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External morphology of the quiescent instars of trombiculid mites (Acariformes: Trombiculidae) with notes on their moulting processes

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Abstract

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The morphology and cuticular ultrastructure of the quiescent instars prelarva, proto-and tritonymph of the trombiculid mites, *Hirsutiella zachvatkini*, *Leptotrombidium orientale* and *Leptotrombidium schlugerae* (Trombiculidae) were investigated with transmission electron microscopy (TEM) and scanning electron microscopy (SEM). Their post-embryonic life cycle is considered in terms of their moulting processes. The existence of the quiescent instars in mite ontogenesis is determined by the presence of the cuticle being closely appressed to the hypodermis irrespective of whether the old cuticle occurs or not, and is limited to a relatively short time, approximately at the middle of the entire inactive period. The quiescent instars possess non-articulated reduced legs and mouth parts as well as slightly tuberculate and folded cuticle without setae.

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Introduction

The life cycle of mites from the order Acariformes is comprised of an egg and six active instars, or stages of post-embryonic development – two hexapod larvae, three octopod nymphs and adult mites (see Grandjean 1938b; Lange 1960; Hammen 1964; Travé 1976; Böttger 1977; Kethley 1990b). In some groups of trombidiform mites (suborder Actinedida), such as Nanorchestidae (Kethley 1991), Ereynetidae (Fain 1972; Baker 1973; Andre and Fain 1991; Andre 1992), Pterygosomatidae (Newell 1973; Kethley 1991), Trombidiidae (Singer 1971; Robaux 1974), Trombiculidae (Ewing 1944; Michener 1946; Wharton 1946; Neal and Barnett 1961; Johnston and Wacker 1967) and water mites belonging to the phalanx Hydrachnidia (Bottger 1977) there is a regression, or 'calyptostatic inhibition' (Coineau 1974), not only the first larval instar (pre-larva), but also the first (protonymph) and the third (tritonymph) nymphal instars. Such inhibition results in the alternation of active and quiescent (inactive, regressive) instars and has been thought to perform the progressive phylogenetic compression and reduction of development (Reuter 1909; Knülle 1961). This phenomenon, which has been variously called as 'developmental canalization' (Newell 1973; Kethley 1991), 'alternating calyptostasy' (Kethley 1991), 'complex life cycle' (André 1991a) and 'developmental constraints' (André 1992), is often quite complicated due to the parasitic habits of the larva as in Parasitengona or of all active instars as in Pterygosomatidae.

Little is known about real processes during ontogenesis and moulting of these arthropods. The essential problem that concerns the developmental peculiarities of trombidiform mites is not only the clear detection of quiescent instars, or calyptostases (Andre 1992), but also the demonstration of precise mechanisms, principles and causative motives of such a pattern of ontogenesis. The use of electron microscopy might be useful in estimating the exact duration of the quiescent instars and their ontogenetic role. Resolution of the problem could give us

a key to the real comprehension of 'the unique complexity of these developmental systems' of trombiculid mites and other Parasitengona (Johnston and Wacker 1967).

In a previous publication (Shatrov 1995), some problematical questions concerning characteristic peculiarities of ontogenesis and moulting of arthropods were discussed with special regard to trombidiform mites. I have postulated that, apart from the naming the stages, the presence of the replacement cuticle during moulting manifests the successive developmental steps, either being active or inhibited (Shatrov 1995). The purpose of this investigation is to report morphological and ultrastructural observations of the quiescent instars of *Hirsutiella zachvatkini*, *Leptotrombidium orientale* and *Leptotrombidium schlugerae*.

Materials and Methods

Prelarvae, proto- and tritonymphs of *H. zachvatkini* (Schluger, 1948) and prelarvae of *L. orientale* (Schluger, 1948) and *L. schlugerae* (Emeljanova et Gorbatcheva, 1960) were obtained from a laboratory culture that has been maintained at the Laboratory of Parasitology, Zoological Institute of the Academy of Sciences, St Petersburg, Russia, according to procedures described in Shatrov (1993). Prelarvae were examined within the egg-shell and also in more developed conditions. Mites during the inactive nymphal periods were taken at daily intervals from the moment of inactivation of the active instars (fed larva or deutonymph) until the completion of ecdysis of the subsequent instar (deutonymph or adult mite). For comparative analysis, eggs of different ages of each species as well as their quiescent instars were also examined with a dissecting microscope.

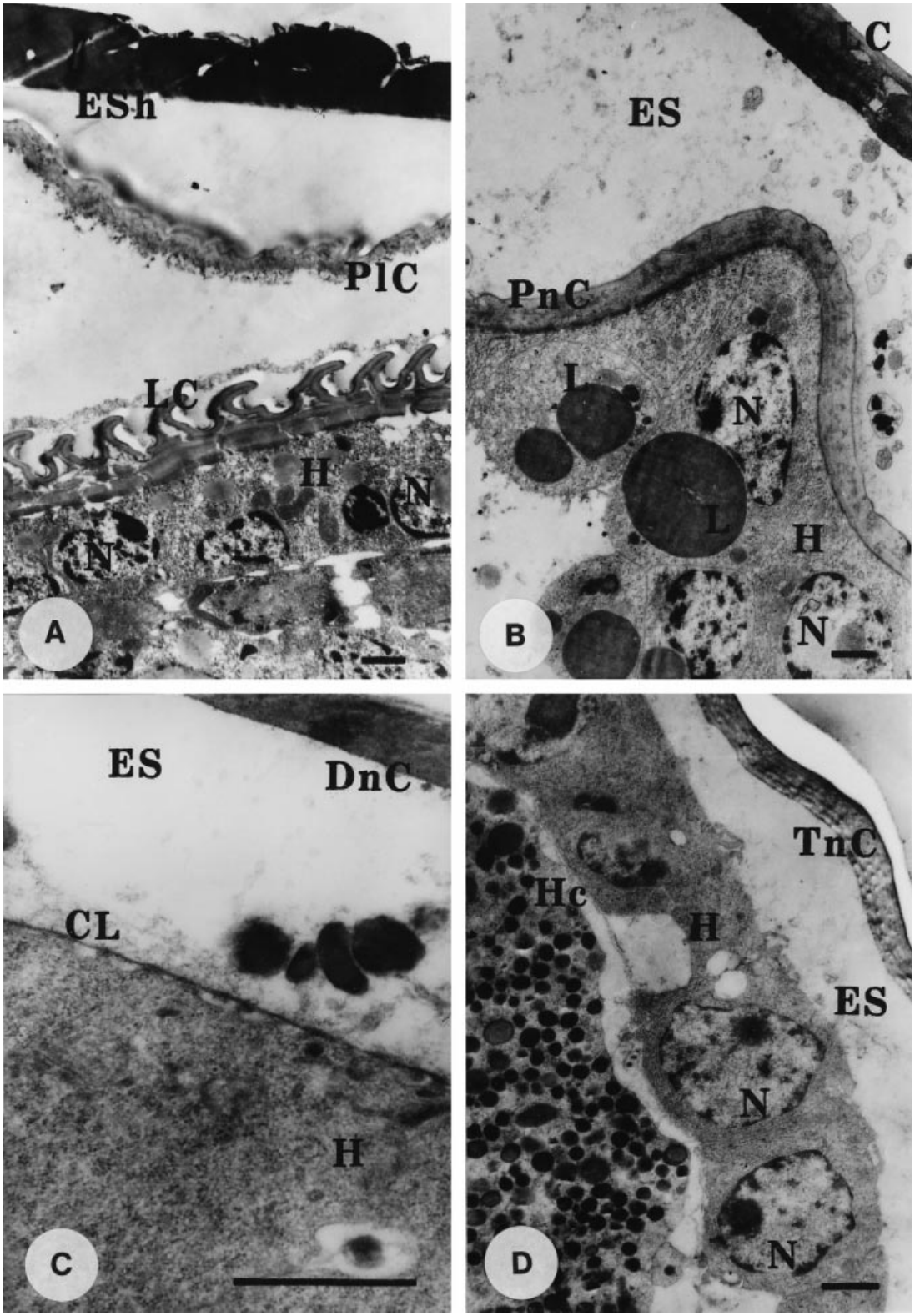
For scanning electron microscopy (SEM), specimens were alcohol fixed and dehydrated, then critical point dried in a Hitachi HCP-2. After coating with platinum in an Eiko-5 apparatus they were examined with a Hitachi S-570 electron microscope at 20 kV.

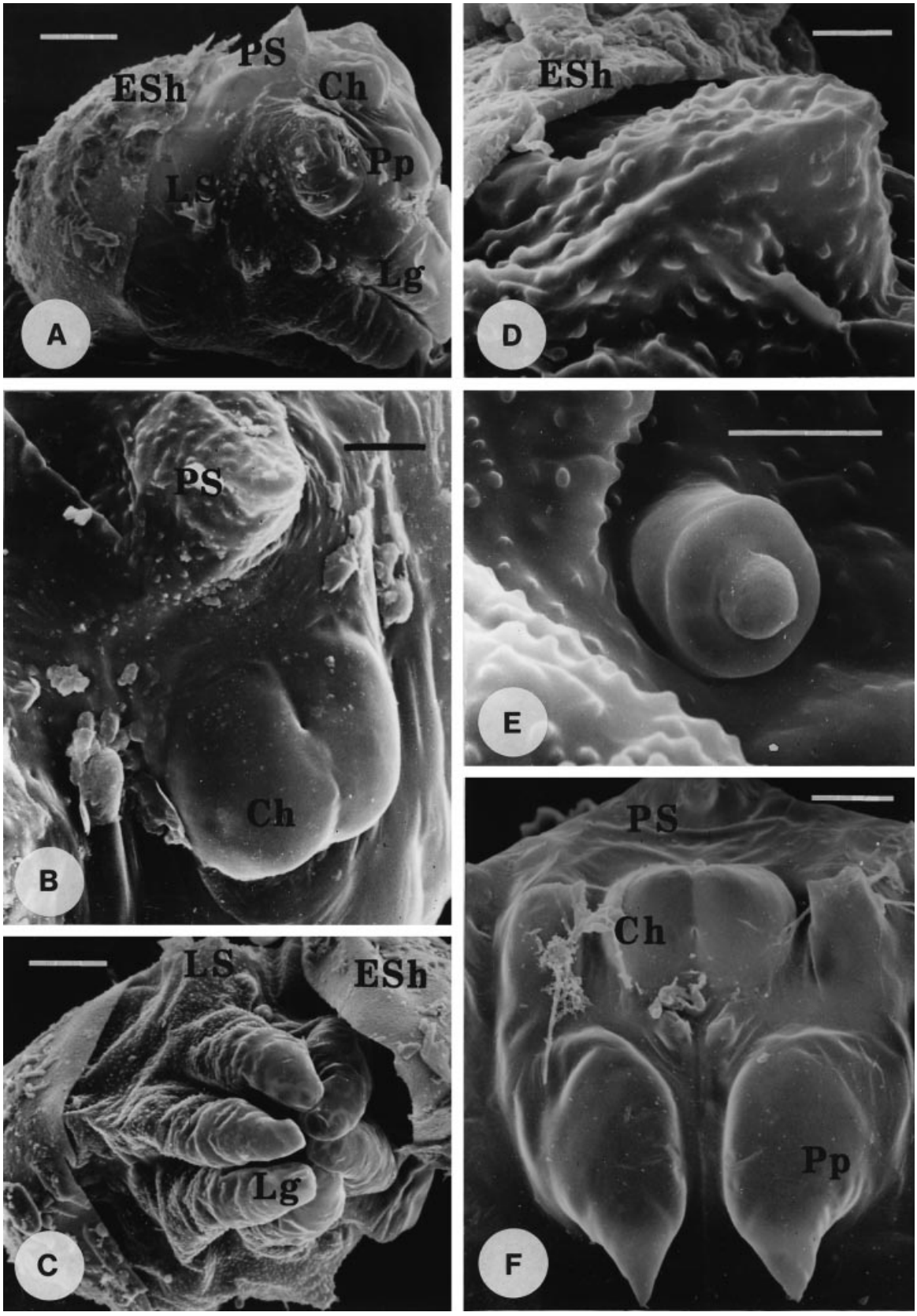
For transmission electron microscopy (TEM), eggs, prelarvae as well as proto-and tritonymphs were initially fixed in 2.5% glutaraldehyde in 0.1 m phosphate buffer (pH 7.2–7.4) for 2–4 h. For better penetration of the fixative, quiescent nymphs were carefully pierced in the middle part of the body, whereas eggs and prelarvae either were pierced or left intact. Mites were then washed in several changes of 0.2 m phosphate buffer, post-fixed in 2% osmium tetroxide in phosphate buffer containing 8.56% sucrose for 1–6 h to overnight, dehydrated in alcohol and acetone series, and finally embedded in an araldite mixture. Serial sections were cut on a LKB-III ultramicrotome, and, after staining with uranyl acetate and lead citrate, were examined with a Tesla BS-500 or a JEM-100 CX transmission electron microscope at 60–90 kV. For preliminary observations, semithin sections were stained with toluidine blue and examined with an Amplival light microscope.

Results

The quiescent regressive instars of trombiculid mites undergo moulting both at the beginning and also at the end (Fig. 1). The moulting processes are very gradual, so that it is difficult to determine the limits of the ontogenetic stages precisely by using observations of external characteristics of the living organism. The whole quiescent protonymphal period ('nympho-chrysalis' of the earlier authors) consists of the resting larva preparing to moult, the moulting larva, the protonymph proper (Fig. 1B), the moulting protonymph and, finally, the pharate deutonymph within the protonymphal covering. The same processes occur during the tritonymphal period ('imago-chrysalis'), except for the identity of phases (Fig.1 C,D). The exact duration of the true proto- and tritonymphal instars is limited to a relatively short time at the middle of the quiescent periods. Externally well developed proto- or tritonymphal coverings, near the end of these periods, are found to conceal only the pharate deutonymph or adult mite preparing to eclose (ecdysis). The prelarval cuticle is closely appressed to the hypodermis and exists for a very short time, almost completely within the egg-shell (chorion). The detection of this instar is possible only through examination of the eggs and only the apoderma (Reuter 1909; Grandjean 1938b), or secondary cuticle (deutovum) (Wharton 1946), may be seen after the splitting of the egg-shell. The rapid changing of prelarval

Fig. 1.—TEM micrographs of the integument of different instars of *Leptotrombidium orientale* and *Hirsutiella zachvatkini* demonstrating various relationships between coverings and hypodermis. — **A,** Cross-section of a 10-day old egg of *L. orientale* showing the egg-shell (ESh), prelarval covering (PlC) and the folded larval cuticle (LC) developed beneath them; note the larval hypodermis (H) with nuclei (N). — **B,** Cross-section of the integument in the ventro-lateral region of a 4-day-old quiescent protonymph of *H.zachvatkini*. Note the old larval cuticle (LC), distended due to feeding and pressure of the exuvial fluid, and the slightly folded hypodermal layer (H) of the protonymph with the own protonymphal cuticle (PnC). ES, exuvial space; L, lipid droplets; N, nuclei of hypodermis. — **C,** Cross-section of the apical part of hypodermis (H) of a three-day old quiescent tritonymph of *H. zachvatkini*. Note the cuticulin layer (CL) being deposited above the short microvilli of the tritonymphalhypodermal cell. ES, exuvial space; DnC, old deutonymphal cuticle. — **D,** Cross-section of the integument of a more developed three-day old quiescent tritonymph of *H. zachvatkini*. Note that the tritonymphal cuticle (TnC) is detached and a newimaginal cuticle will be deposited over the hypodermal layer (H) with its closely arranged nuclei (N). ES, exuvial space; Hc, haemocyte. Scale bar: 1 μm.





cuticle to larval one, often being within the chorion (Fig. 1A), may be referred to as an 'embryonic moult', the phenomenon that is also found among insects (Dorn and Hoffmann 1981).

The life cycle of trombiculid mites begins with the egg and under the chorion is not only an embryo and prelarva, but often a developing larva, often found situated (Fig. 1A). The egg diameter of *L. orientale* is about 235 μm and is similar in the other species.

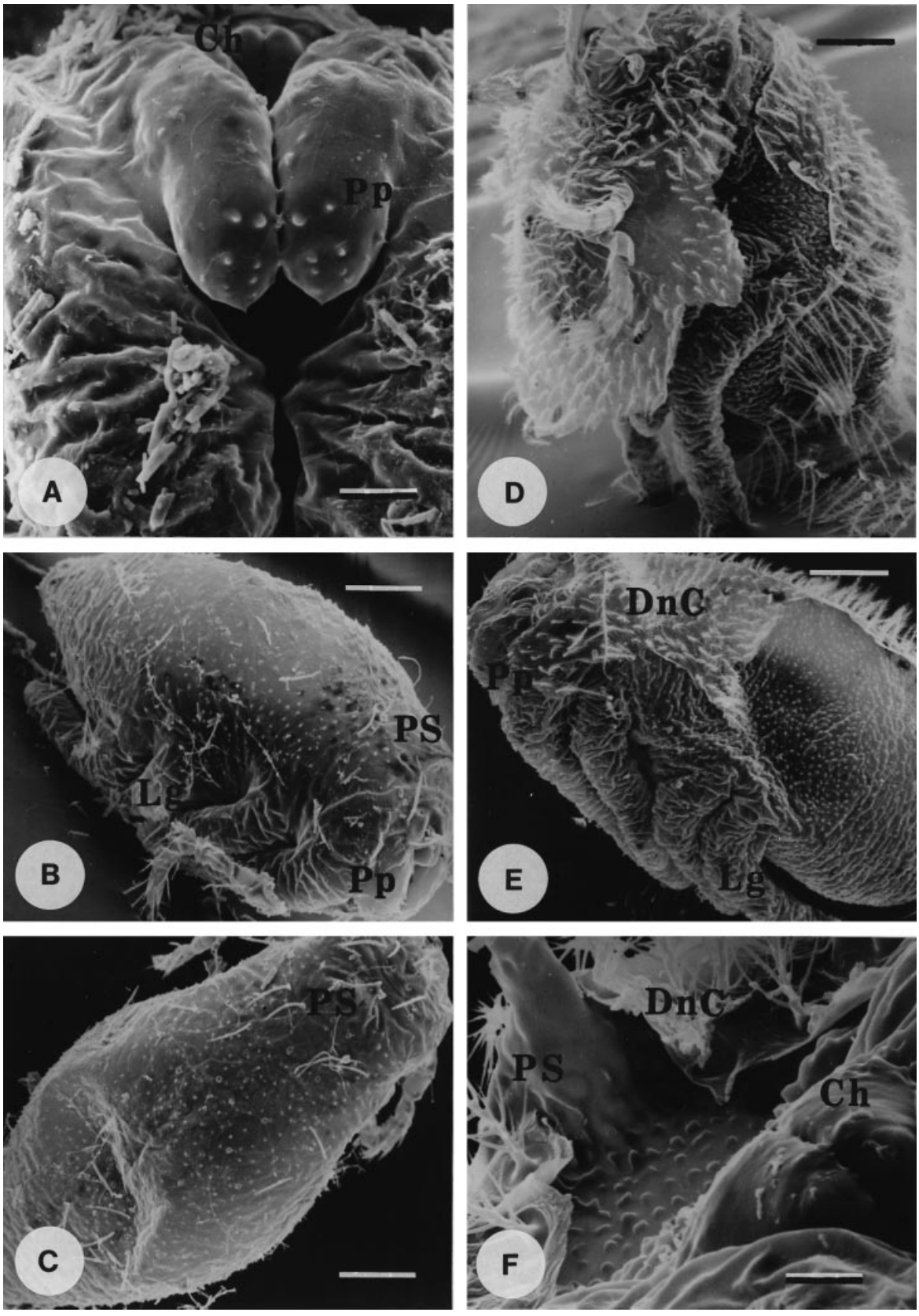
As embryonic development proceeds, the egg becomes larger and slightly elongated, and the prelarva is formed within the chorion due to deposition of the cuticle by the thin ectoderm of the embryo. The growing prelarva splits the chorion into halves along a transverse cleft-line from the anterior venter to the mid-dorsum of the mite (Fig. 2 A,C) similar to what was earlier observed in the trombiculid mite *Ascoschoengastia indica* (Wharton 1946). This prelarva has been termed traditionally as 'deutovum' by earlier authors on trombiculids (Ewing 1944; Michener 1946; Wharton 1946; Neal and Barnett 1961), although it has been known for a long time that this organism represents the first but strongly regressed larval instar (Grandjean 1938b). The observations made with a dissecting microscope show a smooth and shiny prelarva, while its colour changes from light yellow to deep orange as the development progressed. The SEM observations show a tuberculate cuticle at the base of appendages and on the dorsum especially in *L. schlugerae* (Fig. 2C), but in *H. zachvatkini*, the tubercles are smaller and the area, covered with them, is less extensive. The cuticle of prelarvae and quiescent nymphal instars completely lacks setae. However, on the lateral surfaces of the body, approximately at the level of the base of leg III, prelarvae of each species have a pronounced and sharp cuticular spine facing posteriorly (Fig. 2 A,C). These lateral cuticular spines closely resemble the 'poil residuel' of trombidiid prelarvae (Robaux 1974) and represent the homologue of the latter. It is interesting to note that these spines are situated immediately beneath the cleft-line of the chorion, and after splitting the chorion they remain in close proximity to the border of its back half. A structure resembling latero-frontal groove (ecdysial line) has not been observed in trombiculid and trombidiid (Robaux 1974) mite prelarvae.

The elongate prelarva of *L. orientale* measured about 257 μm long and possessed two pairs of reduced mouthparts (chelicerae and palps) anteriorly and three pairs of reduced non-articulated appendages (legs) in the anterior-ventral position (Fig. 2 A-C, F). Initially, just after the egg splitting, the legs are tightly adjoined and form a cone with the apex facing ventrally. On the dorsal surface behind the chelicerae is situated an unpaired prodorsal spine (Fig. 2 A,B,D), or horn (Wharton 1946), formed from a cuticular fold. It is found in nearly all prelarvae studied so far. This structure, often termed as 'naso' (Alberti 1975; Coineau 1979; Kethley 1990a), has long been attributed in trombidiform prelarvae as specialized 'egg-burster' developed for piercing the chorion (Moss 1962). However, its precise function is still unclear, because the main force for splitting the chorion in trombiculid prelarvae appears to be the strongly protruded legs, situated on the ventrum, or the above-mentioned lateral cuticular spines. In prelarvae of *L. orientale* and *L. schlugerae*, the prodorsal spine has a pyramidal form and is covered with tiny tubercles (Fig. 2 B,D), like the ones on the surface of the mite, whereas in *H. zachvatkini* the prodorsal spine is narrower and more pointed and has no tubercles (Fig. 2E).

The chelicerae are represented by two short cone-like structures situated on the frontal surface of prelarvae (Fig. 2 B, F). These structures are probably homologous to the basal segments of the larval chelicerae. They are mesally fused with each other and the groove between them is most conspicuous in *L. schlugerae* and less noticeable in *H. zachvatkini*. In the latter, in contrast to other species studied, two small folds of cuticle, which often take the form of slightly sharpened tubercles, are observed situated just beneath the reduced chelicerae (Fig. 2F). These structures probably represent the remnants or vestiges of the lost movable digits.

The palps are located beneath and topographically in front of the chelicerae, face downwards and are separated in the mid-line (Fig. 2 A,F). In prelervae of *H. zachvatkini*, the palps are also distally separated from the body wall and have the sharpened tips (Fig. 2F). The cuticle of palps is smooth with indistinct transverse grooves (Fig. 2F), that may indicate traces of the segmentation. Such a sign of segmentation may be also seen in the limbs of prelarvae of Eupodidae (Coineau 1976) and Trombidiidae (Robaux 1974). In prelarvae of *L. orientale* and *L. schlugerae* the palps are found tightly adjoined to the body wall, particularly in *L. schlugerae*, and do not have conspicuously sharpened tips as compared to *H. zachvatkini*. Urstigmae or Claparede's organs, which are

Fig. 2.—SEM of prelarvae of *Leptotrombidium orientale*, *L. schlugerae* and *Hirsutiella zachvatkini*. — **A,** Lateral view of *L. orientale*. Note half of the egg-shell (ESh) and a lateral cuticular spine (LS). Ch, chelicerae; Lg, legs; Pp, palps; PS, prodorsal spine. Scale bar: 43 μm. — **B,** Dorsal view of the chelicerae (Ch) and prodorsal spine (PS) of *L. orientale*. Scale bar: 12 μm. — **C,** Ventral view of *L. schlugerae*. Note the two halves of the split egg-shell (ESh) and the legs (Lg) grouped into a cone. LS, lateral cuticular spine. Scale bar: 37 μm. — **D,** The pyramidal prodorsal spine of *L. schlugerae*. Note small tubercles covering the prelarva and a split egg-shell (ESh) behind the prodorsal spine. Scale bar: 5 μm. — **E,** Urstigma, or Claparede's organ, of *L. schlugerae*. Scale bar: 7.5 μm. — **F,** Frontal view of the mouth appendages of *H. zachvatkini* showing chelicerae (Ch), palps (Pp) with the pointed tips and also a prodorsal spine (PS). Scale bar: 20 μm.



thought to function in osmoregulation (Alberti 1979; Fashing 1988), are found both in prelarvae and larvae of Actinedida (Kethley 1990b) and Oribatida (Sitnicova 1960; Grandjean 1962). The urstigmae are located between the bases of legs I and II (Fig. 2C) and have a nearly similar organization, consisting of a short and stout cuticular rod with a semispherical cap (Fig. 2E). No evidence of genital and anal openings is observed in prelarvae of the species examined.

As larval hatching proceeds, the prelarval exuviae are always found splitting into halves along an oblique transverse cleft line as in the case of the chorion. This type of dehiscence may be classified as merodehiscence (Norton and Kethley 1994). The average time from the appearance of prelarva from the chorion to the ecdysis of the unfed larvae in culture lasted about 19–22 days in these species.

The quiescent nymphal instars, namely proto- and tritonymphs, are organized similarly. They are initially formed within the detached covering of previous active instars (Fig. 1C) with the deposition of their cuticulin layer. Morphologically, this is the starting point of every instar in arthropod development, in particular, the quiescent ones, while externally mites retain the organization of larva and deutonymph. The apolysis in the active instars takes place approximately a day or even earlier after the loss of their activity. About 2–4 days later, the outlines of the regressive inactive instars may be distinguished through the old cuticle. Later on, the formation of proto- or tritonymphs becomes well pronounced. The shedding of the old cuticle occurs fragmentarily and nearly imperceptibly, especially in protonymphs (Fig. 3 B,C), however, the whole frontal region, bearing the mouthparts and sometimes legs, may be shed simultaneously (Fig. 3D). Taking into account the transvers ecleavage line in these cases, it becomes clear that the merodehiscence mode of splitting of the old cuticle (Norton and Kethley 1994) is found in the active and also in the inactive instars of trombiculid mites. Apart from the frontal region,the dried legs of the previous active instar remains, usually attached to the ventral body wall of quiescent one (Fig. 3 B,C).

The quiescent nymphal instars moult under the old cuticular covering and this can be detected only through the use of transmission elecron microscopy. This process consisits of a gradual detachment of the cuticle (Fig. 1D), reorganization of the hypodermis and deposition of the new cuticle of the next active instar. The cuticular deposition takes place entirely within the covering of the quiescent instar and occupies a relatively long period (the pharate period). It terminates by the ecdysis of active instars deutonymph or adult mite. In culture, the time from the immobilization of the previous active instar to the appearance of the next one takes about 18–26 and 14–16 days in proto- and tritonymph, respectively.

The proto- and tritonymph are slightly flattened laterally and have more or less spindle-shaped outlines with a sharpened caudal tip from the dorsal view (Fig. 3 B,C,E). When viewed with a dissecting microscope, the mites appear yellowish-white in colour and have a shiny appearance due to the translucent exuvial fluid. SEM of the quiescent nymphal instars of *H. zachvatkini* reveals a folded cuticle, particularly in the legs and relatively little tuberculation of the body and palps (Fig. 3 A, B, E). The integument completely lacks setae and other specialized cuticular structures such as spines and teeth. Near the end of the quiescent period, the cuticle is flattened due to pressure of the exuvial fluid of the second moulting cycle. The length of the proto- and tritonymph of *H. zachvatkini* is about 800 and 1200 μm, respectively.

The appendages (Fig. 3 A, B, E) occupy the anterior and ventral portions of the body and are distinctly separated from the latter. The palps are positioned anteriorly, and touch the inner surface of legs I (Fig. 3A); hence these structures form the frontal portion of the proto- and tritonymph (Fig. 3 B, E). In addition, palps in these instars of *H. zachvatkini*, as in the prelarva, possess a tiny sharpened tip (Fig. 3A). The legs are arranged with a distinct gap between the bases of legs II and III, and are bent posteriorly (Fig. 3E).

The chelicerae, composed only of the supposed basal segments, are completely adnate mesally and are situated on the anterior dorsum between the bases of the palps (Fig. 3 A, B, F). Each chelicera possesses a wide base and bears one small tubercle on its dorsal surface near the rounded distal end (Fig. 3F). The characteristic prodorsal spine, which is formed of an integumental fold, is situated behind the cheliceral bases as in the prelarvae (Fig. 3 B, C, F). In proto- and tritonymphs of *H. zachvatkini*, the prodorsal spine bears no tubercles and is pointed forward.

Fig. 3.—SEM of the quiescent nymphal instars of *H. zachvatkini*. — **A,** Frontal view of an 89-day-old quiescent protonymph showing chelicerae (Ch) and palps (Pp) with few small tubercles and slightly pointed tips. Scale bar: 30 μm. — **B,** Lateral view of an 11–12-day-old quiescent protonymph showing palps (Pp) and pairs of legs (Lg). Note the tuberculate and folded protonymphal covering and the larval dried leg applied to protonymph. Scale bar: 88 μm. — **C,** Dorsal view of an 11–12-day old quiescent protonymph of a spindle shape with the retained larval setae and legs. PS, prodorsal spine. Scale bar: 88 μm. — **D,** Lateral view of a 12-day-old moulting tritonymph with the splitted old deutonymphal skin. Scale bar: 125 μm. — **E,** Lateral view of a 4–5-day old quiescent tritonymph showing its tuberculate and folded cuticle and also the shedding of the deutonymphal covering (DnC). Note the palps (Pp) and legs (Lg) arranged in pairs like in protonymph. Scale bar: 125 μm. — **F,** Frontal view of the pointed prodorsal spine (PS) and the chelicerae (Ch) of a 4–5-day-old quiescent tritonymph. Note the deutonymphal covering with setae (DnC). Scale bar: 15 μm.

As is seen from Fig. 3B and D, the prodorsal spine in trombiculid mites probably does not play a role as a burster of the covering of the previous active instar.

The internal organization of the mouthparts of the inactive proto- and tritonymphs consists mainly of a reduced labrum, an epistome as well as the atrial cavity. The pharynx is represented in the form of a cuticular fold near the ventral sides of the sunken cheliceral bases.

There are other important structures that characterize both nymphal instars and adult mites such as the genital papillae. The presence of genital papillae in postlarval instars and the presence of Claparede's organs in prelarva and larva in trombidiform mites, are generally thought to obey the Oudemans-Grandjean rule (Johnston and Wacker 1967; Kethley 1990b; André 1992) with some sporadic exceptions (André 1991b). Unfortunately, views of the genital opening and genital papillae with SEM of quiescent instars was difficult to obtain due to the position of their legs. Nevertheless, light microscope slide preparations reveal that protonymphs possess one pair of genital papillae, whereas deutonymphs (an active instar) as well as tritonymphs have two pairs and the adult, three pairs. In quiescent instars these structures are situated approximately at the level of the base of legs III.

The ecdysis of the active postlarval instars in trombiculid mites is achieved by a transverse dorsal split (cleavage line) in the old covering, approximately at the juncture of proterosoma and hysterosoma (merodehiscence) (Norton and Kethley 1994). Active movements of the mouthparts and legs also help in the shedding of the old cuticular covering.

Discussion

From the material presented here, it is clear that the major time of the inactive periods of trombiculid mites is occupied by two moulting cycles. The first one occurs entirely under the cuticle of previous active instar without visible termination, e.g. without ecdysis of the quiescent instar. Later on, near the middle of the inactive period, the old covering is lost by passive means through its gradual shedding. About this time, a new active instar starts to develop within the covering of the quiescent one during the second moulting cycle. The latter terminates by the ecdysis of the active instar. Moulting is quite an important morphogenetic process (Sehnal 1985). A moulting process had not been admitted previously during the transformation from larva to protonymph and from deutonymph to tritonymph in some trombidiform mites (Böttger 1977). The culmination of the moulting process is referred to as the time of deposition of the most superficial layer (cuticulin layer) of new cuticle. This moment is proposed to be an exact starting point of every new instar in ontogenesis of mites and other arthropods (Shatrov 1995).

The difficulties in detecting the quiescent instars have not allowed previous authors to interpret the post-embryonic development of trombiculid mites clearly (Wharton 1946; Ewing 1944; Jones 1954). However, as is seen from the evidence presented, the organisms at inactive periods of trombiculid life cycle are actual but strongly reduced instars, irrespective of their duration and morphological expression. This finding again emphasizes the thesis that there are no so-called 'missing stases' in trombiculid development as in other trombidiform mites (André 1992). This does not correspond with the assumption of Johnston and Wacker (1967), who regarded the quiescent instars as belonging to the entire inactive periods and, moreover, as being completely within the cuticle of the preceding instars, e.g. as the pharate instars as a whole. However, the pharate period (Hinton 1973, 1976; Sehnal 1985) in trombiculid mite ontogenesis might be refered to as the active instars before their ecdysis, but to a lesser extent as the quiescent instars, being partly or entirely within the old covering of the active one.

This situation is complicated by the prelarval cuticle, which appears deposited not by mature hypodermis, but by the ectodermal layer. As has been observed in some insects, the first embryonic cuticle is a distinct layer and is deposited by the ectoderm during their 'embryonic moult' (Dorn and Hoffmann 1981). In trombiculid mites, the covering of prelarva seems to be a true cuticle. It is deposited above the tiny short microvilli of the probable ectoderm cells, but undergoes apolysis rather quickly often still under the egg-shell. In addition, the ultrastructure of both prelarval and proto- and tritonymphal cuticles is practically the same, despite differences in width. In any case, the cuticle applied to the hypodermis or even to the ectodermal layer in these instars cannot, apparently, be referred to as the only particular provisional cuticle (Jones 1954), or mere apoderma, and is believed to play, in fact, a complex functional role, as in any organism.

It is evident that the quiescent instars either in trombiculid mites, or in other trombidiform groups with such a mode of development (Singer 1971; Newell 1973; Robaux 1974; Böttger 1977; Kethley 1991) cannot really be regarded as a pupa like the specific quiescent stage in the holometabolous insects. This analogy with respect to proto- and tritonymphs of parasitengona mites has been postulated by several authors (Jones 1954; Singer 1971, etc.) and discussed in the work of Johnston and Wacker (1967). Concerning this problem, it needs only to be noted that a 'marked but unspecified anatomical reorganization' (Johnston and Wacker 1967; p. 309) during post-embryonic development of these mites, is just a long moulting process.

The apparent differences in the duration of prelarval and quiescent nymphal instars seem to indicate that the embryonization in trombiculid mites is a longer and more pronounced phenomenon than the reduction of post larval

nymphal instars. Therefore, one can reasonably propose, that embryonization appeared first in evolution of Trombiculidae and, possibly, in Acariformes and may have preceded both the acquisition of the so-called 'alternating calyptostasy' in *Neonanorchestes* (Nanorchestidae), Pterygosomatidae andParasitengona (Kethley 1991) and also the development of parasitism in the latter two groups. In either case, the alternating calyptostasy, or reduction of prelarval as well as proto- and tritonymphal instars, appear to be the answer to complex environmental and physiological challenges of different ways of life in Actinedida and must be regarded as having developed independently. As has been noted previously (Knulle 1961; Böttger 1977), these evolutionary trends manifest the tendency to reduce the duration of post-embryonic development in trombidiform mites. Moreover, the quiescent, in particular, nymphal instars, have been thought to play a stabilizing role in the whole ontogenesis (Newell 1973) which is believed to have evolved as an integral evolutionary unit (Shatrov 1995). The latter has been thought of as a peculiar pattern of 'developmental canalization' (Newell 1973).

This type of ontogenesis is thought to imply not only the proportional duration of active and quiescent instars (Kethley 1991), but also the fixed number of developmental steps in the ancestral acariform life cycle (André 1992). From this point of view, the observed extraordinary adult moults in the Trombidiidae and water mites (Tevis and Imamura 1952; Newell 1952; Kethley 1991) represent a great deviation of the given developmental type and must be verified carefully.

The prelarva in mites live on yolk and have been considered to be a particular foetal instar, as in some other arachnids (Canard and Stockmann 1993). Due to its reduction, the prelarva is referred to as a part of the embryonic development. In any case, either being embryonic or post-embryonic, the prelarva is an instar mostly engaged in the utilization of the embryonic yolk and seems unable to disappear completely from the ontogenesis of trombidiform mites (André 1992). Thus, prelarva expresses a progressive ontogenetic acceleration (Kethley 1991) and is thought to be the first step in the evolution of 'alternating calyptostasy'.

There is another problem concerning morphological and phylogenetical status of the prelarva in mites. Instead of being a regressed instar, the prelarva is considered to be a distinct embryonic stage (Otto 1997). It must be kept in mind, however, that from an ontogenetic position, the prelarva is an instar with organs not yet developed. Nevertheless, phylogenetically, the prelarva must be regarded as a highly regressed first larval instar, being active and capable of feeding in the ancestral acarine forms (Grandjean 1938b). If the prelarva, especially active ones, represent a derived embryonic stage, as Otto (1997) has proposed, they must have specialized embryonic characters needed for embryonic life, and this does not actually take place. Practically all structures of the prelarva, such as palps, legs, setae, etc., are obviously not the embryonic ones, so this instar can ultimately be considered not as a special embryonic stage, but only as a reduced post-embryonic instar. Therefore, it is not surprising that the active prelarva in some groups of trombidiform mites is thought to represent the plesiomorphic condition (Coineau 1976, 1979; Schuster and Pötsch 1988).

The variation in regression of prelarvae, especially in early derived free-living Actinedida, with their motility in some groups (Grandjean 1938a, 1948, 1957; Lindsay 1972; Coineau 1976; 1979; Schuster and Pötsch 1988; Kethley 1990a; Otto and Olomski 1994; Otto 1997), may be due to environmental and physiological factors acting separately or in combination over a long period of time. The apparent convergence of prelarvae among different taxa of trombidiform mites (Kethley 1990a) may be interpreted as a consequence of the monophyletic origin of this branch of Acari.

The comparative analysis of the quiescent instars in Trombiculidae and Trombidiidae reveals distinct similarities between them (Wharton 1946; Robaux 1974) with the exception of some small details. In the trombidiid prelarva the 'organ ecuticulaire' was observed on the lateral sides of the body (Robaux 1974), but was not identified in trombiculid mites. The lateral cuticular teeth and 'poil residuel' of Robaux (1974) are obviously homologous structures and both supposedly function for the initial piercing of the egg-shell. It might be proposed therefore that there will be no significant variations in organization type among other representatives of these two families. Thus, in both cases, the long evolution in rather conservative environmental conditions, such as soil, is thought to give rise to similar morphology and ontogenesis.

In early derived taxa, such as Nanorchestidae and some others, there may be diverse and unstable environmental conditions that supposedly influenced the diverse morphological expression of the quiescent instars even within a given family (Alberti 1975; Coineau 1976, 1977, 1979; Kethley 1990a, 1991). Nevertheless, the same situation can be seen within the Pterygosomatidae, one of the highly specialized taxa, where the transition to obligate parasitism is, supposedly, a relatively young evolutionary phenomenon that minimizes the environmental pressure and gives rise to quite different morphology of the quiescent instars between genera (Newell 1973).

The variable development (or, more exactly, the variable degree of reduction) of the mouth appendages, in particular palps, in prelarvae of the species studied, may indicate different levels of their embryonization. Thus, the latter is probably less pronounced in *H. zachvatkini* prelarvae because of their more prominent palps with sharpened tips. In general, the prelarvae of Trombidioidea

occupy the same level of reduction as the prelarvae of Caeculidae (Coineau 1974) and the primitive prelarvae of Bdellidae (Cytinae) (Alberti 1975) with the developed prodorsal spine (naso), highly reduced chelicerae, and moderately reduced palps and three pairs of legs. The legs II and III, often crossed on the ventral side of the body of prelarvae in some Caeculidae and Bdellidae (Coineau 1974; Alberti 1975), are grouped, together with the first pair, forming a cone in trombiculid prelarvae. The prodorsal spine (naso) has been considered to develop in close association with the median eye (Coineau 1970; Alberti 1975) which often occurred without a visible cuticular termination (cornea). The presence of this eye was not confirmed by the author in trombiculid mites. The homologization of the naso with the acron (Alberti 1975) needs to be proven more conclusively. I tend to think that the prodorsal spine, developed equally in prelarvae and quiescent nymphal instars, may be a more probable homologue of the tectum, which is also represented in deutonymph and adult mites. The acron, as the embryonic head lobe, is completely lost in the trombidiform, in particular, in the parasitengona mites. The latero-frontal groove, found in many trombidiform prelarvae, either developed or regressive (see Grandjean 1938c; Coineau 1974, 1976, 1979; Alberti 1975; Kethley 1990a; Otto 1997), is absent in Trombidioidea. It should be noted that both the lateral spines and the ecdysial line typically occur in the strongly regressed prelarvae of Oribatida (Sitnicova 1960; Grandjean 1962; Lions 1973)

The trombiculid mites, possibly together with other Parasitengona, are thought to show the most generalized pattern of ontogenesis among Acariformes within the developmental phenomenon of alternating calyptostasy. The 'developmental constraints' (André 1992) may be expected as the only consequence of the constant environmental conditions acting through the relatively long period of time and also as a result of the supposedly progressive parasitism of the only specialized larval form. Such an ontogenetic pattern seems to develop independently of the systematic relationships of different trombidiform taxa and also of their starting evolutionary points. Whereas only few random representatives of the order possess motile prelarvae (Coineau 1977, 1979; Schuster and Pötsch 1988; Otto and Olomski 1994; Otto 1997), the other trombidiform groups, as well as Oribatida and Acaridida (Lange 1960; Sitnicova 1960; Fain and Herin 1979; Grandjean 1962; Trave 1976) have, typically, much more derived and regressed prelarvae than trombiculid one. Unfortunately, until now, little is known about either the organization of the quiescent nymphal instars, or about the fine developmental processes in these mites. Therefore, there is a great need for obtaining representative morphological data on acariform ontogenesis in order to understand the exact meaning of alternating calyptostasy.

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References

Alberti, G. 1975. Prälarven und Phylogenie der Schnabelmilben (Bdellidae, Trombidiformes). – *Zeitschrift fur zoologische Systematik und Evolutionsforschung* **13**: 44–62.

Alberti, G. 1979. Fine structure and probable function of genital papillae and Claparede organs of Actinotrichidae. In Rodriguez, J. G. (Ed.): *Recent Advances in Acarology, Vol. 2,* pp. 501–507. Academic Press, New York.

André, H. M. 1991a. Stase, metamorphosis and competition in insects and other arthropods. – *Belgian Journal of Zoology* **121**: 3–25.

André, H. M. 1991b. The Tydeoidea: a striking exception to the Oudemans-Grandjean rule. In Dusbabek F. and Bukva V. (Eds): *Modern Acarology, Vol. 2,* pp. 293–296. Academia, Prague and SPB Academic Publishing bv, The Hague.

André, H. M. 1992. Calyptostases as indicators of developmental constraints in mites and other arthropods. – *Acarologia* **33**: 223–231.

André, H. M. and Fain, A. 1991. Ontogeny in the Tydeoidea (Ereynetidae, Tydeidae and Iolinidae). In Dusbabek F. and Bukva V. (Eds): *Modern Acarology, Vol. 2*, pp. 297–300. Academia, Prague and SPB Academic Publishing bv, The Hague.

Baker, R. A. 1973. Notes on the internal anatomy, the food requirements and development in the family Ereynetidae (Trombidiformes). – *Acarologia* **15**: 43–52.

Böttger, K. 1977. The general life cycle of fresh water mites (Hydrachnellae, Acari). – *Acarologia* **18**: 496–502.

Canard, A. and Stockmann, R. 1993. Comparative postembryonic development of Arachnids. – *Memoires of the Queensland Museum* **33**: 461–468.

Coineau, Y. 1970. A propos de l'oeil antérieur du naso des Caeculidae. – *Acarologia* **12**: 109–118.

Coineau, Y. 1974. Eléments pour une monographie morphologique, ecologique et biologique des Caeculidae (Acariens). – *Memoires du Museum National d'Histoire Naturelle, Ser. A* **81**: 1–299.

Coineau, Y. 1976. La première prélarve connue du genre *Eupodes: Eupodes strandtmanni*, n.sp. – *Acarologia* **18:** 56–64.

Coineau, Y. 1977. La première prélarve élattostatique connue chez les Acariens. – *Acarologia* **19**: 46–54.

Coineau, Y. 1979. Les Adamistidae, une étonnante famille d'acariens prostigmates primitifs. In Piffl, E. (Ed.): *Proceedings of the 4th International Congress of Acarology*, pp. 431–435. Akadémiai Kiadó, Budapest.

Dorn, A. and Hoffmann, P. 1981. The 'embryonic moults' of the milkweed bug as seen by the S.E.M. – *Tissue & Cell* **13:** 461–473.

Ewing, H. E. 1944. The Trombiculid mites (chigger mites) and their relation to disease. – *Journal of Parasitology* **30**: 339–365.

Fain, A. 1972. Développement postembryonnaire chez les Acariens de la sous-famille Speleognathina (Ereynetidae: Trombidiformes). – *Acarologia* **13**: 607–614.

Fain, A. and Herin, A. 1979. La prélarve chez les Astigmates (Acari). – *Acarologia* **20**: 566–571.

Fashing, N. J. 1988. Fine structure of the Claparede organs and genital papillae of *Naiadacarus arboricola* (Astigmata: Acaridae), an inhabitant of water-filled treeholes. In Channabasavanna G. P. and Viraktamath C. A. (Eds): *Progress in Acarology*, *Vol. 1*, pp. 219–228. Oxford and IBH Publishing Co, New Delhi.

Grandjean, F. 1938a. Description d'une nouvelle prélarve et remarques sur la bouche des Acariens. – *Bulletin de la Societe Zoologique de France* **63**: 58–68.

Grandjean, F. 1938b. Sur l'ontogenia des Acariens. – *Comptes Rendus des Séances de l'Academie des Sciences (Paris)* **206**: 146–150.

Grandjean, F. 1938c. Observations sur les Acariens (4e Série). – *Bulletin du Muséum National d'Histoire Naturelle*, *Ser.* **210:** 64–71.

Grandjean, F. 1948. Quelques caractères des Tétranyques. – *Bulletin du Museum National d'Histoire Naturelle, Ser. 2*, **20**: 517–524.

Grandjean, F. 1957. Les stases du développement ontogénétique chez *Balaustium florale* (Acarien, Erythroide). Premiere Partie. – *Annales de la Societe Entomologique de France* **125**: 135–152.

Grandjean, F. 1962. Prélarves d'Oribates. – *Acarologia* **4**: 423–439.

Hammen, L., van der 1964. The morphology of *Glyptholaspis confusa* (Foa, 1900) (Acaria, Gamasina). – *Zoologische Verhandelingen (Leiden)* **71**: 1–56.

Hinton, H. E. 1973. Neglected phases in metamorphosis: a reply to V. B. Wigglesworth. – *Journal of Entomology (A)* **48**: 57–68.

Hinton, H. E. 1976. Notes on neglected phases in metamorphosis, and a reply to J. M. Whitten. – *Annals of the Entomological Society of America* **69**: 560–566.

Imamura, T. 1952. Notes on the moulting of the adult of the water mite, *Arrenurus uchidai* n. sp. – *Annotationes Zoologicae Japonenses* **25**: 447–451.

Johnston, D. E. and Wacker, R. R. 1967. Observations on postembryonic development in *Eutrombicula splendens* (Acari Acariformes). – *Journal of Medical Entomology* **4**: 306–310.

Jones, B. M. 1954. On the role of the integument in Acarine development and its bearing on pupa-formation. – *The Quarterly Journal of Microscopical Science* **95**: 169–181.

Kethley, J. B. 1990a. The prelarva of *Alycus roseus* Koch (Bimichaeliidae: Acariformes: Acari). – *Canadian Journal of Zoology* **68**: 1058–1061.

Kethley, J. B. 1990b. Acarina: Prostigmata (Actinedida). In: Dindal D. L. (Ed.): *Soil Biology Guide*, pp. 667–756. John Wiley & Sons Inc.

Kethley, J. B. 1991. Calyptostasic nymphs of *Neonanorchestes* (Nanorchestidae): A third example of alternating calyptostasy. In Dusbabek F. and Bukva V. (Eds): *Modern Acarology*, *Vol. 2*, pp. 279–282. Academia, Prague and SPB Academic Publishing bv, The Hague.

Knülle, W. 1961. Primitivstruktur und Evolution einiger Charaktere der Acariformes. – *Zoologischer Anzeiger* **167**: 10–15.

Lange, A. B. 1960. Praelarva of mites of the order Acariformes and its peculiarities in Palaeacariformes. – *Zoologitchesky Zhurnal* **39**: 1819–1834 (In Russian with English summary).

Lindsay, K. L. 1972. *Nanorchestes antarcticus* Strandtmann; a taxonomic description of the instars. – *Acarologia* **13**: 593–606.

Lions, J.-Cl. 1973. Quelques prelarves nouvelles d'Oribates. – *Acarologia* **15**: 356–370.

Michener, C. D. 1946. Observations on the habits and life history of a chigger mite, *Eutrombicula batatas* (Acarina: Trombiculinae). – *Annals of the Entomological Society of America* **39**: 101–118.

Moss, W. W. 1962. The immature stages of the red velvet mite *Allothrombium lerouxi* (Acari. *Trombidiidae)*. – *Annals of the Entomological Society of America* **55**: 295–303.

Neal, T. G. and Barnett, H. C. 1961. The life cycle of the scrub typhus chigger mite, *Trombicula akamushi*. – *Annals of the Entomological Society of America* **54**: 196–203.

Newell, I. M. 1973. The protonymph of Pimeliaphilus (Pterygosomatidae) and its significans to the calyptostases in the Parasitengona. In Daniel M. and Rosicky B. (Eds): *Proceedings of the 3rd International Congress of Acarology*, pp. 789–795. Junk, Prague, The Hague.

Norton, R. A. and Kethley, J. B. 1994. Ecdysial cleavage lines of acariform mites (Arachnida, Acari). – *Zoologica Scripta* **23**: 175–191.

Otto, J. C. 1997. Observations on prelarvae in Anystidae and Teneriffiidae. In Mitchell R. D., Horn D., J., Needham G. R. and Welbourn W. C. (Eds): *Acarology IX, Proceedings*, pp. 343–354. Ohio Biological Survey, Columbus.

Otto, J. C. and Olomski, R. 1994. Observations on a motile prelarva in *Chaussieria venustissima* (Berlese, 1882) (Acari: Anystidae), with a description of the larva. – *Canadian Journal of Zoology* **72**: 287–292.

Reuter, E. 1909. Zur Morphologie und Ontogenie der Acariden. – *Acta Societatis Scientiarum Fennicae* **36**: 1–287.

Robaux, R. 1974. Recherches dur le développment et la biologie des acariens 'Thrombidiidae'. – *Mémoires Muséum National D'Histoire Naturelle Ser. A*, **85**: 1–186.

Schuster, R. and Pötsch, H. 1988. Another record of an active prelarva in mites. In: Channabasavanna G. P. and Viraktamath C. A. (Eds): *Progress in Acarology*, *Vol. 1*, pp. 261–265. Oxford and IBH Publishing Co, New Delhi.

Sehnal, F. 1985. Growth and life cycle. In Kerkut G. A. and Gilbert L. I. (Eds): *Comprehensive Insect Physiology, Biochemistry and Pharmacology, vol. 2*, pp 2–86. Pergamon Press, New York.

Shatrov, A. B. 1993. Culture and life cycle of the trombiculid mite *Leptotrombidium orientale* (Schluger, 1948) (Acariformes, Trombiculidae). – *Entomological Review* **72**: 138–158.

Shatrov, A. B. 1995. Ontogenesis and peculiarities of its differentiation in Arthropoda. – *Entomological Review* **74**: 84–99.

Singer, G. 1971. *Neotrombidium leonardi* (Acarina: Trombidioidea). Part 2: Biology of *N. beeri* and other species of the genus. – *Acarologia* **13**: 143–151.

Sitnicova, L. G. 1960. Les prelarves des Oribates. – *Parasitologitchesky Sbornick* **19**: 220–236 (In Russian with French summary).

Tevis, L. and I. M. Newell 1962. Studies on the biology and seasonal cycle of the giant red velvet mite, *Dinothrombium pandorae.* (Acari, Trombidiidae). – *Ecology* **43**: 497–505.

Travé, J. 1976. Les prélarves d'Acariens. Mise au point et donnees recentes. – *Revue d'Ecologie et de Biologie Du Sol* **13**: 161–171.

Wharton, G. W. 1946. Observations on *Ascoschöngastia indica* (Hirst, 1915) (Acarinida: Trombiculidae). – *Ecological Monographs* **16**: 151–184.

