinth, subostial space and tracheal atrium. The arrows point to the cuticular channels of the spiracle glands. LM image (toluidine blue), Scale bar: 50 μ m. e) *Ixodes laguri*, female: The spiracle plate is smaller and bears fewer pores than that in the female of *I. ricinus*. The pelage covering most of the cuticle around the spiracle plate is most likely a product of type 1 glands. SEM image, Scale bar: 50 μ m. f) *Ixodes ricinus*, female: Razor blade section through the spiracle

showing the subostial space and its connection to the labyrinth (arrow head). Within the subostial space a structure (asterisk) is visible that might be a remnant of a nymphal trachea. SEM image, Scale bar: 20 μ m.

Av, atrial valve; Bpl, baseplate; Fen, fenestrations; Lab, labyrinth; Mac, macula; Ost, "ostium"; Pac, primary atrial chambers; Pch, peripheral chamber; Ped, pedicels; Pel, pelage; Por, pores; Se, setae; Sos, subostial space; Tr, tracheae; Tra, tracheal atrium

Semithin sections through the spiracle (spiracular) plates reveal their main features.

A thick cuticular plate (baseplate) covers the hypodermal layer and is connected to a thinner cuticular plate on the surface of the spiracle by numerous cuticular rods (pedicels). Electron micrographs reveal that the pedicels of the labyrinth are densely packed leaving narrow openings (fenestrations) between them (Figures 3d and 4c). The star shaped pedicels are not solid but contain several holes in cross-section (Figure 3d). Usually a hole close to the centre of column is surrounded by few smaller ones. They are round to oval in shape but form sometimes radial extensions (of different length). It is not clear whether they are interconnected with each other.

The space occupied by the pedicels is the labyrinth in which circular cavities (chambers) of different diameter and height are obvious (Figures 3c and 4d). Larger bell shaped cavities (primary atrial chambers) surrounded by smaller ones (secondary atrial chambers) can be distinguished in longitudinal sections (Figure 4d). Cross sections through the spiracles (Figure 3c) reveal a regular arrangement of the different sized primary and secondary atrial chambers. In addition small peripheral chambers are present only below the outermost row of pores (Figure 4c). The distal ends of the bell shaped primary atrial chambers are larger than the proximal ones. In the thick cuticular baseplate are cuticular channels containing cytoplasmic extensions from the hypodermal layer (Figures 3c and 4d). Between the last row of primary atrial chambers and the first circular row of cuticular channels is another row containing sections of cuticular channels with a conspicuous ovoid to slit like outline (Figure 3c).

This row contains the most distal parts of the spiracle gland. The cuticular channels as well as the chambers of the labyrinth are arranged in rows corresponding to the superficial pores (aeropyles) of the spiracle plate.

The large subostial space is a cavity in the baseplate below the pore-less area of the macula. It is connected to the labyrinth by fenestrations between the pedicels (Figure 4f). The subostial space leads into the tracheal atrium which connects to the tracheal system of the tick (Figure 4d). The pipe-like structure with its loose apical end in the subostial space of a female *I. ricinus* might be a residual of a nymphal trachea (Figure 4f).

3.3 Spiracle gland structure

Each spiracle gland consists of a cuticular and a cellular portion (Figures 5 and 6). The cuticular part is a pear-shaped channel through the baseplate, with a simple tube opening on the hypodermal side. From the primary atrial chamber a short duct of small diameter leads into the cuticular channel. This duct widens into the large horizontal cavity, an elongated space. The horizontal cavity has a small opening leading into the duct of the cellular portion of the spiracle glands. This opening of the horizontal cavity is flanked by a proximal and a distal pair of cuticular flaps (calyx). Electron-lucent fibres which are anchored in the ground of the horizontal cavity extend towards the distal flaps. The distal pair of flaps facing the horizontal cavity is electron-dense and they form irregular projections. Similar structures surround the horizontal cavity and its connection to the chamber in the labyrinth. The proximal flaps have a smooth outline. Muscles are neither attached to the distal nor to the proximal pair of flaps (Figure 5).



Figure 5: *Ixodes ricinus*, female: Longitudinal section through the distal part of a spiracle gland. The cuticular channel in the baseplate widens distally to make room for the characteristic calyx structure of dermal glands. TEM image, Scale bar: 1 μ m. Insert: *Ixodes ricinus*, male: Cross-section below the horizontal cavity showing the cells of the spiracle gland in the distal region. Scale bar: 1 μ m. TEM image from Suppan et al., 2013, *Acarologia* 53(2): 127-136.

Bpl, baseplate; CtPac, connection to primary atrial chamber; D, duct; Dc, duct cell; Df, distal flap; Fib, fibres; Hc, horizontal cavity; M, mitochondria; Mt, microtubules; Pf, proximal flap; Shc, sheath cell; Suc, support cell

The cellular portion of a spiracle gland is made of six cells belonging to four different cell types. Two secretory cells each forming a sac shaped reservoir lined with numerous microvilli are the centre of the gland within the hypodermal layer. These reservoirs are usually filled with secretory material (Figures 6 and 7c). The open side of each reservoir forms a neck which elongates into a single duct cell originating between the two secretory cells. The duct cell surrounds the necks of the secretory cells and also sends narrow prolongations into the reservoirs where they overlap the ascending neck regions and finally end attached to the wall of the reservoirs. The resulting two ascends are collected to a single lumen within the duct cell. In the contact zone between the duct cell and the secretory cell necks septate desmosomes are present (Figures 6 and 7a). A single support cell in close proximity to the secretory cells enters the cuticular channel in the baseplate together with the duct does not surround the duct cell completely (Figure 6, insert). In the distal and wider portions of the cuticular duct (i.e. closer to the labyrinth or the body surface) the extensions of the two cells are getting smaller or disappear. In the region with the cuticular flaps the support cell encloses the whole duct cell and makes up the largest part of cross-sections in this region (Figure 5, insert). The duct cell ends between the proximal cuticular flaps connecting its lumen to the opening in the horizontal cavity (Figure 5). The support cell extends around the tips of the distal cuticular flaps to their upper surface. Two sheath cells originate laterally from the duct- and support cell within the hypodermal layer. They surround the duct cell and the support cell. Close to the hypodermis they form lateral processes which interconnect with each other and with the processes of the duct cell and the sheath cells (Figure 6, insert). In



Figure 6: *Ixodes ricinus*, female: Longitudinal section through the proximal part of a spiracle gland. The diameter of the cuticular channel is smaller than in the distal part. The characteristic reservoir of the spiracle glands is visible in the hypodermal layer. TEM image, Scale bar: 2 μm . Insert: *Ixodes ricinus*, male: Cross-section showing the cells of the spiracle gland in the proximal region. Scale bar: 1 μm . TEM image from Suppan et al., 2013, *Acarologia* 53(2): 127-136.

Bl, basal lamina; Bpl, baseplate; D, duct; Dc, duct cell; M, mitochondria; Mt, microtubules; Mv, microvilli; N, nucleus; Sc, secretory cell; Scn, secretory cell neck; Shc, sheath cell; Sm, secretory material; Suc, support cell

the distal part of the cuticular duct, where the support cell encloses the duct cell completely the cell extensions are only present between the neighbouring sheath

cells (Figure 5, insert). Along the major length of the cuticular duct the sheath cells are narrow but widen in the distal portion to enclose the horizontal cavity and its ascending part to the primary atrial chamber. The cells continuously narrow again and end close to the labyrinth of the spiracle (Figure 5).

The duct cell often forms irregular cellular protrusions within its lumen which lack the uniform appearance of microvilli. Occasionally the latter are visible isolated or in small groups, but not in the dense and regular fashion of the secretory reservoirs. The lumen is getting larger between the lower parts of the cuticular flaps changing into a narrow channel. The duct cell contains a dense array of microtubules getting obvious near the horizontal cavity (Figure 5). The cell often appears electron-denser than the neighbouring support cell.

The latter also contains numerous microtubules and high numbers of large mitochondria. Between the proximal and distal flaps there are homogenous (fine granular) areas conspicuously lacking any larger cell organelles (e.g. mitochondria). Since no membrane separates these regions from the cytoplasm of the support cell they seem to be part of it (Figure 5).

The secretory cells contain basal, elongated nuclei and only little rough endoplasmic reticulum, few Golgi bodies, vesicles with contents similar to those in the duct lumen, and numerous mitochondria. No nuclei are present in the cellular portions of the duct in the baseplate.

With exception of a single male specimen the sac-shaped reservoirs of the secretory cells contain large amounts of secretory material (in unfed specimens of adult *I. ricinus*). Its contents are mainly granular and of different electron density. The reservoirs and parts of the duct occasionally also contain fibrous structures. Within the secretory deposits often dense droplets and an electron-lucent material can be distinguished. The confluence of the droplets results in aggregations of different size

and shape surrounded by a rim of the electron-lucent material. Larger aggregations of the electron-lucent material are also present in the periphery of the reservoir filling the narrow crevices between the densely arranged microvilli (Figure 7c). The dense secretions are also visible in the duct lumen and in the horizontal cavity. In these parts it appears aggregated and no longer separated in droplets. Within the duct lumen the electron-lucent portions are not obvious. No secretions are present in the primary atrial chambers of the labyrinth.

The glandular cells of the single male specimen without material in the reservoir appear intact. Several vesicles close to the cellular border of the reservoir seem to have lost their contents (Figure 7a). In one case two intact vesicles are present within the reservoir. They are close to the apical membrane of a secretory cell bearing circular depressions of similar size and open towards the lumen. The vesicle membranes are covered by secretory cell fragments (Figure 7b).

The basic structure of the spiracle glands in *Ixodes laguri* is similar to that of *I. ricinus*. The secretory reservoir from a fed female *Ixodes laguri* contains material. The granular contents are not as fine as in the unfed *Ixodes ricinus* specimens but also an electron-dense and –lucent compound can be distinguished. The dense material appears concentrated to a single mass which is penetrated by numerous different sized intrusions of the electron-lucent material surrounding it (Figure 7d). No convincing signs of neuronal innervations of spiracle glands can be reported.

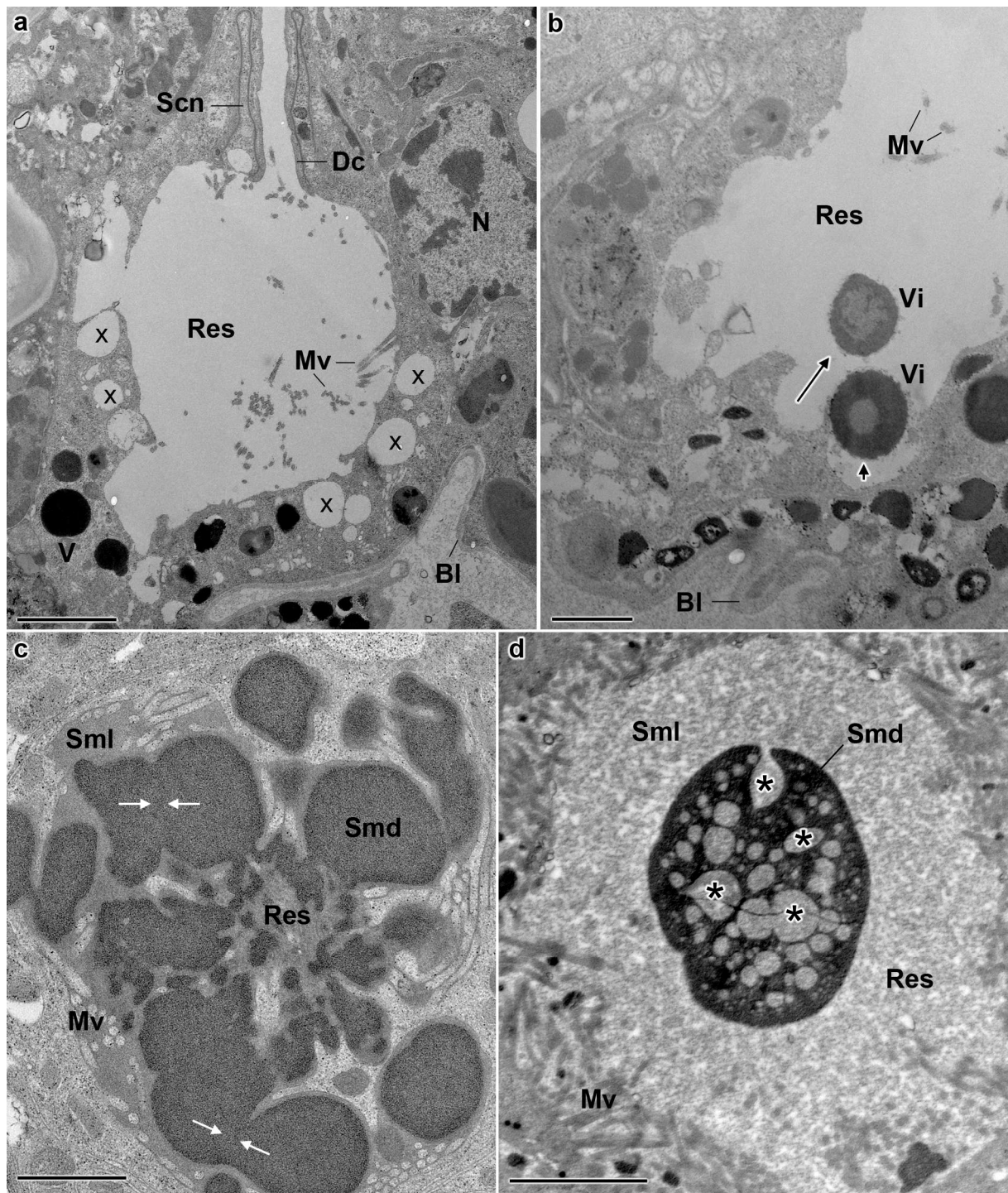


Figure 7: Apocrine secretion of spiracle gland and secretory material in its reservoir (TEM images)

a) *Ixodes ricinus*, male: Overview of a secretory reservoir with vesicles around the lumen. Scale bar: 2 μm . b) *Ixodes ricinus*, male: Secretory reservoir of the same specimen as in figure 7a. Two intact vesicles are discharged into the lumen from depressions in the secretory cell (arrows). Scale bar: 1 μm . c) *Ixodes ricinus*, female: Secretory reservoir filled with granular secretory material of different electron-density. Portions of electron-dense material aggregate (arrows facing each other). The electron-lucent material is around the dense portions and in the periphery of the secretory reservoir. Scale bar: 1 μm . d) *Ixodes laguri*, female: Secretory reservoir of a fed female with “coarsely grained”

secretory material. The dense material is interspersed with electron-lucent secretory material (asterisks). Scale bar: 1 μ m.

Bl, basal lamina; Dc, duct cell; Mv, microvilli; N, nucleus; Res, reservoir; Scn, secretory cell neck; Smd, electron-dense secretory material; Sml, electron-lucent secretory material; V, vesicles; Vi, intact vesicles; X, discharged vesicles

4 Discussion

4.1 Dermal gland openings

Following previous descriptions the openings of the different dermal gland types can be distinguished by their morphology and their position (Axtell and LeFurgey, 1979; Coons and Alberti, 1999; Klompen et al., 1996; Walker et al., 1996; Yoder et al., 1993; Yoder et al., 2009a).

Secretory activity for type 1 glands is reported during and after feeding in *Rhipicephalus appendiculatus* (Walker et al., 1996) and *Amblyomma cooperi* (Labruna et al., 2004). This agrees with the overall closed condition of type 1 glands observed in the unfed specimens of *Ixodes ricinus*.

The function of type 1 glands is uncertain but suggestions include the production of a water proof substance, excretory products from hypodermal cells (intense metabolism to form new cuticle) and the synthesis of Mounting Sex Pheromone (MSP) (Appendix, Table 2). If the dense coverage observed on the fed females of *Ixodes laguri* is a dermal gland product, it presumably originates from type 1 glands. Type 1 glands are the only dermal glands which are able to produce such a large-scale pelage on the ticks' body because of their wide distribution on the body surface. The appearance of this layer makes an additional waterproof possible, but does not exclude an excretory function. It might be argued that the deposition of waste product on the cuticle which will be shed (in larval and nymphal stages) is an advantage. The latter assumption might be further supported by *A. cooperi* which

produces a conspicuous black secretion in the pre-moulting period (Labruna et al., 2004). An additional pheromone role in adult ticks might be a later adaptation of the secretions.

The foveal glands with their typical slit-like openings (Axtell and LeFurgey, 1979) are the source of ASP (Attractant Sex Pheromone) in females of several genera within the Amblyomminae (Metastriata) (Sonenshine, 2006). In immature life stages these glands may serve desalination by the storage of di- and tri-chlorophenols after a blood meal. It has to be stressed that the foveal glands only store but do not secrete their contents in immature stages and males. Secretion of 2,6-DCP to the body surface only occurs in females where it acts as ASP (Yoder et al., 2002). This could explain the closed appearance of the surface openings in the examined nymph. On the other hand it raises the question why the elongated depressions (presumably the later slit-like openings) are present in nymphal stages at all.

Nothing new can be added to type 2 glands and porose areas. The often observed material protruding from the ground of type 2 glands is most likely the secretory product of these glands and is in agreement with the fact that secretion is easily induced (Yoder et al., 2009a; Yoder et al., 2009b).

4.2 Spiracle morphology

The morphology of the spiracles from *Ixodes ricinus* agrees with that of other ixodid ticks (e.g. Pugh et al., 1988; Pugh et al., 1990; Schöl et al., 1995). Therefore only additional or controversial features will be discussed.

The serrations on the spiracle margins from *Ixodes* specimens and their lack in the male of *Haemaphysalis concinna* indicate that these structures are typical for *Ixodes*.

The functionality of the “ostium” in the ixodid spiracles was a matter of dispute for a long time. According to authors like Arthur (1956) the pores (goblets) of the spiracle plate are covered by a thin cuticular layer and are closed. Instead the “ostium” opened and closed by muscle action is thought to be the functional opening for gas exchange. Alternately other scientists described pores (aeropyles) permanently open in the spiracle plate (Hinton, 1967; Woolley, 1972) and a sealed condition of the “ostium”, which is explained as scare from the moult of the nymphal to the adult stage. In none of the presently examined specimens the numerous pores of the spiracle plate appeared closed. They are permanently open. Ticks lack structures for tracheal ventilation and gas exchange is only driven by diffusion (Pugh, 1997). Taking into account the connection between labyrinth and subostial space a functional ostium (which also leads into the subostial space) will only have minor influence on total air flow. The ostium is structurally not suitable to separate the tracheal system from ambient air. Such a mechanism exists in form of the atrial valve (movable by muscle action) between subostial space and tracheal atrium (Pugh, 1997). This explains the prolonged survival in cyanide gas observed in *Haemaphysalis longicornis* (Roshdy and Hefnawy, 1973). Based on this and the presence of a single open pore within the maculae of nymphal stages it is most likely that the “ostium” in adult ixodid ticks indeed constitutes a moulting scare (Hinton, 1967). An incomplete closure instead of totally sealed “ostia” might be the result of individual differences during the moulting process.

The relative increase of pore (aeropyle) numbers in the spiracle plates from nymphal to male and female stages of *I. ricinus* is in agreement with other tick species. Higher pore numbers in females of the same species are thought to reflect an increased demand for gas exchange, not only in relation to the often larger body size of females but also due to energy intensive metabolic processes (e.g. digestion of enormous

blood meals, production of large egg masses) requiring more oxygen. Physiological processes are highly dependent on the microclimate of a particular tick species (Baker, 1997). Compared to the female of *I. ricinus* the smaller pore (aeropyles) number of the female *I. laguri* is likely caused by the smaller size of this species but might be also the result of its nidicolous lifestyle. Species living this way spend their life largely in sheltered environments (nests, burrows, caves used by their host animals). The conditions are more uniform throughout the year than in the external macroenvironment (Sonenshine, 2005). Therefore energy consuming changes between suitable places to get in touch with a host and places with sufficient air humidity (Needham and Teel, 1991) are not necessary. The larger spiracle plates of the male specimen from *Haemaphysalis concinna* most likely is a result of its larger body size compared to the male of *I. ricinus*. Both were collected in the same region and exhibit a similar life-style as non nidicolous (exophilic), three-host ticks (Cringoli et al., 2005). Another explanation for the observed differences between *I. laguri* and *I. ricinus* will be discussed below (linking pore and gland number).

4.3 Hypothetical development of the spiracle glands from type 1 glands

Type 1 glands are also present in Argasidae (Coons and Alberti, 1999) and therefore seem to be the oldest dermal gland type in ixodid ticks. As obvious from ventral plates in male *I. ricinus* a great number of type 1 glands are on the tick's body. Spiracle glands most likely developed from a precursor similar to recent type 1 glands because the latter are morphologically similar (Coons and Alberti, 1999). Glands associated with the spiracles are restricted to Ixodidae which have larger spiracle plates than the Nuttalliellidae and Argasidae (Pugh, 1997). An enlargement of the slightly elevated spiracle plates might have covered the precursor glands in the

vicinity of the original smaller spiracles. Associated with the spiracle labyrinth but no longer opening directly on the body surface the glands may have lost or modified their original function and specialized.

4.4 Spiracle gland structure

The spiracle glands of *Ixodes ricinus* meet the morphological characteristics for dermal (integumental, cuticular) glands given by Coons and Alberti (1999): They are located within the hypodermal layer (below the spiracles), consist of few cells only (six cells in *I. ricinus*) and a duct opening on the body surface by a pore. Even though the duct actually leads into the labyrinth, a connection to the surface is given by the pores in the spiracle plate. Typical for dermal glands in Anactinotrichida the spiracle glands of *I. ricinus* are equipped with a cuticular apparatus (the calyx).

The hypodermal origin of the spiracle glands of *Ixodes ricinus* is in agreement with descriptions in other tick species. Unfortunately the cell number of the spiracle glands in species other than *I. ricinus* is not known. Therefore a comparison cannot be made. But there is agreement that several “duct cells” (duct-, support- and sheath cells in the present study) and in most cases two “gland cells” (secretory cells in the present work) (Sixl and Sixl-Voigt, 1974; Walker et al., 1996) are components of the spiracle glands.

The lack of neuronal innervations of the glands is in agreement with findings in *Rhipicephalus appendiculatus* (Walker et al., 1996) and *Amblyomma americanum* (Baker, 1997). The description of sensory cells in the spiracle gland tissue from *Haemaphysalis inermis* (Sixl and Sixl-Voigt, 1974) is probably based on the assumed main function of the glands as sensory organ (Schulze, 1942). Nevertheless it is uncertain how secretion of the spiracle glands is induced. In addition a nerve is

described in another dermal gland type, the foveal glands of Amblyomminae (Metastrata) for which at least partially a neurosecretory regulation is hypothesized (Sonenshine et al., 1981). Observations that secretion of these glands can be inhibited by treatment with catecholamine antagonists (Sonenshine, 2004) support this hypothesis.

A restriction of the spiracle glands to the cuticular channels is described in *A. americanum*. Therefore the spiracle glands are termed “basal plate glands” in this species (Baker, 1997). This restriction is not observed in *I. ricinus* as well as in *Haemaphysalis inermis* (Sixl and Sixl-Voigt, 1974) and *R. appendiculatus* (Walker et al., 1996).

Apart from basal plate glands additional cells with presumed secretory activity are described in the hypodermis below the spiracles of *A. americanum*. They are thought to produce a granular substance observed in the narrow gap between the hypodermal layer and the cuticle as well as in channels (basal plate channels) traversing the baseplate. These channels, smaller in diameter than those harbouring the basal plate glands continue into the labyrinth as central (and secondary?) channels of the pedicels (Baker, 1997). The basal plate channels seem to be identical with the pore canals described in the spiracles of *R. appendiculatus* (Walker et al., 1996). Pore canals are not exclusively found in the cuticle of spiracles but are characteristic for the (pro-) cuticle of ticks in general. These canals are thought to allow the exchange of material, e.g. for the restoration of the wax layer after abrasion (Coons and Alberti, 1999; Walker et al., 1996). The spiracles of *I. ricinus* also contain pore canals (basal plate channels) and pedicel channels (central- and secondary) but the pore canals are not as pronounced as in *A. americanum* (Baker, 1997). Varying numbers of those canals are known from different species and particular body regions (Coons and Alberti, 1999). The lower number of pore canals in *I. ricinus*

might also reflect different physiological stages (specimens of the present study were unfed and collected soon after winter inactivity).

One of the most conspicuous differences of described spiracle glands concerns the number of flaps in the calyx region. Bearing in mind the basal position of the genus *Ixodes* within the Ixodidae (Sonenshine, 1991) and the emergence of spiracle glands within this tick family (Pugh, 1997) it can be assumed that four flaps as in *I. ricinus* and *I. laguri* are a basal feature of spiracle glands. Within the Amblyomminae (Metastrata) the same number of flaps is also visible in images of *Haemaphysalis inermis* (Sixl and Sixl-Voigt, 1974). Only two sclerites are described in *R. appendiculatus* (Walker et al., 1996), which are presumably identical to a single pair of the original flaps. The “basal plate glands” in *A. americanum* are lacking cuticular projections and flap-like structures (Baker, 1997) even though a small remnant of them might be present (Suppan et al., 2013). These disparities may point to a gradual reduction of the complex cuticular apparatus but they do not reflect if a species/genus is basal or derived within the Amblyomminae (Metastrata): The genus *Haemaphysalis* contains some relatively basal species, but *Rhipicephalus* is one of the most derived genera of ixodid ticks and *Amblyomma* instead is thought to be one of the most basal genera (Sonenshine, 1991).

In *A. americanum* the reduction of the glands seems to include parts (approximately one half) of the horizontal cavity resulting in the “L-shaped duct” described by Baker (1997). So far it is not clear if these reductions functionally impair the glands because only speculation is possible on the mechanism of the calyx. Several aspects of the complex structure of the cuticular apparatus indicate motility and/or flexibility. Resilin is known to be present in the cuticle of *Ixodes ricinus* (Dillinger and Kesel, 2002) and might be also part of the calyx (Alberti and Seeman, 2005). Since muscles are lacking these structures must be moved by other means. Pressure induced motility

would be obvious. Pressure differences can be expected by extending cells of the hypodermis during feeding but may also develop from the secretory material itself. A continuously filling secretory reservoir should lead to forces directed towards the surrounding tissue. Finally secretions are forced by different pressure gradients into the duct and through the only opening of the structure into the horizontal cavity and the labyrinth. This transport depends on density of the secretory material. It can be facilitated by gravity if the tick's spiracles are directed to ground. The cuticle of the baseplate surrounding the cellular extensions of each gland might act as antagonist to pressure forces and prevent lateral torsions of the involved cells. On cellular level the dense microtubuli can protect cellular integrity and septate desmosomes may help to stabilize the position of cells in the tissue.

Homogenous (fine granular) areas conspicuously lacking any larger cell organelles as found in the support cell between the proximal and distal flaps are also known from foveal glands of *Hyalomma dromedarii* where they are described to contain minute granules and microfibrils (Sonenshine, 1991). Similar regions are also known in dermal glands from other anactinotrichid species and described to consist of an electron-lucent cuticular material (Alberti and Seeman, 2005; Alberti, 2010).

The great number of microvilli lining the secretory reservoirs is a feature often found in dermal glands of ticks. They might prevent the secretory material from sticking to the cell membranes or facilitate transport within the lumen. An additional function might be the recovery of compounds (or transport in general) over the enlarged cell surfaces.

Compared to descriptions of different dermal gland types the spiracle glands from *Ixodes ricinus* show the highest congruence with glands associated with the porose areas in *Haemaphysalis longicornis* (Kakuda et al., 1995). Even though these glands consist of more than two secretory cells and more than one duct cell they show

several similarities with the spiracle glands: Their calyx regions are also endowed with one pair of proximal flaps and one pair of distal flaps (termed “flap-like structures” and “cuticular projections”). They have the characteristically formed connections between the secretory and duct cells strengthened by septate desmosomes (Suppan et al., 2013) which are compared to “mandibles of a stag beetle” (Kakuda et al., 1995). Interestingly the glands associated with the porose areas are delocalized from the *basis capituli* into the anterior idiosomal cavity during maturation because the size of the fully developed glands exceeds the available room in the *basis capituli*. In the course of this the ducts extend extremely and the great number of microtubuli within these cells is thought to maintain cellular integrity (Kakuda et al., 1995).

4.5 Secretory product and mode of secretion

Nearly all of the examined unfed adult specimens of *I. ricinus* have well developed secretory reservoirs. Only a single male specimen obviously discharged the contents of its glands prior to fixation. The numerous empty spaces close to and the few vesicles within the reservoir of the secretory cell suggest that the secretory products are delivered into the lumen as intact membrane bound vesicles (apocrine secretion). Later these vesicles rupture and discharge their contents. The different non granular structures within the secretory deposits are probably parts of broken vesicle membranes as described in foveal glands (Sonenshine et al., 1981) or secretory cell fragments transported along with the vesicles. Based on observations that the secretory product usually reveals an electron-lucent and an electron-dense portion within the reservoir at least two different compounds of the secretion are assumed. The electron-lucent compound appears less viscous because it also fills the narrow

spaces between the densely placed microvilli. Since it usually surrounds the electron-dense portions it may act as transport medium. Probably the secretory product undergoes a kind of maturation within the reservoir. The electron-dense portions aggregate prior to transport through the duct in the baseplate.

The empty secretory reservoirs in the unfed male specimen make a direct context between the time of secretion and feeding unlikely. This is further supported by the presence of material of different appearance in a fed female of *Ixodes laguri*. The filled reservoirs in the other specimens examined might indicate that the secretory material is quickly replaced by apocrine secretion or that a complete discharge of secretion is a rare event. For example the male specimen (collected in March) might have overwintered as fed nymphal stage and moulted to an adult not long before collection. Extended periods during which fed life-stages do not develop (morphogenetic or developmental diapause) are known from *I. ricinus* (Sonenshine, 1993). If this is the case for the male examined, the contents of the secretory reservoirs are probably lost during apolysis and have to be renewed. In contrast to this apocrine mode of secretion exocytosis is described from the glands associated with the porose areas in *Haemaphysalis longicornis* (Kakuda et al., 1995) and holocrine secretion is documented in type 2 dermal glands (Yoder et al., 2009a).

Presumably the destination of the spiracle gland products is the body surface even though there is no evidence for such transport in the specimens examined. Secretion in the chambers of the labyrinth as in *Hyalomma marginatum* (Schulze, 1942) is not observed. Either the secretory material disperses to such an extent in the large room of the labyrinth that it is no longer detectable with certainty or it is washed out during processing for electron microscopy. On the other hand it is unlikely that secretory products in living ticks fill the primary atrial chambers over a longer period of time because this would result in a blockage of the respiratory way.

4.6 Possible functions of the spiracle gland secretions

According to Pugh (1997) the appearance of spiracle glands (“sub-atrial glands”) may not be related to spiracle function but it correlates with ostial closure. This is a major difference in the spiracle structure between these two families: The Argasidae have a functional ostium, in the Ixodidae the ostium is functionless. Pugh suggested that the spiracle glands may produce hygroscopic substances to retard water vapour transpiration.

If the spiracle gland secretions fulfil this function it is unlikely that they should reach the outer spiracle surface of the tick. It makes more sense to retain the secretory product inside the spiracle structure. A deposition of the secretory product in the labyrinth would lead to a blockage of the respiratory way. Alternately secretions can be stored in the horizontal cavity. This assumption is supported by the lack of secretory material in the labyrinth of specimens investigated in this study but is contrary to descriptions of granular material in chambers of the labyrinth by Schulze (1942).

For a hygroscopic function a salt-rich solution, probably similar to the secretion of the salivary glands used for water vapour uptake can be expected (Bowman and Sauer, 2004; Rudolph and Knülle, 1974). Blood is a salt-rich diet. Therefore ticks have to deal with high amounts of ions (mostly Na^+ and Cl^-) after a meal. The production of chlorophenols by the foveal glands in several genera of the Amblyomminae (Metastrata) is explained as mechanism for the desalination of the ticks’ body fluids. Foveal glands are present in all life-stages and produce 2,6-DCP (2,6-Dichlorophenol). This substance is only secreted to the body surface in females acting as pheromone (ASP, Attracting Sex Pheromone) but is retained in immature individuals and males (Sonenshine, 2006; Yoder et al., 2002).

A similar mechanism might be effective in the spiracle glands even though it must be based on other chemical substances. There is no evidence of 2,6-DCP reported in Ixodinae (Sonenshine, 2006). Also similar to the foveal glands the primary function of the spiracle glands might be the storage of substances. Additional other functions might be fulfilled in certain life stages. In the case of *I. ricinus* secretion of the spiracle glands might additionally act as tracer pheromone. Behavioural experiments have shown that walking females leave cues on the substrate for attracting males. These cues vanish within 24 hours suggesting that the compounds either evaporate or degrade (Zemek et al., 2007). Due to the ventral position of the spiracles it might be possible that they are the source of the cues left on the substrate (Suppan et al., 2013). The use of chemicals as pheromones resulting from normal metabolism is not unusual (Sonenshine, 1991). For example *I. ricinus* responds to the excretory product guanine (and other waste compounds) acting as assembly pheromone. This behaviour is also known from several other tick species (Grenacher et al., 2001). As long as the chemical composition of the spiracle glands' products is not known none of these functions can be ruled out.

4.7 Linking pore- and gland number of the spiracles

Spiracle glands are described in connection with labyrinth chambers opening with a pore to the surface of the spiracle plate (Pugh et al., 1988; Pugh et al., 1990; Schöl et al., 1995). It is known from *I. ricinus* that all pores (with the exception of the peripheral row) in the spiracle plate belong to primary atrial chambers with spiracle glands in their bases (Pugh et al., 1988). Consequently the number of (internal) pores reflects the number of glands. The arrangement of pores (aeropyles) in distinct rings is a characteristic of the spiracles from Ixodinae (Prostriata) (Pugh, 1997).

Therefore a determination of the gland number should be possible in all species of the genus *Ixodes*. In species of the Amblyomminae (Metastrata) the pores (aeropyles) are evenly spaced (Pugh, 1997) and additional chambers with surface pores occur which are not associated with glands (e.g. *Rhipicephalus sanguineus*, Pugh et al., 1990; *Hyalomma truncatum*; Schöl et al., 1995). Nevertheless the pore number informs about the maximum possible number of glands.

The approximate numbers of spiracle glands for each spiracle of the examined *Ixodes ricinus* specimens are therefore 120 in the female, 88 in the male and 57 in the nymph. The spiracle plate of the female *I. laguri* contains about 87 spiracle glands. If the spiracle glands are associated with water balance their different numbers in the life-stages of *Ixodes ricinus* can be explained by the body size, resulting in different body volumes.

Ixodes ricinus as well as *I. laguri* are three-host ticks (Barker and Murrell, 2008) which means that each mobile life stage leaves the host after feeding and hides in the environment to digest and moult, or in the case of females to oviposit. But *I. ricinus* is a free-living (exophilic) tick species whereas *I. laguri* is a nidicolous species (Babos, 1964). Therefore *I. ricinus* is more likely confronted with different environmental conditions while *I. laguri* lives under more uniform conditions (higher relative humidity, cooler air temperature) throughout the year (Needham and Teel, 1991; Sonenshine, 2005). This is also supported by the seasonality of *I. ricinus* (two activity waves annually separated by diapauses) and an unchanged activity throughout the year in *I. laguri* (Babos, 1964). These differences in life style will also influence the total spiracle gland number if they help to save water.

The tick *Rhipicephalus* (formerly *Boophilus*) *annulatus* is known for the highest water loss rates among ticks examined. This deficit is most likely explained by its nearly continual access to blood because it is a one-host tick (Benoit and Denlinger, 2010).

All mobile stages of one-host ticks feed on the same host individual and only fed females leave the host to lay their eggs in the environment. Therefore off-host periods and their challenges are largely avoided by species with this life style (Needham and Teel, 1991). Spiracle plates of this species contain low pore numbers in nymphs (about 24 pores) and adults (around 59 pores in males and 73 pores in females) (Baker, 1997).

Descriptions and SEM images of different tick species from the literature sometimes reveal extremely different numbers of pores (aeropyles) in spiracle plates. The smallest number found is in the one-host tick *Dermacentor* (formerly *Anocentor*) *nitens*. Females of this species only possess about seven pores (of large diameter) arranged in a single row (Yunker et al., 1986). Large numbers of pores are in contrast found in the spiracle plates from the already mentioned three-host tick *Amblyomma americanum*. There are about 2.395 pores in spiracles of the female individuals (Baker, 1997).

Further examples of one-host species with low pore numbers in adults are *Dermacentor dissimilis*, *D. albipictus* and *Margaropus winthemi* (Hoogstraal, 1956; Yunker et al., 1986). Of the few one-host tick species known (all five *Rhipicephalus* (*Boophilus*) sp., *Margaropus winthemi*, *Dermacentor* (*Anocentor*) *nitens*, *D. albipictus* and *D. dissimilis* (Kolonin, 2007; Barker and Murell, 2008) at least five have only small numbers of spiracle glands. Since these ticks belong to three different genera the small numbers of pores might reflect their special life-style.

Scanning electron micrographs of *Cosmiomma hippopotamensis* (Apanaskevich et al., 2013) also show only few pores (13 could be detected) in the nymphal stage but numerous pores in adults. Remarkably this is a two-host species (Apanaskevich et al., 2013) which spends the unfed period on the host as nymphal stages but not as adults (Needham and Teel, 1991).

Even though it has to be investigated if species like *D. (A.) nitens* compensate their small pore numbers by aggregating more secretory cells in the spiracle glands the above mentioned examples might support Pugh's idea of a hygroscopic function of the spiracle glands' secretion (Pugh, 1997). However care has to be taken for two reasons: Even though *Amblyomma americanum* (three-host tick) clearly shows lower water loss rates than *Rhipicephalus annulatus* (one-host tick), species like *Rhipicephalus sanguineus* and *Dermacentor variabilis* (both three-host ticks) reach even lower water loss rates (Benoit and Denlinger, 2010) although they have less pores (Baker, 1997). Additionally it has to be stressed that water balance in ticks is influenced by several factors: e.g. the amounts of cuticular lipids, the ability/disability for water vapour uptake or drinking liquid water (Benoit et al., 2007; Needham and Teel, 1991).

Nevertheless in general it can be assumed that the spiracle glands' product might be of high importance for the off-host period in the life of free-living (exophilic) ticks. An analysis of the chemical composition of the secretions will be necessary to elucidate the function(s) of the glands.

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6 Appendix

Table 1: Systematic of ticks (Ixodida) based on Barker and Murrell, 2008; Guglielmone et al., 2010; Mans et al., 2012 and Oliver, 1989

Family Nuttalliellidae			
	subfamily	genus	Additional information
	Nuttalliellinae	<i>Nuttalliella</i>	Single species <i>N. nam-aqua</i> , oldest extant tick species
Family Argasidae			
	subfamilies	genera	Additional information
	Argasinae	<i>Argas</i>	---
	Ornithodorinae	<i>Ornithodoros</i>	---
		<i>Carios</i>	---
		<i>Otobius</i>	---
Family Ixodidae			
Collective terms	subfamilies	genera	Additional information
Ixodinae or Prostriata	Ixodinae	<i>Ixodes</i>	Not monophyletic: <i>Ixodes</i> (Australasia) and <i>Ixodes</i> (Rest of the world)
Amblyomminae or Metastrata	Bothriocrotoninae	<i>Bothriocroton</i>	Five species previously in <i>Aponomma</i>
	Amblyomminae	<i>Amblyomma</i>	Including other <i>Aponomma</i>
	Haemaphysalinae	<i>Haemaphysalis</i>	---
	Rhipicephalinae	<i>Anomalohimalaya</i>	---
		<i>Cosmiomma</i>	---
		<i>Dermacentor</i>	Including <i>Anocentor</i>
		<i>Hyalomma</i>	---
		<i>Margaropus</i>	---
		<i>Nosomma</i>	---
		<i>Rhipicentor</i>	---
		<i>Rhipicephalus</i>	Including <i>Boophilus</i>

Table 2: Synonyms of dermal gland names and their assumed or proofed functions in ticks

Name	Synonyms	Proofed or implicated production of
Type 1 glands	Sensilla hastiforme, spear-shaped organs, small glands type B, small glands [1; 3; 7; 9]	Mounting Sex pheromone (MSP); additional waterproof to the wax layer [9]; a secretion function in moulting [4]
Type 2 glands	Sensilla sagittiforme, arrow-shaped organs, large glands type A, large wax glands [1; 3; 7; 9]	Attraction-Aggregation-Attachment-Pheromone (AAA) [2]; allomone (defence against ants) [11]; antimicrobial substances [5]
Spiracle glands	Spiracular glands, Sub-atrial glands; Sensilla hastiforme and Sensilla laterniforme [6; 7, 9]	hygroscopic substances to reduce water loss (over the spiracles) [6]
Porose areas	Areae porosae (consisting of Sensilla hastiforme) [7]	a lubricant for the movements of Gené's organ during oviposition; antioxidants to protect egg shells [1]
Foveal glands	Foveae dorsales (consisting of Sensilla hastiforme) [7]	Attractant Sex Pheromone (ASP) → 2,6-Dichlorophenol (2,6-DCP) [8]; di- and trichlorophenols (2,6-DCP or 2,4-DCP and 2,4,6-TCP) for water conservation by elimination of chloride (after a blood meal) [10]

Summary of the synonyms (second column) for the dermal gland names used in the present work (in bold, first column). Also included is an overview of proofed or indicated functions of each gland type (third column). The numbers given in square brackets refer to the literature below.

"Sensilla laterniformia" have not been confirmed (Coons and Alberti, 1999). "S. hastiforme", "S. sagittiforme" and "S. laterniformia" are collectively referred to as "Krobylophoren" by Schulze who thought they fulfil sensory and glandular functions. He also described "Sensilla auriformia" which are assumed to function as proprioceptors and termed Iyrisfissures more generally (Klompén et al., 1996; Schulze, 1942).

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|----------------------------|-------------------------|------------------------|-------------------------|
| 1) Coons and Alberti, 1999 | 4) Klompén et al., 1996 | 7) Schulze, 1942 | 10) Yoder et al., 2002 |
| 2) Diehl et al., 1991 | 5) Pavis et al., 1994 | 8) Sonenshine, 2006 | 11) Yoder et al., 2009a |
| 3) Dinnik and Zumpt, 1949 | 6) Pugh, 1997 | 9) Walker et al., 1996 | |

Zusammenfassung

In Nymphen und adulten Zecken erfolgt der Gasaustausch über komplex gebaute Stigmen die das Tracheensystem im Körperinneren mit der Außenwelt verbinden. In der Familie Ixodidae enthalten die Stigmen zusätzlich eine Vielzahl an Drüsen, die Stigmendrüsen (engl. spiracle/spiracular glands). Die Funktion dieser Drüsen ist ungeklärt, doch möglicherweise verringert ihr Sekret den Verlust von Wasser über die Atemöffnungen. In der vorliegenden Arbeit wird der Aufbau der Drüsen und das in ihnen enthaltene Sekret bei ungesogenen Adulten von *Ixodes ricinus* mit dem Transmissionselektronenmikroskop untersucht. Da die Gattung *Ixodes* innerhalb der Ixodidae eine basale Stellung hat, zeigt diese Art vermutlich den ursprünglichen Bau der Drüsen. Dies ist umso wichtiger da Beschreibungen der Drüsen von anderen Gattungen auf strukturelle Unterschiede hinweist. Die Ergebnisse werden vor allem in Bezug auf eine mögliche Beteiligung der Drüsen am Wasserhaushalt der Zecken diskutiert. Da angenommen wird, dass die Toleranz gegenüber Trockenheit in nidicolen Arten geringer als bei freilebenden Zecken ist, wird in dieser Arbeit auch auf *Ixodes laguri* eingegangen, einer Art die in Hamster- und Zieselbauten lebt. Die höchste nachgewiesene Wasserverlustrate innerhalb der Zecken stammt von der einwirtigen Art *Rhipicephalus (Boophilus) annulatus*. Deshalb werden auch bestehende Beschreibungen von Stigmen einwirtiger Arten in die Diskussion aufgenommen. Wird davon ausgegangen, dass die Drüsen immer mit Poren in der Oberfläche der Stigmen verbunden sind, kann von der Porenzahl auf die maximale Anzahl der Drüsen rückgeschlossen werden. Dieser Ansatz weist zumindest auf die Möglichkeit einer Beteiligung der Drüsen am Wasserhaushalt der Zecken hin.

Danksagung

Ich bedanke mich herzlich bei Prof. Waltraud Klepal für die Annahme und freundliche Betreuung dieser Masterarbeit und einer damit verbundenen Publikation. Ebenso großer Dank gilt Prof. Manfred Walzl (Integrative Zoologie, Univ. Wien) der die Arbeit maßgeblich unterstützt hat. Besonders hervorheben möchte ich die Anfertigung von Schnitten mit der Rasierklinge für das Rasterelektronenmikroskop und die Gelegenheit, einen Teil der Ergebnisse im Rahmen eines Symposiums zu präsentieren. Außerdem bedanke ich mich bei Mag. Carina Siutz (Verhaltensbiologie, Univ. Wien) für das Absammeln der Zecken von Hamstern und Mag. Christoph Hörweg (Naturhistorisches Museum Wien) für die rasche Bereitstellung von zusätzlicher Literatur. Großer Dank gebührt auch Mag. Daniela Gruber, Mag. Norbert Cyran und Dr. Marieluise Weidinger für die technische Unterstützung. Außerdem danke ich meiner Zimmergenossin Mag. Vanessa Zheden für ihre Hilfe und wertvollen Vorschläge. Ebenso bedanke ich mich bei allen Mitgliedern von CIUS (Core Facility of Cell Imaging and Ultrastructure Research) für das freundliche Arbeitsklima. Zuletzt möchte ich mich bei meinen Freunden und meiner Familie für die ungebrochene Unterstützung bedanken.

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Poster: The spiracle glands in *Ixodes ricinus* Linnaeus, 1758

Publikation: Suppan J., Walzl M., Klepal W. 2013 --- The spiracle glands in *Ixodes ricinus* (Linnaeus, 1758) (Acari: Ixodidae) --- In: Schausberger P. (Ed). Acari in a changing world, 7th Euraac symp. Vienna, 2012. Acarologia. p. 127-136.