2009. The Journal of Arachnology 37:292–298

Palpal urticating hairs in the tarantula *Ephebopus*: fine structure and mechanism of release

Rainer Foelix, **Bastian Rast** and **Bruno Erb**: Neue Kantonsschule Aarau, Biology Department, Electron Microscopy Unit, Zelgli, CH-5000 Aarau, Switzerland. E-mail: rainer.foelix@hotmail.de

Abstract. The tarantula genus *Ephebopus* is exceptional with respect to its urticating hairs: they are located on the palps rather than on the abdomen, as is the rule for other Neotropical tarantulas. These urticating hairs occupy a small field of 1–2 mm^2 on the medial side of the palpal femora. Each urticating hair measures 500–600 µm in length and 5–6 µm in diameter. Almost the entire hair shaft is studded with little barbs that point toward the hair tip. Urticating hairs arise from a slipper-shaped socket in the cuticle, at an angle of 25–30°. When the spider is threatened, it shows a brief palpal flick as a defensive reaction, whereby many urticating hairs are brushed off and fly through the air. These hairs do not have a preformed breaking point but become detached at the very base and are then pulled out from their sockets, like an arrow from a quiver. The actual release behavior occurs too quickly (0.1 s) to be followed by the naked eye. Video film analyses reveal that a single upward movement of the palps rubbing against the lateral surfaces of the spread chelicerae causes the dispersal of urticating hairs into the air.

Keywords: Morphology, defensive behavior, Neotropical tarantulas

Many Neotropical tarantulas defend themselves by brushing off special urticating hairs from their bodies (Bates 1863; Bertani & Marques 1995/96). When these hairs come in contact with the skin, eyes, or respiratory tract of a threatening animal, they can cause serious irritations (Cooke et al. 1972) or allergic reactions (Castro et al. 1995). Commonly, urticating hairs are located on the abdomen and are brushed off with the hind legs. However, there is one exception in the genus *Ephebopus*, a colorful South American tarantula, where the urticating hairs occur on the palps rather than on the abdomen (Raven 1985). These hairs form distinct fields, so-called "pedipalpal brushes" on the medial surfaces of the femora; the hairs are released into the air by a flick of the palps. Marshall & Uetz (1990) provided a first description of the morphology of the urticating hairs in *Ephebobus*, their release after provocation, and their effect on laboratory mice. However, several points remained unclear: 1) structure, size, and total number of urticating hairs, 2) attachment and detachment of these hairs from their sockets, and 3) details about the actual release mechanism during the defensive reaction. We addressed the morphological questions using light and scanning electronmicroscopical techniques. For analyzing which body parts were involved in releasing the urticating hairs, we performed frame-by-frame analysis on several video recordings of defensive reactions.

METHODS

Exuvia of subadult *Ephebopus cyanognathus* West & Marshall 2000 were used for all microscopical studies. Isolated urticating hairs were inspected and measured under the light microscope. Entire fields of urticating hairs were excised and embedded in Epon; 1–2 µm sections were stained with methylene blue and examined in a phase contrast microscope. For scanning electron microscopy palpal brushes were sputtered with gold and viewed from various directions in a Zeiss DSM 950. Digital photographs were taken at magnifications from 20× to 5000×. For a more three-dimensional representation of the attachment sites, we cut several palpal brushes into longitudinal strips with a razor blade and then mounted them sideways before examination in the scanning electron microscope. For comparative purposes we also studied nearby sensory hairs.

In order to observe the defensive reaction, a transparent glass vial was moved toward the spider from in front. Slightly touching the front legs often triggered a palpal flick and a concomitant release of urticating hairs. Since this happened very fast, we used a video camera (Sony DCR PC107E) with 25–30 pictures/s and frame-by-frame analysis. Overall we elicited about 20 defensive reactions and captured three of them on video film.

All six *Ephebopus cyanognathus* spiders used in this study were bred in captivity by one of the authors (BR). They were 18 mo old, subadult, and still about two molts away from maturity. With a body length of 3 cm they were slightly smaller than the adults.

RESULTS

The palpal brushes are located on the medial sides of the femora, rather distally and near the patellar joint. They occupy a patch of 1–2 mm^2 that is easily visible with the naked eye (Fig. 1). The term "pedipalpal brush" is quite descriptive since the hairs are tightly packed and run parallel to each other, like a paint brush (Fig. 2). In contrast to the surrounding sensory hairs, which are dark brown or black, and the ornamental hairs, which are deep blue, the urticating hairs have a golden-reddish color. Their orientation is almost parallel to the axis of the femur, but slightly deflected ventrally. A single urticating hair is 500–600 µm long but only 5–6 µm thick. The ratio of length/width is thus around 100:1, giving the hair a needle-like appearance. As is typical for urticating hairs, the hair shaft bears numerous pointed barbs, each about 5 µm long (Figs. 2, 5). They are lacking at the very base, but appear as small cuticular extensions, just after the hair comes out of its socket (Fig. 8). The barbs are spaced rather regularly at an interval of 10 µm along the entire hair shaft, which also bears fine longitudinal ridges. The distal end of the urticating hair is acutely pointed, suggesting that it represents the penetrating tip. However, since all the barbs are pointing distally, this would quickly prevent any further penetration into the skin (see Discussion).

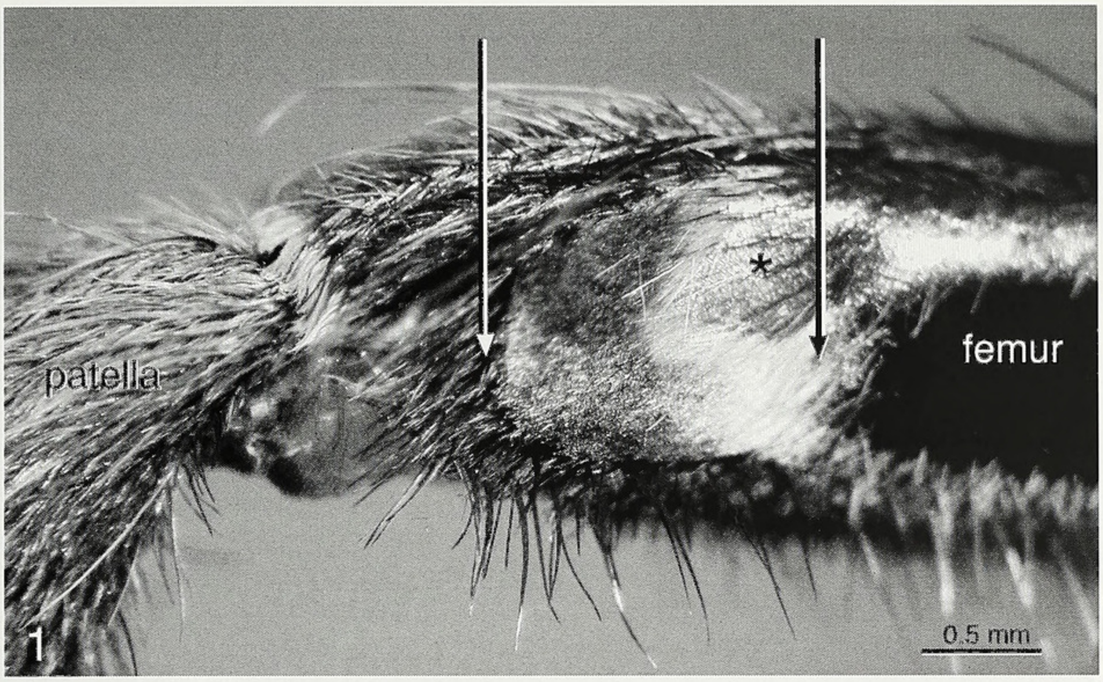

Figure 1.—Medial surface of the femur of *Ephebopus cyanognathus*, near the patella joint. The two arrows mark the location of the "pedipalpal brush," a region that consists of thousands of urticating hairs. A small area (asterisk) is devoid of such hairs, because they were brushed out during a defensive reaction.

At the proximal end the hair shaft becomes very smooth and decreases in diameter from 5 to 2–3 µm. It also exhibits a slight bend where it disappears into the socket (Figs. 4, 8). The angle at which the hair shaft emerges from the socket lies between 25 and 30°, which means that the urticating hairs are lying rather flat on the surface of the femur.

The sockets are slipper-shaped, about 10 µm long and 5–6 µm wide, rising 3–4 µm above the leg cuticle (Figs. 3, 4, 7). The

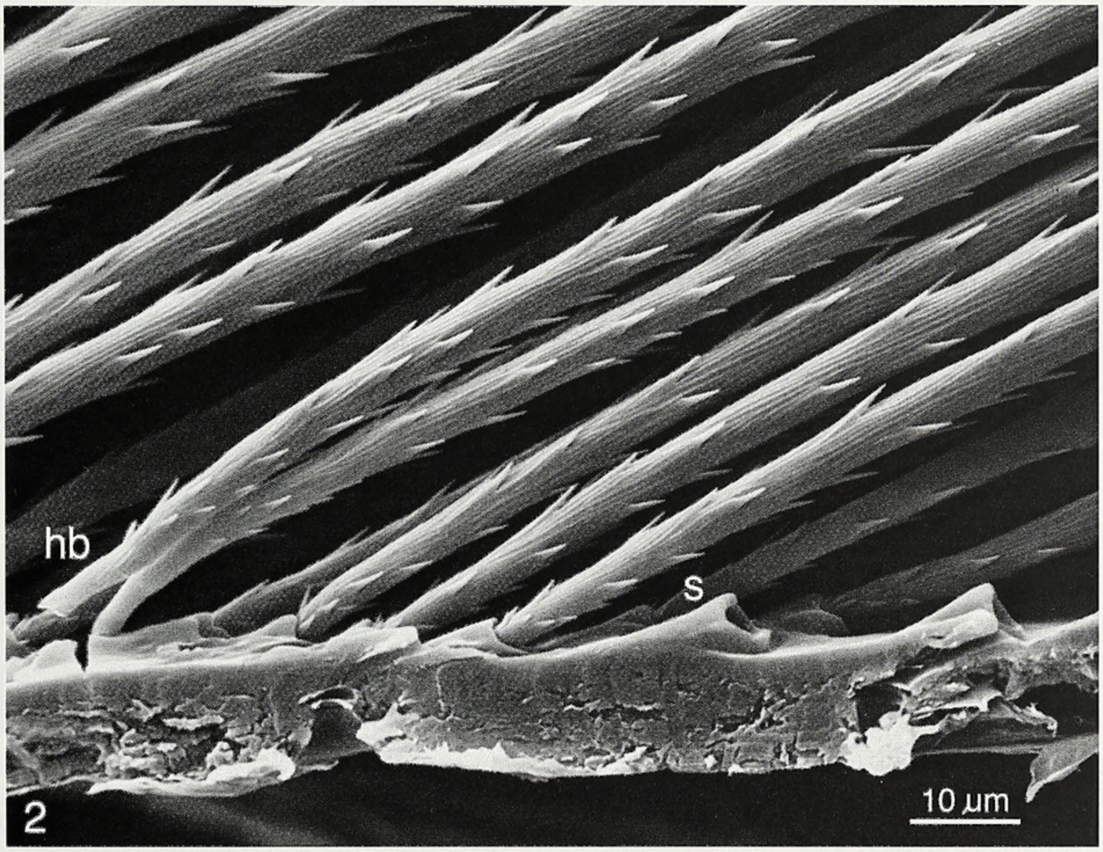

Figure 2.—The pedipalpal brush cut longitudinally: the barbed urticating hairs are seen from the side. Each hair arises from a slipper-shaped socket (s) at an angle of 25–30°. Some urticating hairs have become detached from their sockets, and their free hair base (hb) is visible on the left.

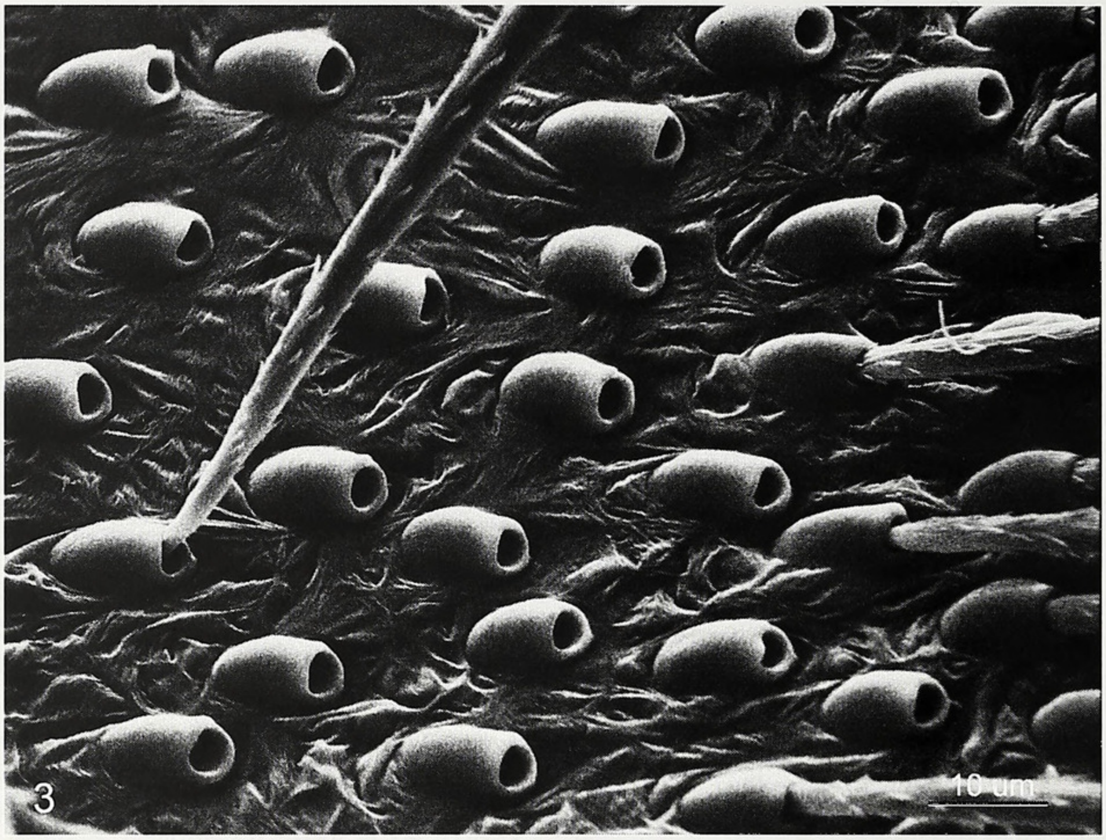

Figure 3.—A marginal region of the pedipalpal brush showing mostly empty sockets, because the urticating hairs had been brushed off in a defensive reaction. A few hairs (on the right) are still held within their sockets, but the single hair on the left has been pulled out partially. Note that there is no breaking point at the hair base.

opening for the hair shaft measures 3–4 µm, allowing a bit more movement vertically than horizontally. The basal part of the hair shaft fits snugly into its socket and ends at a basal ring (Figs. 9, 10). Below that hollow, circular structure a canal of about 5 µm in diameter traverses the cuticle vertically (Figs. 4, 7, 9).

How is the urticating hair attached to its socket? It appears that only the most proximal rim of the hair base is connected to the basal ring, via a thin cuticular membrane. This membrane can be seen in its original position in Fig. 9, and remnants are often found attached to isolated hairs at the very

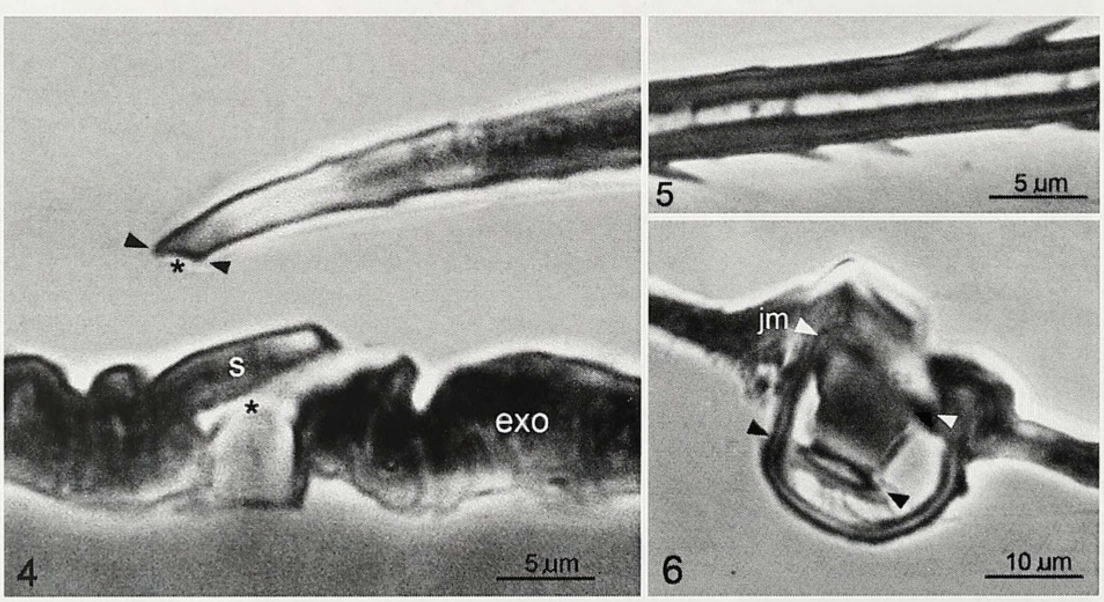

Figure 4.—Longitudinal section of a socket (s) and the detached base (arrowheads) of an urticating hair. The two asterisks mark the original position of the hair base inside the socket. The exocuticle (exo) of an exuvium is only 5–10 µm thick.

Figure 5.—For most of its length, the hair shaft is barbed on the outside and hollow inside. The central lumen is only 1–2 µm wide and exhibits small struts crossing the lumen.

Figure 6.—Section of the base of a large sensory hair showing fine membranous connections to the socket (black arrowheads). A much stronger joint membrane (jm) is responsible for the firm attachment of the hair shaft to the socket (white arrowheads).

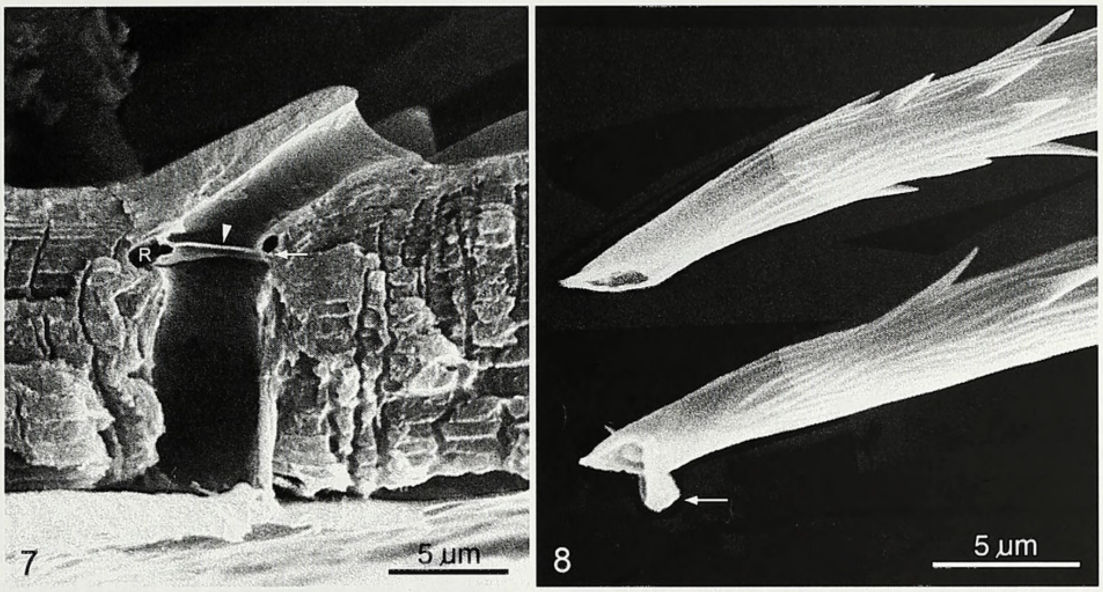

Figure 7.—A sagittal section of an empty socket of an urticating hair. The oblique upper part of the canal, which normally houses the base of the hair shaft, ends in a horizontally running membrane (arrowhead). That is the spot where the hair base is attached laterally to a ring-like structure (R); it appears as two small circles, because the ring is seen in cross-section (arrow). Further below, the canal continues in a vertical direction through the exocuticle.

Figure 8.—Two hair bases of detached urticating hairs. Note the obliquely pointed endings reminiscent of a hypodermic needle. The part of the hair shaft that is normally concealed within the socket has been shaded. Fragments of the attachment membrane (arrow) often still adhere to the hair base.

base (Fig. 8). The hair shaft seems to be hollow and thus very fragile at the attachment site; it quickly becomes more solid distally, with a wall thickness of 2 µm and a central lumen of 1 µm (Fig. 5). The delicate connection via a membrane is probably the key factor when the hair becomes dislodged from its socket. A corresponding membrane connecting the hair base to the socket is also present in the large sensory hairs (Fig. 6). However, sensory hairs have an additional and much stronger membrane, the joint membrane, that anchors the hair shaft movably yet firmly in the socket.

It is noteworthy that we never found broken urticating hairs. Any released urticating hair (detached either naturally by the spider or artificially with a needle) remains in one piece. We found no stumps of broken hair shafts inside the socket. An exception is pictured in Fig. 9, but there the hair shaft had been crushed by the razor blade when we cut the cuticle.

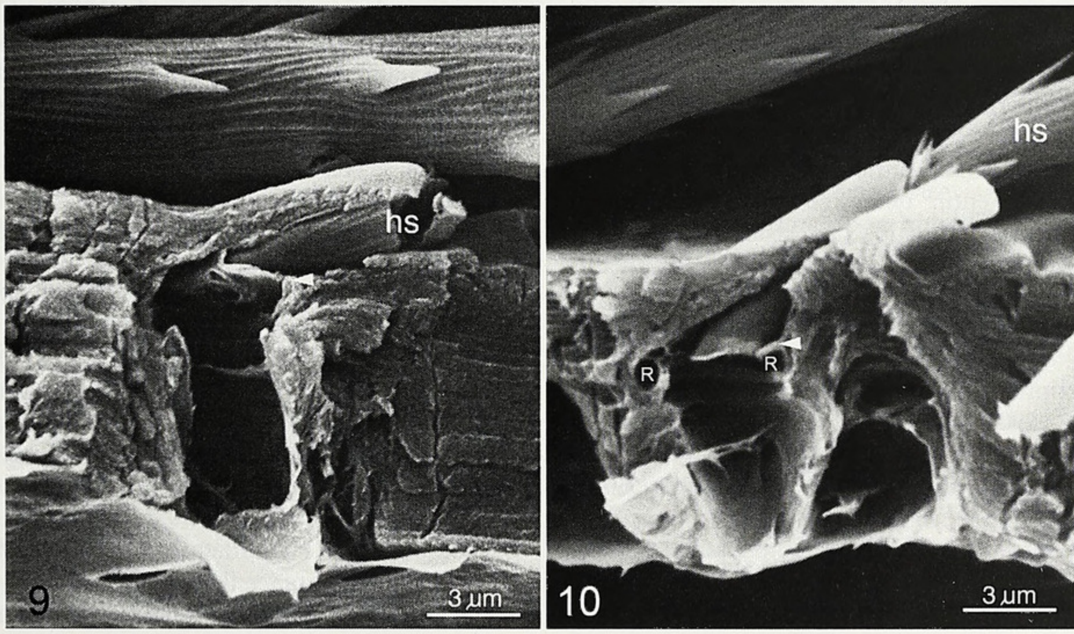

Figure 9.—Parasagittal section of the attachment of an urticating hair. In this case the hair shaft (hs) had been broken inadvertently when the cuticle was cut with a razor blade. However, the attachment membrane (arrowhead) at the base of the socket is clearly visible. Note the narrow diameter of the hair shaft inside the socket (2–3 µm), compared to a more distal portion (top of figure; 5–6 µm).

Figure 10.—A similar parasagittal section, but with the hair shaft (hs) still intact. The arrowhead marks the ending of the hair base and the surrounding ring-like structure (R; *cf.* Fig. 7).

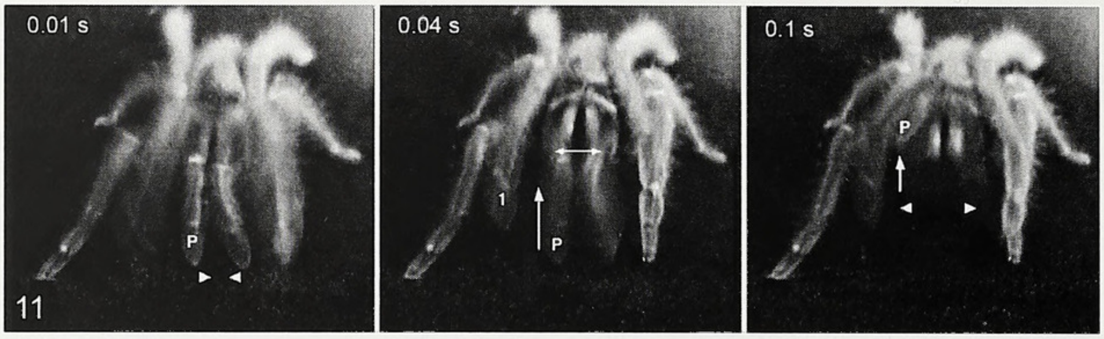

Figure 11.—Three consecutive still pictures from a video film of the defensive reaction in *Ephebopus cyanognathus*. Left: Immediately following a mechanical stimulus, both palps (P) are held close together (arrowheads) and are touching the substrate. Middle: Only 40 milliseconds later, the first legs (1) and the palps (P) are rapidly moved upward (vertical arrow); at the same time, the chelicerae are spread apart (horizontal arrows), thus exerting more friction upon the pedipalpal brushes on the femora. Right: The palps are now in an Up-Position and kept much farther apart (arrow heads). It is at this point that a small puff of urticating hairs is released.

In order to determine how many urticating hairs there are within a palpal brush, it is best to examine areas where the urticating hairs have been brushed off and only the empty sockets remain. The sockets are rather evenly spaced at a distance of about 20 µm, a bit closer in the vertical direction than in the horizontal (Fig. 3). On scanning electron micrographs we counted 55 sockets on an area of 0.01 mm^2, which corresponds to a density of 5500 urticating hairs/mm^2. Since the entire palpal brush measures around 1.5 mm^2, the total number of urticating hairs on one palp is about 8250.

How does *Ephebopus* actually release the urticating hairs from its palpal brushes? Is it an interaction between the two palps or between the palps and chelicerae? Since the defensive reaction (palpal flicking) happens so fast, we used a video camera and analyzed the movements of the involved body parts in consecutive frames.

At the onset of the defensive reaction, the spider holds both palps close together, touching the substrate (Fig. 11, left). About 30–40 ms later the first legs and the palps are rapidly thrust upward and at the same time the chelicerae are spread sideways (Fig. 11, middle). This apparently creates friction between the inside of the femora (pedipalpal brush) and the outside of the chelicerae. After 100 ms the palps are seen in the Up-position and are held much farther apart than at the beginning (Fig. 11, right). It is at this moment that a little puff of urticating hair is released into the air. The entire defensive reaction consists of only one upward stroke of the palps that lasts approximately 0.1 s. Most of the time, the spiders are reluctant to repeat this behavior and instead tend to flee.

DISCUSSION

The previous descriptions of the urticating hairs in *Ephebopus* sp. (Marshall & Uetz 1990; West et al. 2008) were very brief and did not give any dimensions. We found that most hairs are 500–600 µm long but only 5–6 µm in diameter; thus the shape corresponds more to a long knitting needle, which is in contrast to the description "*short and stout*" given by Marshall & Uetz (1990). Only the rather long and thin urticating hairs, with a length to diameter ratio of 100:1 or 200:1, are considered to float through the air (Bertani & Marquez 1995/96).

Barbs along the urticating hair shaft pointing distally have been discussed with regard to other tarantulas, and it was argued that the acute distal end could not be the penetrating tip (Bertani et al. 2003; Pérez-Miles 1998). This makes sense, since the barbs would quickly get stuck in the skin if pushed in the wrong direction. The basal end of the hair is less pointed but very thin (2 µm) and squared off obliquely like a hypodermic needle. One could well imagine that the basal end functions as the penetrating tip, yet experimental proof is lacking so far.

Urticating hairs in tarantulas were classified into four different types by Cooke et al. (1972), based on morphological differences. Marshall and Uetz (1990) added a "fifth type" for *Ephebopus*, but did not describe the typical features. Our definition of type V urticating hairs would read as follows: Straight hairs of 0.5 mm length and 5 µm diameter, slightly bent at the base (socket region), fine barbs (0.5 µm) along the entire hair shaft pointing toward the distal end. Overall, type V hairs are similar to type II urticating hairs, except for the distinct basal stalk of the latter.

Based on the density of empty sockets we calculated about 5500 urticating hairs for 1 mm^2, or around 8250 hairs for one palpal brush. This seems a modest number when compared to over 10,000 hairs/mm^2, as was claimed for abdominal urticating hairs (Cooke et al. 1972). Unfortunately, the authors did not provide any measurements for the diameter of those hairs (type I), but deducing from their micrographs, it should be around 7 µm. In order to achieve that high density the hair sockets would have to be spaced at 10 µm or less. Since this leaves almost no space between the hair shafts, it would mean a veritable "solid forest" of urticating hairs. In *Ephebopus*, sockets are spaced at 20 µm, which itself makes for a very dense packing (Fig. 3).

It was surprising to find only small denuded areas (with empty sockets) within the palpal brushes of the exuvia. It could be that these spiders had hardly ever defended themselves, or that they shed only a very limited amount of urticating hairs during one palpal flick. It seems necessary, of course, that *Ephebopus* be thrifty with its urticating hairs, since they can grow back only between molts. In those animals filmed repeatedly for their defensive reaction, the palpal brushes showed large denuded patches. We estimated that those spiders had reacted with a palpal flick approximately 5–10 times. In one of these animals we found almost 5000 empty sockets on the proximal side of each palpal brush. This would correspond to more than 500 urticating hairs released with a single palpal flick.

Another interesting aspect is that the palpal flick has to be a controlled action; otherwise all urticating hairs might get lost

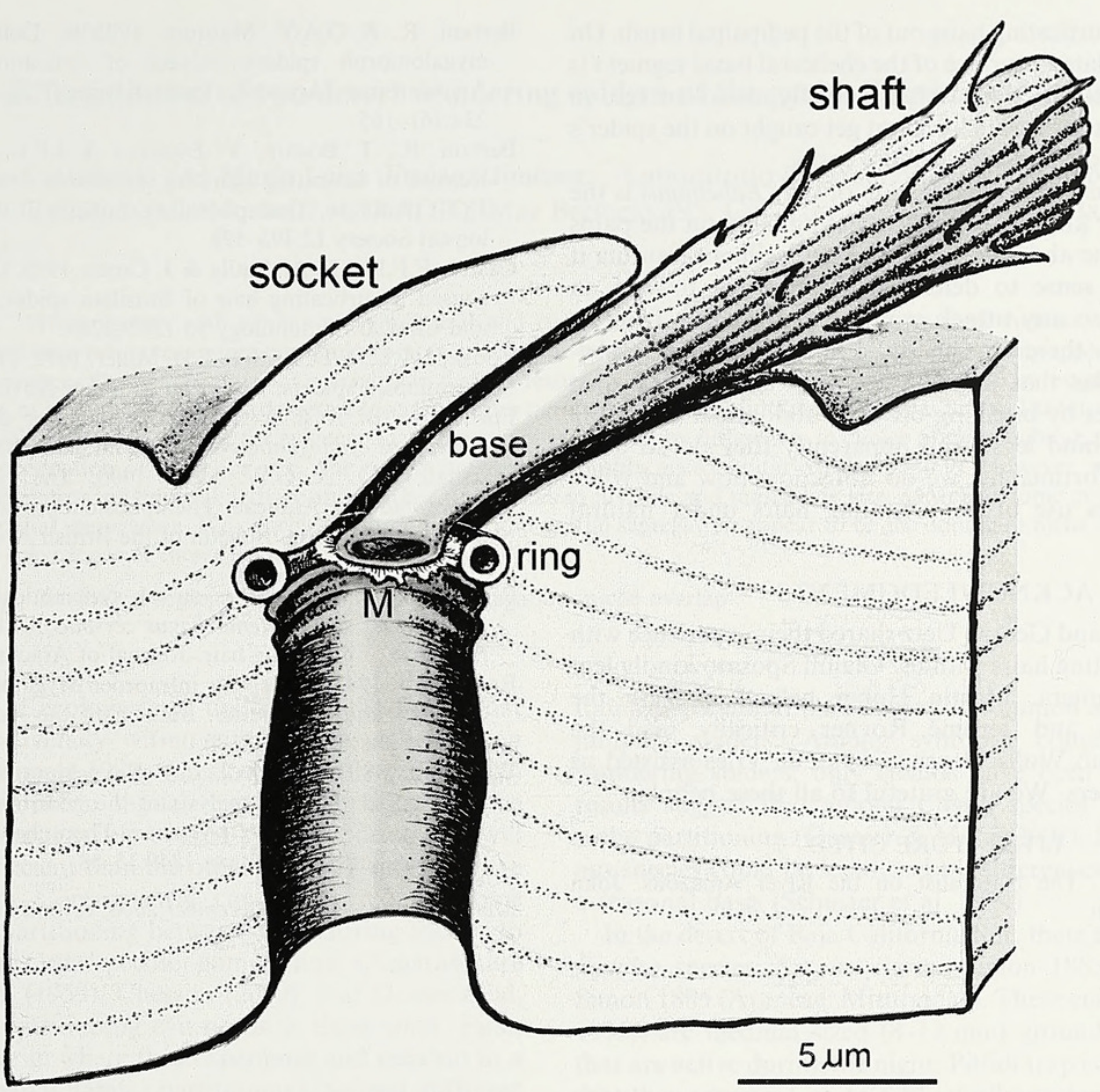

Figure 12.—Cut-away view of the attachment of an urticating hair in *Ephebopus cyanognathus*. The hair is only held by a thin membrane (M) between the hair base and a ring-like structure beneath the socket. This is the site of detachment when the urticating hair is released.

with one vigorous stroke. A mechanical feedback indicating the number of urticating hairs could be transmitted by sensory hairs that are interspersed among the palpal brush. These hairs can hardly be seen in an intact hair field, but stand out in denuded areas. In contrast to the urticating hairs, they remain in their sockets after a palpal flick.

The main questions of this study were: how are those urticating hairs attached, and how can they be detached? The hair shaft tapers down to 2–3 µm near the base and then disappears in a tightly fitting socket (Figs. 2, 3). Initially we assumed that any forcible movement of the hair against the rim of the socket would break the hair shaft right there and leave a little stump inside the socket (Foelix et al. 2009). However, this is not the case. All released hairs remain practically in one piece and there are no stumps in the sockets. So, how is the hair base actually connected inside the socket? We were expecting to get an answer from longitudinal sections of palpal brushes embedded in hard Epon. Unfortunately this failed because during sectioning with the microtome the embedding medium separates from the leg cuticle and practically all urticating hairs are torn out (Fig. 4). We were more successful by simply cutting strips out of the pedipalpal brushes with a razor blade and then inspecting those longitudinal sections under the scanning electron microscope. Again, most sockets were empty due to the manipulation with the razor blade, but if the sockets were only grazed by the blade, the hairs remained in place (Figs. 9, 10). In such cases it was clearly seen that the hair base is connected by a very thin cuticular membrane to a ring structure lying horizontally beneath the socket. This delicate attachment is apparently the only connection of the urticating hair to the socket (Fig. 12). In contrast, sensory hairs were found to be held by strong joint membranes in their sockets; additionally, they also have a fine connecting membrane at the very base of the hair (Fig. 6). It thus seems that it is the lack of a joint membrane that makes it easy for urticating hairs to become detached.

How does *Ephebopus* actually release its urticating hairs? The only description available states that spiders "*were observed to bring the pedipalps down across the basal segments of the chelicerae in a brief scrubbing motion*" (Marshall & Uetz 1990). Our analyses show that it is indeed a single motion; however, it seems to be the upstroke of the palps against the spread chelicerae that causes the release. The entire reaction lasts only 0.1 s.

It is also interesting that there is no obvious counterpart present on the chelicerae, such as marked ridges or a comb that would scrape the urticating hairs out of the pedipalpal brush. On the contrary, the lateral surface of the cheliceral basal segment is surprisingly smooth. Perhaps this is actually an advantage, so that the barbed little "spears" will not get caught on the spider's body but can fly off into the air more easily.

Finally, we must raise the question of why *Ephebopus* is the only genus so far known to have urticating hairs on the palps rather than on the abdomen. For a tube-dwelling tarantula it certainly makes sense to defend itself frontally toward an aggressor, because any attack would usually occur from in front. However, there are many other Neotropical tube-dwelling tarantulas that first have to turn around and then defend themselves by brushing off their abdominal urticating hairs with their hind legs. And apparently they do so quite successfully. Unfortunately, we do not know how and when *Ephebopus* makes use of its urticating hairs under natural conditions.

ACKNOWLEDGMENTS

Sam Marshall and George Uetz shared their experience with *Ephebopus* urticating hairs with us. Gianni Sposato kindly lent us his video camera. Martin Huber helped us with the literature search and Jerome Rovner critically read the manuscript. Benno Wullschleger and Jan de Vries assisted us in technical matters. We are grateful to all these people.

LITERATURE CITED

Bates, H.W. 1863. The Naturalist on the River Amazons. John Murray, London.

Bertani, R. & O.A.V. Marques. 1995/96. Defensive behaviors in mygalomorph spiders: release of urticating hairs by some Aviculariinae (Araneae, Theraphosidae). Zoologischer Anzeiger 234:161–165.

Bertani, R., T. Boston, Y. Evennou & J.P.L. Guadanucci. 2003. Release of urticating hairs by *Avicularia versicolor* (Walckenaer, 1837) (Araneae, Theraphosidae). Bulletin of the British Arachnological Society 12:395–398.

Castro, F.F.M., M.A. Antila & J. Croce. 1995. Occupational allergy caused by urticating hair of Brazilian spider. Journal of Allergy and Clinical Immunology 95:1282–1285.

Cooke, J.A.L., V.D. Roth & F.H. Miller. 1972. The urticating hairs of theraphosid spiders. American Museum Novitates 2498:1–43.

Foelix, R., B. Rast & B. Erb. 2009. Über die Brennhaare von *Ephebopus cyanognathus* West & Marshall 2000. Arachne 14:4–13.

Marshall, S.D. & G.W. Uetz. 1990. The pedipalpal brush of *Ephebopus* sp. (Araneae, Theraphosidae): evidence for a new site for urticating hairs. Bulletin of the British Arachnological Society 8:122–124.

Pérez-Miles, F. 1998. Notes on the systematics of the little known theraphosid spider *Hemirrhagus cervinus*, with a description of a new type of urticating hair. Journal of Arachnology 26:120–123.

Raven, R.J. 1985. The spider infraorder Mygalomorphae (Araneae): cladistics and systematics. Bulletin of the American Museum of Natural History 182:1–180.

West, R.C., S.D. Marshall, C.S. Fukushima & R. Bertani. 2008. Review and cladistic analysis of the neotropical tarantula genus *Ephebopus* Simon 1892 (Araneae, Theraphosidae) with notes on the Aviculariinae. Zootaxa 1849:35–58.

Manuscript received 28 December 2008, revised 20 May 2009.