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Modus operandi of oviposition in *Dermacentor reticulatus* (Acari: Ixodidae)

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Abstract. The process of oviposition in *D. reticulatus* was observed and found to be a sequence of exactly coordinated, interlocking events independent of the phase of oviposition. The average period of oviposition in the investigated ticks was 31.6 days at 20 °C and 95% relative humidity. The number of eggs deposited on each day increased until reaching a maximum on the fifth day of oviposition and then decreased continuously. As a result, most of the eggs were deposited during the initial phase of oviposition. The total number of eggs was proportional to the ticks' weight replenishment. Egg-laying commenced with the lowering of the capitulum and the simultaneous spread of the pedipalps which were lowered to the body wall embracing the genital aperture on both sides. Immediately afterwards the cuticular sac of Gene's organ was pushed out and retracted several times. At the cuticular sac's maximum extension, the vestibulum vaginae prolapsed, forming the ovipositor as an extended tube which handed over an egg to the two horns of the cuticular sac after a brief, but intensive, contact with the cuticular sac. Then the vestibulum vaginae invaginated, the pedipalps closed, and the cuticular sac was retracted. Finally, the egg was transported onto the dorsal area of the tick by means of a vigorous rising of the capitulum. During the course of oviposition most of the events, especially the period of egg embracement by the cuticular sac, were prolonged, as was the total time for laying one egg. Similarly, the intervals between successive egg-laying processes increased continuously.

The number of eggs deposited was not dependent on the functional ability of Gene's organ, as shown by similar numbers of deposited eggs from ticks with and without mechanical blocking of the cuticular sac. But the participation of the organ in the process of oviposition proved to be a prerequisite for the viability of the eggs. Larvae developed and hatched only from those eggs which were deposited from ticks with an undisturbed Gene's organ. In comparison, eggs without contact to the cuticular sac of Gene's organ dried up and shrivelled immediately after being deposited and did not hatch. Consequently, it strongly suggests, together with the results from other studies, that Gene's organ covers the eggs with a secretion that prevents the loss of water.

Key words: Acari, Ixodoidea, *Dermacentor reticulatus*, oviposition, modus operandi

Introduction

As previously seen (Zahler and Gothe, 1995), adult *Dermacentor reticulatus* ticks have two periods of host-seeking activity annually but with a locally differing range of their amplitudes. Host-seeking may start as early as January in France and

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Germany, not before March in Poland and not until April in the eastern part of the distribution area, and is always finished in June. The second activity period begins in July in western Siberia, during August in Poland and in September in France and Germany and decreases rapidly as the further east this tick species occurs. The seasonality of oviposition in the field has yet to be explored, but laboratory experiments reveal that egg-laying only occurs at ≥ 10 °C. At 10 °C, however, either few females oviposit or the egg index and the larvae index are extremely low. In addition, the preoviposition period is very long at 10 °C averaging 116 d and 153 d at 50% and 100% relative humidity (R.H.), respectively. In contrast, the percentage of egg-laying females, as well as the egg index and the larvae index, are very high at 20 °C even at 15% R.H. Further studies reveal that engorged females also lay viable eggs at 20 °C following exposure to −10 °C for 10 weeks and to 5 °C for more than 20 weeks. Compared with engorged females permanently kept at 20 °C, there were no significant differences in respect of preoviposition period, egg index, larvae index and hatching rate (Zahler, 1994; Zahler and Gothe, 1995).

The total number of eggs deposited, which is directly proportional to the repletion weight of female ticks, was quantified in many species (Gold, 1983; Sonenshine, 1991), but the exact process of oviposition was usually not noted. This, too, applies to *D. reticulatus*, a three-host metastriate tick species spread extensively throughout the Palaearctic fauna region. *D. reticulatus* is an accomplished natural vector of *Babesia canis*, which is highly pathogenic for dogs and has become endemic in Germany (Zahler and Gothe, 1997).

Accordingly, the present investigation examines the mechanism of oviposition in *D. reticulatus* as well as the sequence and coordination of events, the majority of which were photographically and time recorded. In addition, the viability of deposited eggs was investigated with and without mechanical blocking of the Gene's organ. Because at least 80% of engorged females laid eggs, the preoviposition was short and the egg index and the larvae index were very high at 20 °C (Zahler and Gothe, 1995), these studies were also conducted at 20 °C.

Materials and methods

D. reticulatus ticks used in the present study were laboratory-reared offspring of ticks collected from vegetation in Germany near Offenburg (district Freiburg) in 1990. Rabbits were used as hosts for the larvae and nymphs. Adult ticks fed on sheep. All host animals were infested only once. Off-host ticks were kept at 20 °C and 95% R.H. until used. For observing the oviposition process, engorged females, which had repleted together with males on laboratory sheep were used. The weights of the repleted ticks varied between 250 and 410 mg. They were kept singly in glass tubes at 20 °C and 95% R.H. The mean length of the preoviposition period was 22 d. The number of deposited eggs was determined for each day. To observe oviposition

movements, ticks were fixed in a specimen holder and kept at room temperature and 40–60% R.H. After a 24 h phase of adaptation, egg-laying was observed under a binocular microscope with a photographic camera attached (Photoautomat MPS 400, Fa. Wild). The sequence of events during the laying of one egg and the coordination of the organs involved, as well as their surface structures, were analyzed exactly and photographed and the time taken was manually measured with a stop watch. In the process of laying one egg 15 events were investigated as described in Tables 1 and 2.

Table 1. Average duration in seconds (S.D.) of events in the process of laying an egg in the 1st, 2nd and 3rd third of oviposition.

	Third of oviposition		
Event	1st	2nd	3rd
Event 1: Duration of lowering the capitulum	2.7 (0.4)	2.8 (0.2)	3.1 (0.3)
Event 2: Time between the start of lowering the capitulum and the first eversion of the cuticular sac of Gene's organ	1.3 (0.5)	1.6 (0.4)	2.2 (0.5)
Event 4: Duration of the eversion of the cuticular sac	2.9 (0.3)	2.8 (0.3)	3.3 (0.6)
Event 5: Time between the lowering of the capitulum and the maximal eversion of the cuticular sac	25.0 (4.1)	34.2 (10.9)	54.9 (10.1)
Event 6: Time between the maximal eversion of the cuticular sac and its first contact with the vestibulum vaginae	7.3 (1.2)	8.6 (8.6)	12.4 (3.4)
Event 7: Time of contact between the cuticular sac and the vestibulum vaginae	18.2 (2.3)	20.3 (5.2)	24.3 (5.3)
Event 8: Total time of ovipositor eversion	28.7 (2.3)	31.1 (5.7)	39.4 (7.7)
Event 9: Duration of pedipalps' spreading	51.7 (5.5)	64.8 (16.3)	94.4 (19.5)
Event 11: Period of egg-embracement by the cuticular sac	67.2 (11.8)	124.2 (15.2)	200.3 (27.6)
Event 12: Time between the beginning of the retraction of the cuticular sac and the deposition of the egg onto the dorsal surface of the capitulum	31.8 (9.8)	65.9 (20.8)	76.7 (18.4)
Event 13: Time between deposition of the egg onto the dorsal surface of the capitulum and the rising of the capitulum	1.3 (0.8)	1.2 (0.1)	1.1 (0.1)
Event 14: Duration of the rising of the capitulum	1.0 (0.2)	1.1 (0.1)	1.0 (0.1)
Event 15: Duration of the intervals between successive egg-laying-processes	3.1 (1.0)	9.0 (5.7)	60.5 (22.4)

Table 2. Average number (S.D.) of cuticular sac eversion and of circular movements of the pedipalps in the process of laying an egg in the 1st, 2nd and 3rd third of oviposition.

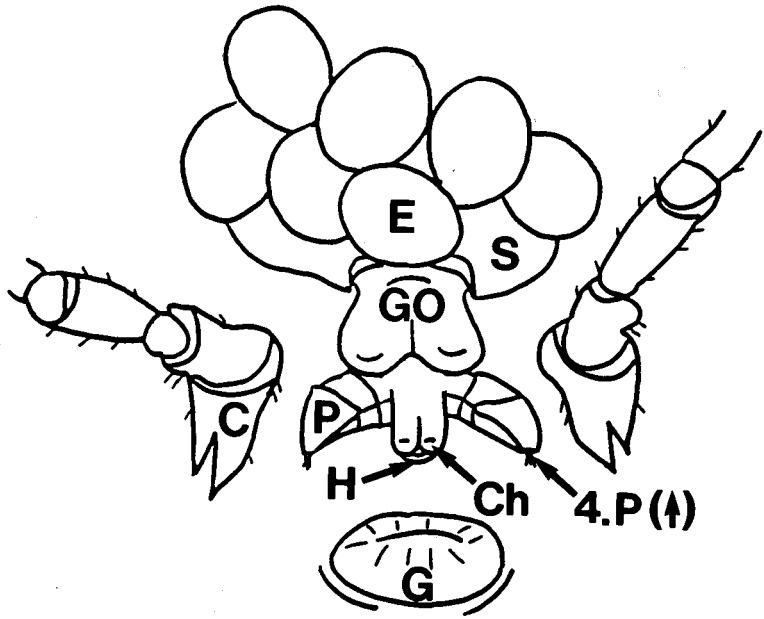
Event	Third of oviposition		
	1st	2nd	3rd
Event 3: Number of eversions of the cuticular sac	5.9 (0.9)	7.6 (2.4)	11.5 (0.9)
Event 10: Number of circular movements of the pedipalps	2.5 (0.5)	1.8 (0.4)	1.8 (0.4)

For this investigation 10 ticks were studied in each third of oviposition. For each tick 30–50 single egg-laying processes were recorded from each oviposition period. After observation the investigated ticks were incubated in glass tubes at 20 °C and 95% R.H. The number of eggs deposited for each day was recorded until the end of oviposition. The eggs deposited in glass tubes or in the object holder were counted separately for each day in a glass dish under a binocular microscope. A group of 10 females with corresponding repletion weights but undisturbed oviposition was used as a control.

For the investigation of oviposition and the determination of the viability of eggs deposited by female *D. reticulatus* with and without blockage of the eversion of the cuticular sac, 10 engorged females were weighed and incubated singly at 20 °C and 95% R.H. After the fourth day of oviposition the camerostomal fold was covered with a double layer of adhesive (cyano-acrylat-adhesive, Uhu™, Henkel, Düsseldorf) to prevent the eversion of the cuticular sac of Gene's organ. Thereafter the ticks were incubated until the 10th day of oviposition under the same laboratory conditions. The ticks were then separated from the eggs deposited so far, which were incubated further. Viability of eggs was determined as the proportion of eggs from which larvae hatched. Untreated engorged females served as a control. These ticks were marked at the caudal region of the body with an equal quantity of cyano-acrylat-adhesive. Eight weeks after the hatching of the first larva the glass tubes were transferred to −18 °C for 24 h to kill the surviving larvae. Thereafter the number of hatched larvae versus undeveloped eggs was determined.

Results

Independent of repletion weight and preoviposition time, the modus operandi of egg-laying did not change during the 1st, 2nd and 3rd third of oviposition. With increasing duration of oviposition, the time needed for most of the events increased (Table 1). Egg-laying commenced with a jerky lowering of the capitulum and the simultaneous spread of the pedipalps with an everted fourth segment. The pedipalps were lowered to the ventral body wall embracing the genital aperture on both sides (Fig. 1). Before starting the process of oviposition, the fourth segment of the pedipalps



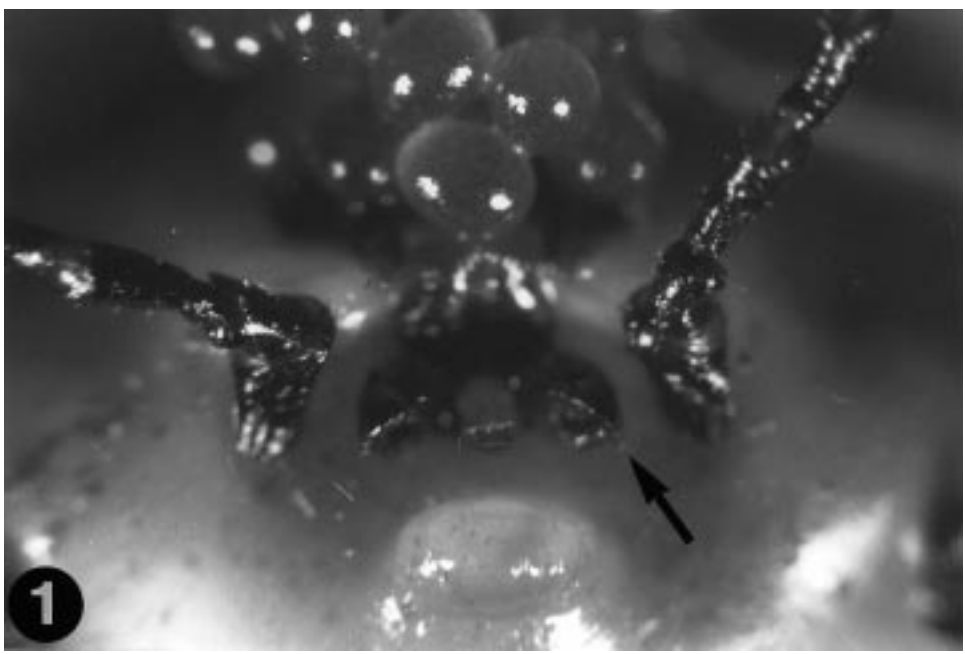


Figure 1. Pedipalps (P) embracing the genital aperture (G) on both sides: chelicerae (Ch); coxa I (C); eggs (E), everted fourth segment of pedipalps (↑4. P); Gene's organ (GO); hypostome (H); scutum (S)

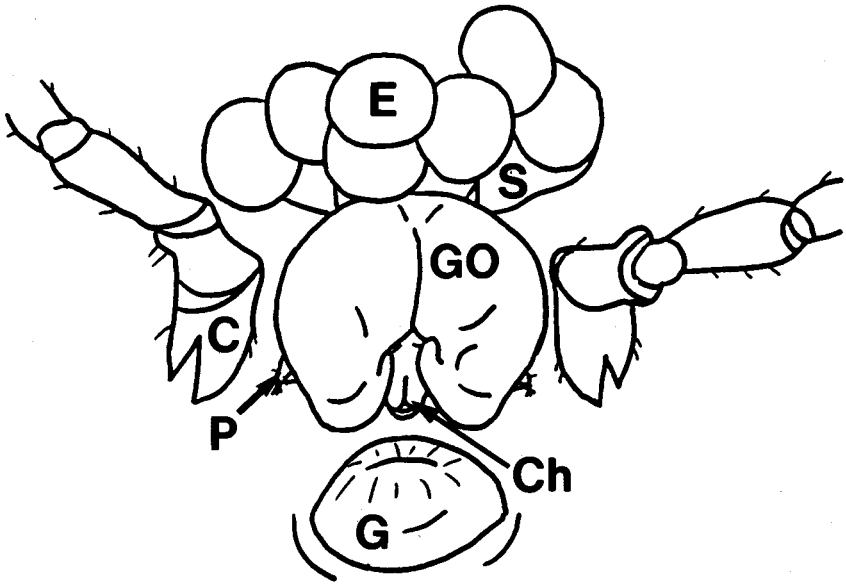
was pushed out and retracted several times. Immediately after the capitulum was lowered, which caused an extension of the camerostomal aperture, the glittering, grey-white Gene's organ emerged at the camerostomal aperture, but was invaginated again immediately, then pushed out and retracted several times. The eversion of the cuticular sac increased continuously.

Initially the cuticular sac only touched the porose areas, and then also the chelicerae and the insides of the pedipalps. At its maximal eversion, with its two horns evaginated, the cuticular sac eventually rose above the pedipalps (Fig. 2). During this maximal eversion of the cuticular sac, the vestibulum vaginae prolapsed from the genital aperture forming an extended tube. The vestibulum vaginae operated as an ovipositor and handed over an egg to the two horns of the cuticular sac after brief, but intensive, contact (Fig. 3). The egg was embraced by the horns of the cuticular sac, except for a small sector (Fig. 4), and the vestibulum vaginae invaginated simultaneously. After the retraction of the ovipositor the pedipalps were positioned nearly parallel to each other and carried out circular movements while the egg was turned slightly. The movements of the pedipalps were accompanied by lowering of the capitulum and up-and-down motions of the chelicerae. The egg was embraced by the horns of the cuticular sac for a short period. Subsequently, the horns were invaginated while the egg was slightly turned (Fig. 5), followed by the slow retraction of the remaining parts of the cuticular sac.

In the final phase of the process the pedipalps were closed. At this time the egg laid freely on the pedipalps and chelicerae for a moment (Fig. 6). Immediately afterwards the egg was transported onto the dorsal area of the tick by means of a vigorous rising of the capitulum. After the deposition of the egg the capitulum remained for a short period in an elongated position relative to the longitudinal axis of the tick body and was then lowered down again, thereby starting the next process of egg-laying. With the increasing duration of oviposition, most of the events, especially the period of egg embracement by the cuticular sac, were prolonged, as were the intervals between successive egg-laying processes (Tables 1, 2). Similarly, the total time of laying one egg increased continuously from 123 seconds (s) (S.D. = 16 s) in the 1st third of oviposition to 192 s (S.D. = 28 s) in the 2nd third and 297 s (S.D. = 27 s) in the 3rd third of oviposition.

The average period of oviposition was 31.6 days. The total number of eggs deposited was on average 3473. Of these, 2197 (63.3%) were deposited during the 1st third, 1071 (30.9%) during the 2nd and 205 (5.9%) during the 3rd third of oviposition. The number of eggs deposited per day increased until reaching a maximum on the fifth day of oviposition and then decreased continuously. No significant differences (t-test, $p > 0.05$) relating to the duration of oviposition and the number of eggs deposited during the 1st, 2nd and 3rd third of oviposition were found between ticks with or without observation during the egg-laying process.

The number of eggs deposited within the first 10 days of oviposition revealed no significant difference (Welch test, $p > 0.05$) between ticks with or without the



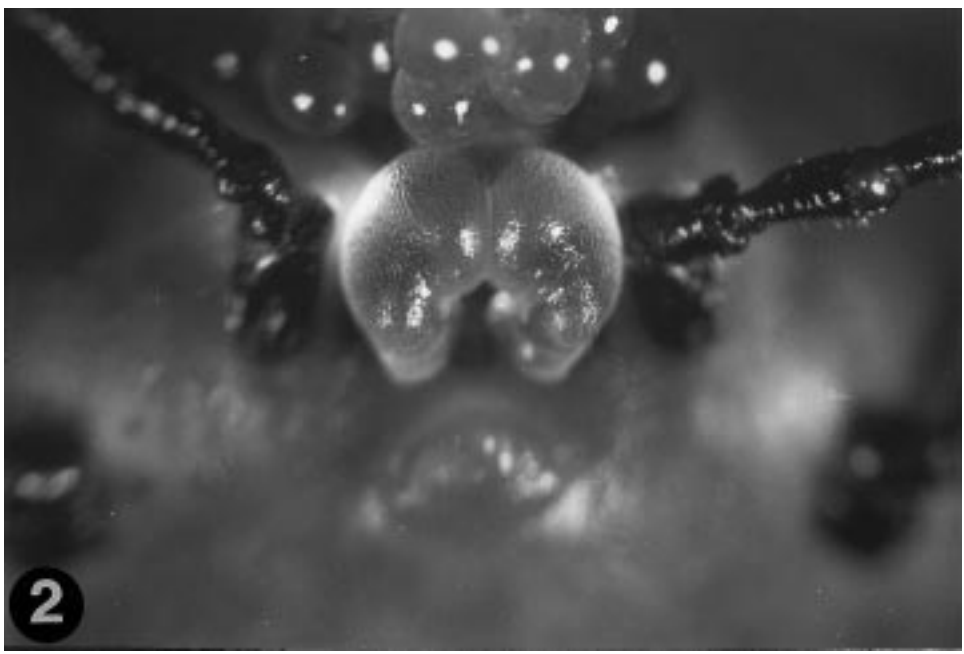
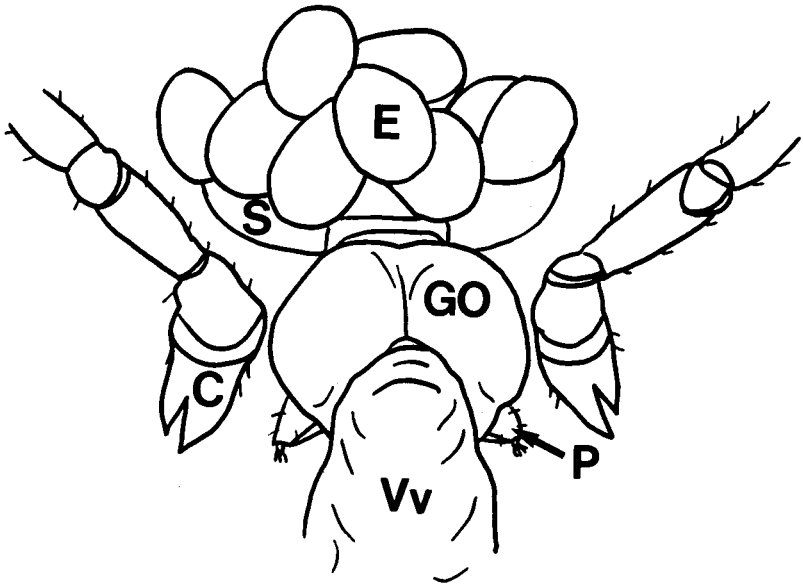


Figure 2. Maximal eversion of the cuticular sac of Gene's organ (GO); chelicerae (Ch); coxa I (C); genital aperture (G); eggs (E); pedipalps (P); scutum (S)



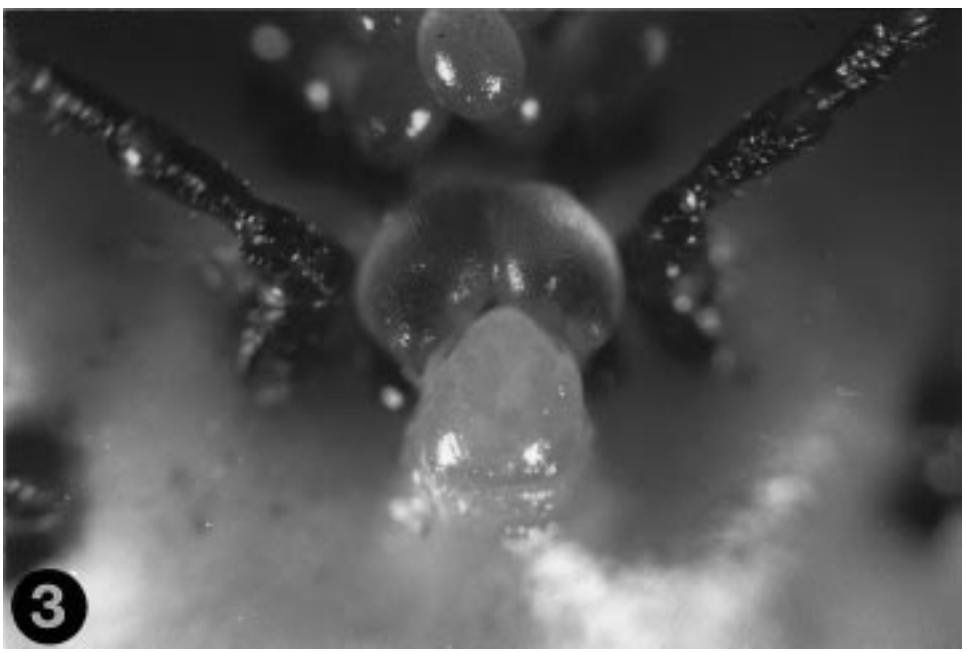
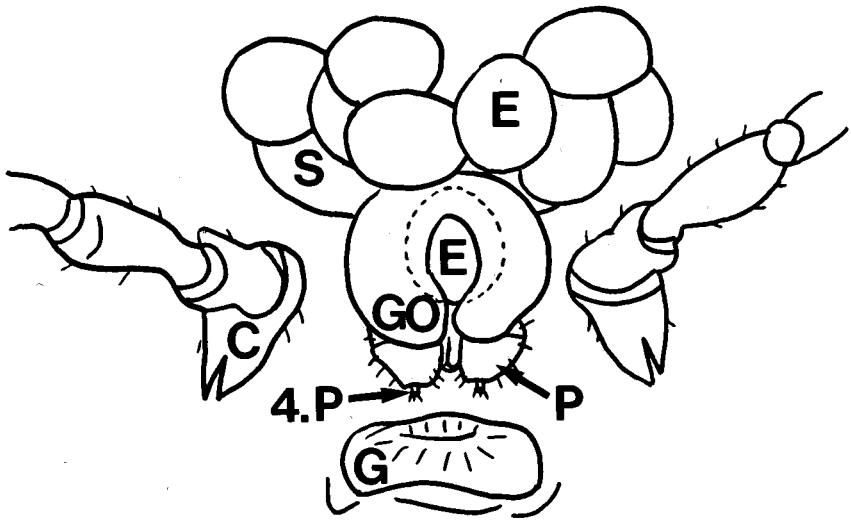


Figure 3. Cuticular sac of Gene's organ (GO) in broad contact with the prolapsed vestibulum vaginae (Vv): coxa I (C); eggs (E); pedipalps (P); scutum (S)



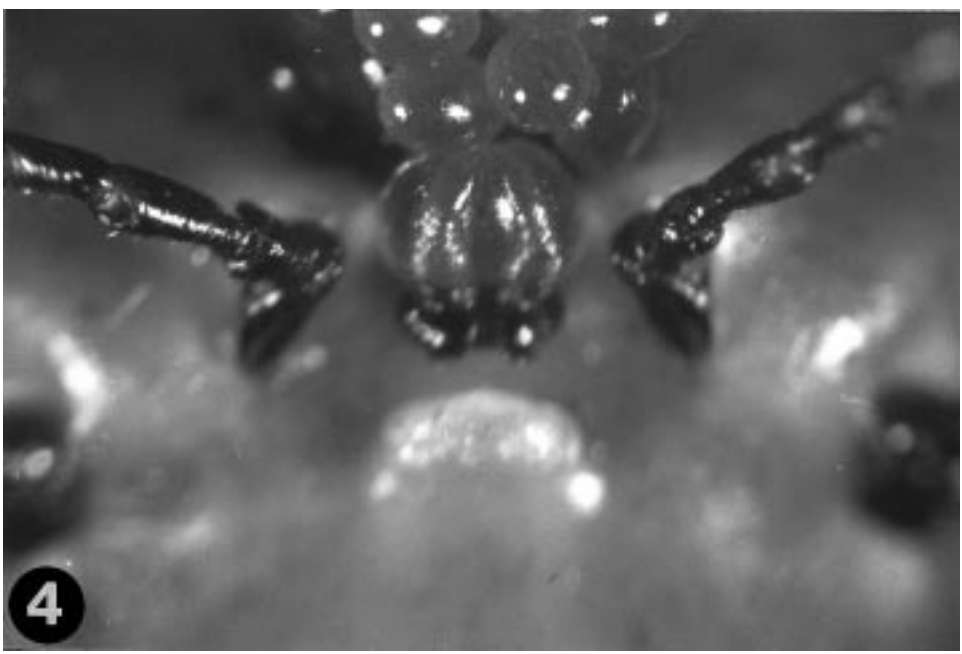
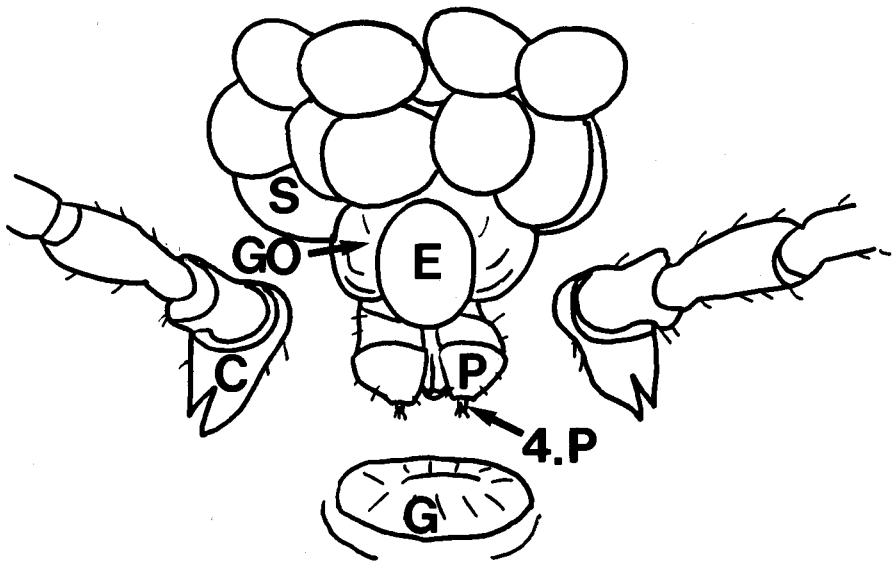


Figure 4. Egg (E) embraced by the horns of the cuticular sac of Gene's organ (GO): coxa I (C); genital aperture (G); pedipalps (P); fourth segment of pedipalps (4.P); scutum (S)



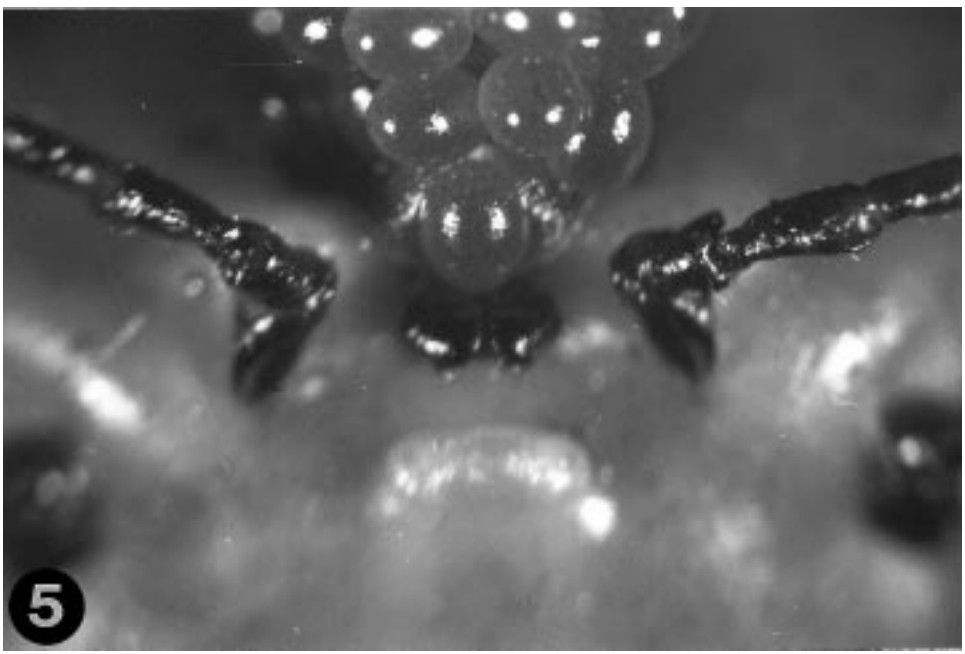
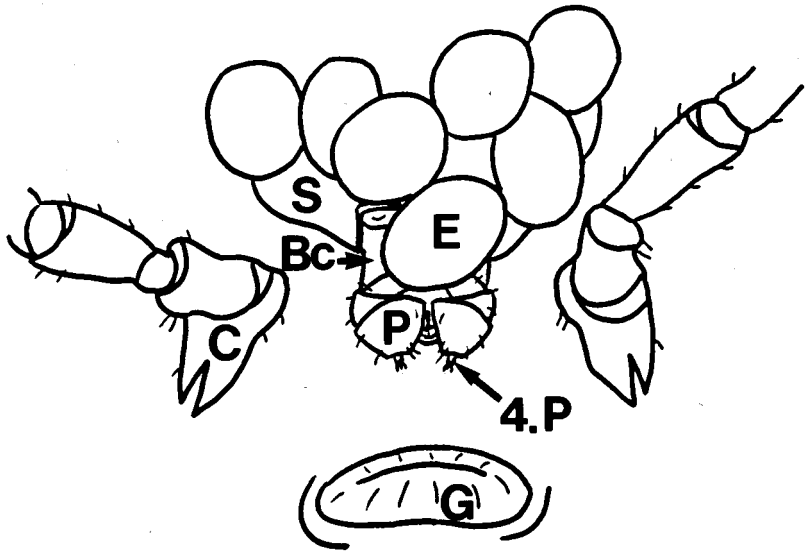


Figure 5. Partially retracted cuticular sac of Gene's organ (GO) with an egg (E): coxa I (C); genital aperture (G); pedipalps (P); fourth segment of pedipalps (4.P); scutum (S)



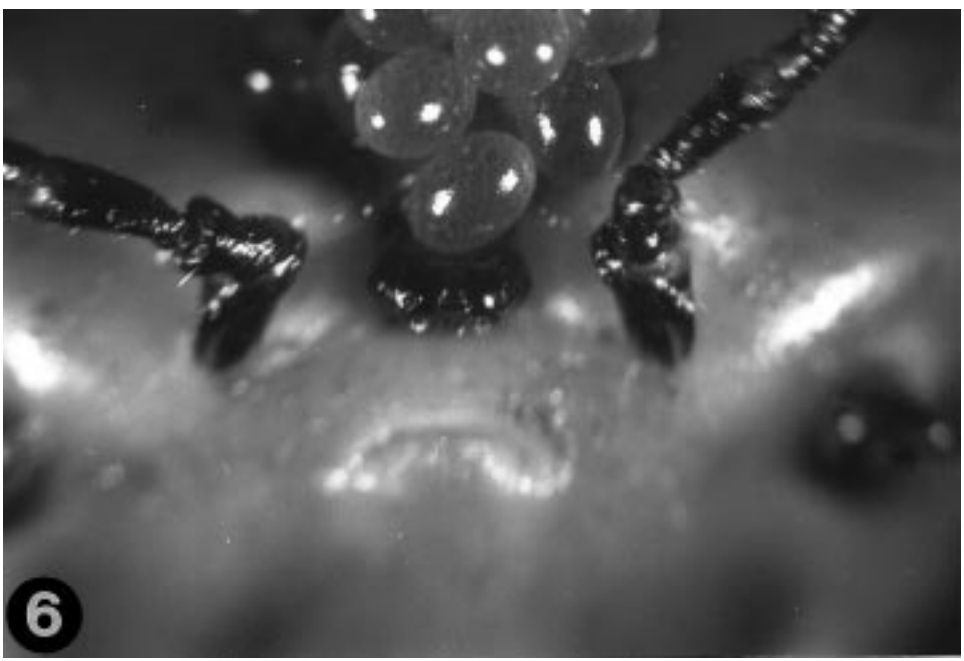


Figure 6. Egg (E) laying freely on the closed pedipalps (P) and chelicerae: basis capituli (Bc); coxa I (C); fourth segment of pedipalps; (4.P); genital aperture (G); scutum (S)

cuticular sac of Gene's organ being blocked on the fifth day of oviposition. Ticks with analogous repletion weights, with and without the blocking of the cuticular sac of Gene's organ, deposited 1759 and 1831 eggs respectively, in this phase of oviposition. The average hatch rate of eggs deposited by those ticks, whose Gene's organ was subsequently blocked on day 5 was 91.8% ($n = 677$) within the first four days of oviposition. The corresponding hatch rate of those ticks not blocked was 92.6% ($n = 649$). Between the 5th and 10th day of oviposition, ticks with and without blockage of the Gene's organ deposited an average 1023 and 1131 eggs, respectively. Larvae developed and hatched only from eggs deposited from ticks with an undisturbed Gene's organ. The mean hatch rate was 93.4%. In comparison, all eggs deposited without contact to the cuticular sac of Gene's organ dried up and shrivelled within a few minutes following deposit and no larvae hatched from them.

Discussion

A significant correlation of the sequence of events during oviposition in *D. reticulatus* and the egg-laying process in other species of ticks can only be drawn from *Rhipicephalus evertsi evertsi.* Only in this two-host ixodid tick has the process of oviposition been documented, analyzed and evaluated in its single events (Gothe and Nadler, 1987).

The process of oviposition in *R. evertsi evertsi* is essentially similar to that of *D. reticulatus.* In contrast to *D. reticulatus*, however, repeated pushing out and pulling in of the fourth segment of pedipalps immediately before the lowering of the capitulum, and the circular movements of the pedipalps during the egg embracement, was not seen in *R. evertsi evertsi*. Likewise, the cuticular sac of *R. evertsi evertsi* was pushing out and pulling in less frequently on average than was the case in *D. reticulatus*. The evagination and invagination of the fourth segment of the pedipalps, and the circular movements of the pedipalps, is unlikely to reflect a difference between the species. Presumably these events were not recognizable, or were overlooked on account of the insufficiency of the technical equipment. In comparison, the frequency of evagination and invagination of the cuticular sac seems to be a genuine difference between the two species. In *D. reticulatus* it seems possible that the egg-impregnating secretion reaches the contact area of Gene's organ and the egg in a lower quantity after one eversion as compared to the situation in *R. evertsi evertsi*. Accordingly, in *D. reticulatus* more evaginations and invaginations must be done to accumulate enough secretion.

The number of eggs deposited was independent of the functional integrity of the Gene's organ, as shown by the similar overall numbers of deposited eggs from ticks with and without mechanical blockage of the eversion of the cuticular sac, but the participation of the organ in the process of oviposition is apparently a prerequisite for

the viability of the eggs. Embracement and impregnation of eggs by the cuticular sac of Gene's organ is indispensable, since eggs without contact to the cuticular sac dried up and shrivelled immediately after deposition and larvae did not hatch. The data suggests that Gene's organ covers the eggs with a secretion that minimizes or decreases the loss of water. This function of Gene's organ has also been proved in other ticks. After the blockage of Gene's organ, eggs of *Ornithodoros moubata* and *Ixodes ricinus* (Lees and Beament, 1948) and *Haemaphysalis longicornis* (Kakuda *et al*., 1992) dried up quickly after deposit and were not viable, or only a minor quota. The chemical nature of the secretion of Gene's organ is still unknown. Results of chemical analyzes of egg shells from *O. moubata* (Lees and Beament, 1948) and *Boophilus microplus* (McCamish *et al*., 1977) with functional Gene's organ are inconclusive with respect to the detected components attributed to the secretion of Gene's organ, since no eggs without a contact with the Gene's organ were investigated.

The function of the secretion of the glands associated with the porose areas, characterized as paired multicellular, alveolar and lobularly arranged glands (Gothe *et al*., 1987), remains to be shown. It is unclear whether, or how far, that secretion is incorporated as a component into the secretion of Gene's organ during the evaginations and invaginations of the cuticular sac, or its potential participation in the impregnation of the eggs. The function of the secretion of the porose areas as a lubricant of the cuticular sac of Gene's organ, as postulated in *B. microplus*, *Dermacentor andersoni*, *Hyalomma* spp. and *R. evertsi evertsi* (Feldman-Muhsam, 1963), can be excluded since the evaginations and invaginations of the cuticular sac for *B. microplus* (Atkinson and Binnington, 1973; Booth *et al*., 1984) and *R. evertsi evertsi* (Gothe and Nadler, 1987) are not impaired after blockage of the areae porosae. HPLC investigations of eggs deposited by females of *R. evertsi evertsi* with either blocked or unblocked porose areas proved that components of the secretion appear on the egg shell. Nevertheless, the chemical identity and the function of these components are still unknown. It has been seen in *R. evertsi evertsi*, that the process of oviposition and the viability of eggs are not influenced by the areae porosae secretion (Gothe and Nadler, 1986). Similarly, the presumption that the secretion is incorporated into the wax layer of the egg, where it prevents the autoxidation of the unstable Δ2,4,6-trien-steroids (Atkinson and Binnington, 1973) is not conclusive. In addition, the quantity of these steroids did not differ in eggs produced by females of *R. evertsi evertsi* deposited with open or cauterized porose areas even 3 months after oviposition (Vermeulen *et al*., 1986).

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