On predation in Epicriidae (Gamasida, Anactinotrichida) and fine-structural details of their forelegs

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Abstract
The present study reveals, based on video-recording, that Epicriidae are predators using their long forelegs provided with a number of long clubbed setae for the capture of small arthropods. The mite walks slowly with raised and probing forelegs. Upon contact with a small isotomid springtail, the forelegs rapidly touch the prey with the tips of the elongated, clubbed setae. The prey evidently adheres to these setae and is drawn back to the mouthparts. The epicriid feeds for a considerable time on its prey whereby the mite can move around until it finds a shelter. The clubs represent spinose setal ends which are loaded with a granular secretion. The origin of the secretion could not yet be clarified definitely. Since the setae contain a lumen it might be that the secretion reaches the club through the seta. The secretion which covers the surface of the mite as a cerotegument is fine structurally distinctly different and is most likely produced by typical anactinotrichid dermal glands, which also occur in the forelegs. Some details of the fine structure of legs I are demonstrated. Since these clubbed setae occur in all Epicriidae it seems likely that all are predators feeding on small, weakly sclerotised arthropods.

Keywords: adhesive setae, dermal glands, mites, sensory setae, video recording

1. Introduction
Epicriidae represent a small family of Gamasida comprising about 35 species (Lindquist et al. 2009a). They are known only from the Northern Hemisphere. In Europe there are two of the four genera present: Epicrius (15 species) and Berlesiana (3 species) (Evans 1955, Evans & Till 1979, Błaszak & Alberti 1989, Karg 1993, Moraza 2005, 2005 [2006]). They are mostly considered to represent an early derivative group of Gamasina (e.g., Evans 1955, Alberti 1980, Moraza & Lindquist 1999, Lindquist et al. 2009a,b; but see Karg, 1993, 2006). Epicriidae are found in mosses, leaf litter, rotten wood and sometimes in nests of rodents and in limestone caves (Lindquist et al. 2009a). Besides others, one characteristic feature of these mites is the feeler-like forelegs which are devoid of a claw and are provided with two or several more or less elongated setae (macrosetae). These setae are apically minutely clubbed and have been interpreted as tactile or sensory setae (e.g., Evans 1955, Karg 1993). Almost nothing is known concerning the biology of these very characteristic mites. It is assumed that they represent tocospermic animals with putative spermatophores (sperm cells are of the vacuolated type; Alberti 1980) transferred into the ventromedially located genital opening of the female with the help of rather unmodified chelicerae of the male. Lindquist et al. (2009a)
suggest based on the morphology of the gnathosoma that they feed on fungi fluids. In the present study direct observations on the feeding behaviour of *Epicrius schusteri* Błaszak & Alberti, 1989 are reported for the first time and some fine structural peculiarities of the forelegs are presented including also observations from other species.

2. Materials and methods

*Collecting and video-recording (VR):* Specimens of *Epicrius schusteri* Błaszak & Alberti, 1989 were obtained from leaf litter (deciduous trees) kindly collected by Prof. Dr R. Schuster near Leutschach (SW-Styria, Austria). The mites were extracted using conventional Berlese-funnels with waterfilled cups underneath. The cups were regularly checked to obtain the mites alive. They were transferred into small plastic-boxes with a bottom made of plaster of Paris, which in some boxes was mixed with charcoal. Other microarthropods (oribatid mites, springtails) from the same sample were added. Observations of the living mites were done using an Olympus SZH10 stereomicroscope provided with a Sony Progressive 3CCD video-camera. The films were later digitalised from the video tapes using the Pinnacle Studio Plus Version 9 program and further processed with Adobe Premiere Pro.

*Light-microscopy (LM):* Specimens were mounted in a modified Berlese fluid (Krantz & Walter 2009) and studied using an Olympus BX60 microscope provided with an Axio Cam MRC – Zeiss digital-camera connected with the Axio Vision Rel. 4.8 program. For additional studies, specimens of *Epicrius mollis* (Kramer, 1876) collected in the vicinity of Kiel (Germany) and *Epicrius canestrinii* (Haller, 1881) and *Berlesiana denticulata* Evans, 1955 collected near Heidelberg (Germany) were also used.

*Scanning electron-microscopy (SEM):* Mites were transferred into 70 % ethanol. Some specimens were more or less cleaned using ultrasonication, others were left untreated. Specimens were dehydrated using graded ethanols (80 %, 90 %, 96 %, absolute ethanol) and transferred into amylacetate, where they were left for several hours. Subsequently the specimens were critical point dried with CO₂ using a Balzer’s critical point drier. The specimens were glued to Al-stubs using conductive silver or (mostly) double-sided conductive carbon tabs and sputter coated with gold-palladium. Scanning electron microscopy was performed with a Zeiss DSM 940A scanning electron microscope.

*Transmission electron microscopy (TEM):* The living mites were transferred into a drop of cold fixative (3.5 % buffered glutaraldehyde; phosphate or cacodylate buffer 0.1 M; pH 7.4) and transversely cut into halves. The specimens were immediately transferred into small vials containing the fixative and placed into a refrigerator for about two hours. They were then rinsed several times with the buffer solution and transferred for postfixation into 2 % buffered OsO₄ solution, where they were left for another two hours. Subsequently they were rinsed several times and finally dehydrated using graded ethanols (see above). Embedding occurred either in Araldite with propylenoxide as intermediate or in Spurr’s mixture (Spurr 1969). Sectioning was done with a Leica ultracut UCT microtome, whereby ultrathin sections were produced (70 nm) – and from time to time semithin sections (400 nm) – using a Diatome diamond knife. The ultrathin sections were stained with uranylacetate and lead citrate according to Reynolds (1963) and studied in a JEM-1011 (80 kV) JEOL-transmission electron microscope. The semithin sections after staining according to Richardson et al. (1960) served for general orientation using a light microscope (for further technical details see, e.g., Alberti & Nuzzaci 1996).
3. Results

Prey capture and feeding (VR)

*Epicrius schusteri*-mites move slowly with raised forelegs on the substratum (Fig. 1). The clubbed setae are directed forward-inward (mediad). The mite touches from time to time particles or other mites and Collembola evidently using these legs as feelers. Sometimes the mites also retract into small holes in the plaster of Paris bottom of the box. The epicriids evidently do not search in a directed manner for food. They just come in contact with materials by chance. Upon contact with a small isotomid springtail (e.g., *Folsomia* sp.) they rapidly touch the prey with the elongated clubbed setae and the collembolan adheres to these setae. The mite raises its forelegs with the attached collembolan. The springtail vividly tries to come free from the setae and may be successful when it gets in contact with the substratum. It then can pull itself off the adhesive setae. However, if this substrate contact has not been achieved, the mite will be the winner. The epicriid retracts the legs by flexing them in such a way that the victim is brought in the reach of the tiny mouthparts. These later hold the prey alone and the chelicerae squeeze the collembolan until it appears as a deformed particle made merely of cuticle, with the dissolved tissues of the collembolan transferred into the mite. The mite may walk around while feeding and may try to capture further prey. This report is based on two observations on only two specimens. In many other cases, nothing happened. However, since the behaviour of these two specimens was so characteristic and the feeding on the collembolan was evident, it seems that these epicriids are certainly predators. Probably, they are quite specialised since other potential prey, such as oribatid mites and other Collembola, in particular onychiurids, were not accepted.

Fig. 1 Figures taken from VR: *Epicrius schusteri* capturing and feeding on an isotomid springtail: a: The mite has just caught the springtail and leaves its shelter; b–e: The mite feeds on its prey while slowly walking around; f: While still feeding, the mite catches another springtail. Note that the collembolan is held with the adhesive clubbed setae only.
Fig. 2  LM of: a: *Epicrius schusteri*, overview. Note elongated forelegs; b: Tibia and tarsus of foreleg. Note clubbed setae (arrows point to two clubs, others are out of focus: and tip of tarsus with numerous setae of differing shape. An ambulacrum is lacking; c: *Epicrius canestrinii*, overview. Note tubercles of the cuticle; d: *Berlesiana denticulata*, overview. *Epicrius* species are easily distinguished from *Berlesiana* species due to their long dorsal setae. Scale bars: a, c, d: 200 μm, b: 50 μm.

Abbr.: Cx: coxa; Fe: femur; Ge: genu; Pu: pustule (= lateral protuberance); Ta: tarsus; Ti: tibia; Tr: trochanter.
Structural peculiarities of the forelegs (LM, SEM, TEM)

General overview:

The four species all are provided with elongated, minutely clubbed setae on the forelegs, which comprise six free segments: coxa, trochanter, femur, genu, tibia and tarsus. The forelegs are thinner than the other legs and distinctly elongated, with femur, tibia and tarsus being the longest segments. They lack an ambulacrum (Figs 1, 2, 3a, b).

The legs of the mite are covered with a secretion layer (cerotegument), which occurs also on the body (Figs 3a, c, d). Small pores were found on the anterior, dorsal border of the genu (Fig. 6a) and also the tibia representing openings of dermal glands similar to those occurring on the body.

Setae:

There are several types of setae on tarsus I to be distinguished based on LM, SEM and TEM (Figs 2b, 3c–k). Most conspicuous are the clubbed setae, to which the longest setae belong (macrosetae). *Epicrius schusteri* bears six clubbed setae on the tarsus and one on the tibia, *Epicrius mollis, Epicrius canestrinii*, and *Berlesiana denticulata* apparently have fewer clubbed setae (see also Evans 1955, Moraza 2005, 2005 [2006]). The club is a tiny knob which shows an irregular outline. It is very tiny in *B. denticulata* and may easily break off. These setae are basally set into a socket and thus may be slightly movable. They are rather straight and directed forward-inward (mediad) (Figs 1, 2b, 3a–d, j, k). An additional set of setae is shorter, slightly curved bowing to the leg surface. These setae are longitudinally divided into two halves. The external half has slight longitudinal ridges and is slightly broader than the part of the seta facing the leg surface (Fig. 3d, g, h). These setae are also set into sockets and thus slightly movable. A third group of setae of different length is represented by smooth and attenuating setae with a basal socket. The longest of these setae is directed straight forward from the tip of the tarsus, where also further similar setae are concentrated. One of these setae has a slightly spatulate tip (Figs 2b, 3a–c, e, f). The fourth type of setae-like structures is present close to the tip of the tarsus representing short sausage-shaped structures which are not set in a socket (Figs 3e, i).

Cross or oblique sections through the setal shafts revealed two populations of setae, which have solid shafts and two others which have shafts containing a lumen. The solid setae represent on the one hand the short curved setae as is evident from their peculiar shape (there frequently appears a lucent core in the section, which certainly is an artifact, however) (Fig. 4a). They are often covered by a homogeneous secretion into which alien material (e.g., bacteria) is embedded. On the other hand there are setae which are of a perfect circular outline (Fig. 4b).

The sausage-shaped setae-like structures are provided with a rather thin cuticular wall penetrated by many pores. The lumen of these setae contains a dense material which obscures further details (Fig. 4d). The other setae containing a lumen have no wall pores and the cuticular wall is much thicker than in the former type. The lumen is divided into two compartments. The setal lumen contains a thin tube bordered by a dense wall which is surrounded by granular material (and the cuticular wall) (Fig. 4c). These sections may belong to either the attenuate setae or to the clubbed setae since these sections are frequently surrounded by the granular secretion which is characteristic of the clubs. At present a definite discrimination of these setae was not possible with regard to the sections, however.
In a few cases it was possible to observe setal bases sectioned longitudinally into which a dendrite and an extension of the receptor lymph cavity entered (Fig. 4e). These bases are considered to represent the bases of the setae just mentioned, i.e. the bases of the clubbed or attenuate setae. At least some of these setae are innervated by three dendrites (Figs 4e, f). The terminal clubs are conspicuous because of their numerous spines, which are embedded into a secretion which is granular (not homogeneous as the cerotegument secretion). Evidently, the setal shaft splits into few main branches from which many short spines arise. The spines are solid structures containing a dense core at best (which might be lost artificially during sectioning). There are no pores (Fig. 5).

Because of the frequent occurrence of tubular bodies, apparently always in the number of two, close to the sockets, it seems likely that all the socketed setae are provided with tubular bodies (Figs 4g, h).

Dermal Glands:

The glands in the forelegs consist of a gland body (vesicle) provided by cell (s; the number could not yet be determined) containing large vacuoles filled with secretion (Fig. 6). The contents of these vacuoles are delivered apically by merokrine extrusion into a small reservoir (also called duct) formed by a ring of few cells (at least two) bearing many, long microvilli and containing many microtubules (Figs 6e, g). The border between the basal, secretory cell(s) and these reservoir cells folds sharply back on itself and is here provided with long dense cell junctions (likely septate junctions) (Figs 6b, f). More distally a further ring of cells (at least two) follows which is bearing a peculiar cuticular structure composed of an electron-lucent material and a dense funnel-like component (close to the lumen), the so-called calyx (Figs 6b, d, e, g). The narrow opening of this calyx-funnel points distally. It opens into a disc-shaped lumen which continues into a short evacuating canal. Both these latter structures are provided with a thin cuticular-lining, which is regularly varying in thickness like a trachea and hence seems to be flexible (Figs 6c, e). This canal leads to the surface, where it opens into a small depression of the leg cuticle. The opening represents a round pore (also called solenostome) surrounded by a cuticular ring located close to the anterior, dorsal border of the genu or tibia, respectively (Figs 6a, c). The secretion within the evacuating canal is homogeneous as is the secretion covering the body and legs as a cerotegument (compare Figs 4a and 6c). It can contain alien structures, e.g. bacteria, which stick to it.
4. Discussion

The observations on the living mites evidently have revealed that these mites feed on small soft-bodied arthropods, e.g. small isotomid Collembola. Although only few observations on the feeding activities were possible, the behaviour is so characteristic, that no doubts on the predatory behaviour remain. It may be, that the epicriids are even specialised on a certain type of prey. Because the behaviour has been seen so rarely, this rarity may indicate that the offered prey was often not acceptable to the mite. In the present study, the mites were brought together with small Collembola and some other mites from the same sample. Oribatid mites and onychiurid Collembola were touched but not accepted, as occurs with many other potential springtail-feeders. Onychiurids are provided with defence structures, so-called pseudocelli from which a secretion is extruded upon attack (Dunger 1983, 2003). It is likely that the mite can recognise the unpalatability of these Collembola and the hardness of oribatid mites encountered through its sensilla on the forelegs. These comprise without doubt mechanosensitive sensilla as is shown by the presence of tubular bodies (see, e.g., Alberti & Coons 1999, Coons & Alberti 1999). Furthermore, there are wall pore sensilla close to the tip of the legs, which likely represent olfactory sensilla. Unfortunately, it could not be detected whether there are also terminal pore sensilla present. However, this seems possible because quite a lot of setae provided with tubular bodies and dendrites entering their shafts were found also close to the tip of the legs. The clubbed setae are also very likely mechanosensitive and may also contain a dendritic process. However, it was not possible to reveal the full structure of these sensilla due to lack of material. From the observations of the living mites it is evident that the clubbed setae are adhesive structures. (The peculiar shape of the club in SEM is likely due to a shrinkage effect of the secretion during dehydration.) The prey can be held by the mite when only adhering to the clubbed setae. Evidently, the adhesive effect is achieved by the granular secretion and the many tiny spines at the tip of the seta. Unfortunately, the origin of the secretion could not be clarified. It may reach the tip of the seta through the hollow shaft, which probably opens apically when the seta branches to form the spiny club. However, this could not be demonstrated with certainty. Also, the typical glands which were found in the genu and tibia were not seen to be in contact with the clubbed setae. These glands are of a structure characteristic of anactinotrichid mites with vesicle/body of gland, duct/reservoir,
calyx and evacuating canal (Alberti & Coons 1999, Coons & Alberti 1999, Alberti 2006) and provide a secretion which is evidently different from that of the clubbed setae. It is obvious that the secretion of these glands contributes to the secretion layer (cerotegument). Glands, likely of the same type, have been described by Moraza (2005 [2006]) also from the coxae of legs I in Epicriidae and on tarsi of other legs. They regularly occur on coxae I in Gamasida (Moraza pers. comm.; see also Alberti et al. 1996, Alberti and Coons 1999 with respect to other Gamasida). They were not yet seen on the tarsi of the forelegs.

Perhaps one could speculate, that the clubbed setae may have evolved from terminal pore sensilla, in which the chemosensitive apparatus has changed its function. A somewhat comparable case has occurred in the very distantly related spider mites (Tetranychidae, Actinotrichida), where in those species capable of spinning a 'hollow' seta (so-called eupathidium su), which represents a spigot connected to a large, unicellular gland, replaces a normal eupathidium (likely a contact chemoreceptor) at the tip of each pedipalp (Alberti & Storch 1974, Alberti & Coons 1999, Lindquist et al. 2009a). However, in these mites which use their spinning capacity to provide a shelter under the leaves where they live, no innervation seems to be present in these modified setae. On the other hand, in snout mites (Bdellidae, Actinotrichida), which use a secretion to fix their prey to the substrate, the secretion comes from prosomal glands and is extruded from the mouth (Alberti 1973, Alberti & Storch 1973, Alberti & Ehrnsberger 1977; see also Krantz 2009).

The collembolan can free itself when it gets contact to the surface, so that it can pull itself apart. To avoid this, the mite raises its legs immediately when contacting a prey. The mite then brings the prey in the reach of the chelicerae and starts to feed on it. Since the mite was seen frequently entering small depressions in the substrate or was walking around only slowly with its forelegs raised and probing around, it might be that the mites are randomly searching their prey or even may sit in ambush waiting for a contact by chance.

The observations presented here provide a remarkable convergence with the numerous clubbed or clavate setae on the pedipalps (!) of Nemastomatidae (Opiliones), which are likely also used as adhesive structures for the capture of small arthropods (Wachmann 1970). The setae of these harvestmen have been studied by Wachmann (1970) and the author could show that they represent structures of much higher complexity than those of the epicriids. But these setae also contain dendrites as well as (a system of) canals leading to the apical club. As here with the mites, the author could not definitely demonstrate the origin of the secretion.
Fig. 6 Dermal glands on genu and tibia I.; a: SEM of genu I of *Epicrius mollis*. Note pore (solenostome) of gland. Note short, curved setae. A similar opening was found at the anterior border of tibia I. Scale bar: 5 μm. b–g: TEM figures of dermal glands of *E. schusteri* on genu and tibia of tarsus I.; b: Overview of gland showing some of the main components. Note secretion vacuoles with the cells of the glandular body (vesicle). Arrows indicate transition between glandular body (vesicle) and reservoir (compare Fig. 6f). Arrowhead points to two dendrites of a sensillum. Scale bar: 5 μm; c: Gland pore (solenostome) and evacuating duct...
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6. References


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