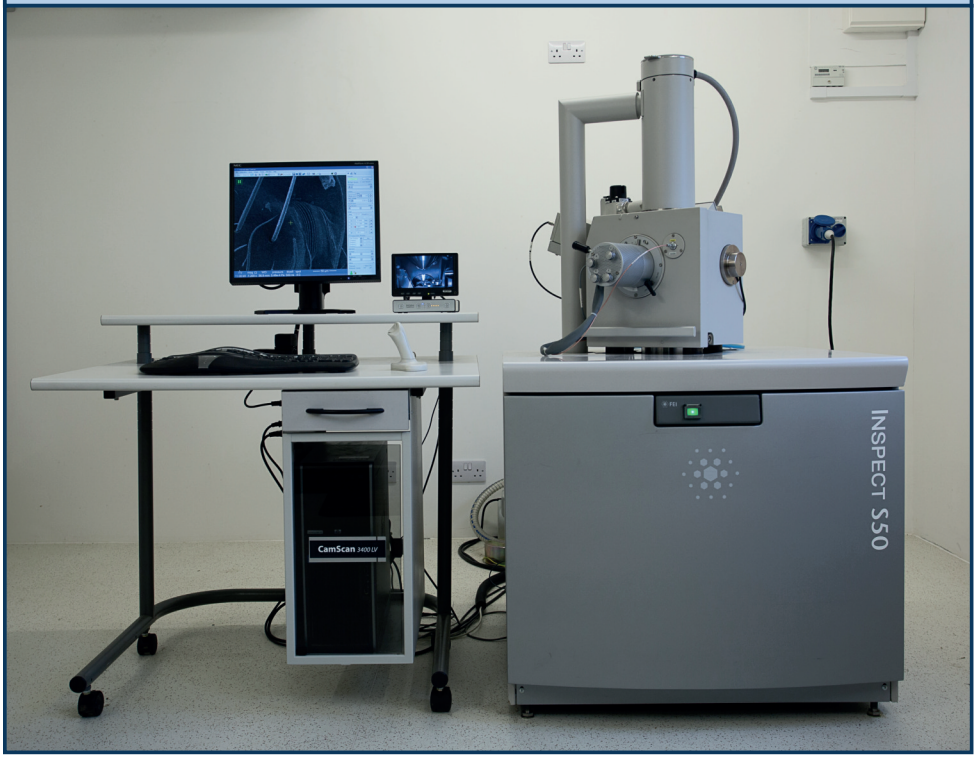
# *SEM Diaries - 4*

## *The Beast Arrives*

Jeremy Poole




My SEM up and running in my laboratory

Readers may well have detected a sense of frustration in Diaries - 3 at the length of time being taken to complete my "laboratory" building. Well, I am glad to say that the frustration has more or less evaporated, with the delivery of my SEM on the 27th January. All that I am waiting for now is for the man with a digger to level off the ground outside the door of the building so I can turf it over and lessen the quantity of mud being trodden inside.

I had intended to record the delivery process photographically, including the column console being wheeled in over boards laid on my lawn, steadied by a couple of labourers and supervised by one of the directors of Tron-Tech, the company

supplying the SEM. But, to be honest, in the excitement of the moment I totally forgot to get the camera out! Suffice it to say that the operation went smoothly and the wooden ramps I had prepared to help the SEM over the threshold and down into the lab survived under the 450 kg load imposed on them.

The SEM was lowered onto the floor in its final resting place and the labourers departed, leaving Don, the other director from Tron-Tech, to complete the installation and setting to work. This generally went smoothly, although my heart was in my mouth for a while as Don tried to locate and fix the cause of a vacuum fault.

Commissioning, together with some basic tuition on using the microscope, continued into the Thursday, when Don eventually left me with instructions to experiment with all possible combinations of the key parameters of high voltage, spot size and working distance. A major milestone had been reached.

Between the 28th January and the end of February, when I am writing this piece, I have made significant progress in gaining familiarity not only with the SEM but also with the critical point dryer (CPD) and sputter coater.

It is all very well having an impressive looking SEM sitting in my lab, but it is worthless unless I have samples to study using it. My intention in procuring this was to record images of as many species of British spider as I can lay my hands on and in particular of their male and female sex organs, which are the main identification clues when attempting to determine the spider to species level. The female organ, or epigyne, is located on the abdomen and is relatively easy to dissect out and mount, as it is effectively two-dimensional. The male organ, or pedipalp, on the other hand, may be viewed from all angles and is an intricate and often fascinating structure. For identification purposes the left palp should be viewed laterally, and compared with the sketches in the standard identification guide [1]. However, I wanted to be able to view it from all angles, at least all angles rotated about the longitudinal axis, so I had to put my mind to designing and building a suitable holder.

Whereas with light microscopy the specimen is almost always mounted on a glass slide, with scanning electron microscopy the specimen is normally mounted on a "stub". This is a disc of aluminium, of diameter ½ inch or greater and about 1/8th inch thick, with a spigot of 1/8th inch diameter sticking out from it for insertion into a mating hole on the specimen table. The specimen is affixed to the stub either by using a conducting glue directly onto the aluminium, or else onto a double-sided adhesive carbon disk (which is also conducting). However, using either of these mounting methods the orientation of the specimen is fixed at the time it is mounted on the stub and cannot be changed later. My plan was to make some stubs with an integral bracket through which a thin rod (of 3/8th inches diameter) could be inserted. The pedipalp would be mounted by gluing its shaft into a small hole in the end of the rod, and the rod would be rotated by means of a lever (broken drill) inserted in one of a pair of holes drilled in the opposite end of the rod.

Figure 2 shows the main part of my special palp stub. The idea of the slot into the side of the flange was to enable me to tighten the fit of the rod in the hole by using the remaining metal as a clamp, using a screw through a clearance hole drilled through the top half and a tapped hole in the bottom half, to adjust the clamping pressure. Having made a couple of these mounts I am now more likely to just drill and tap the top half of the flange and use the screw as a jacking screw to separate the two halves to enable me to insert the rod!

Fig. 2: Bracket type stub to take the pedipalp rod

I now had some palp holders, as well as conventional stubs, so next I needed some specimens, both palps and other body parts. Since I retain almost all the spiders I collect, finding specimens was not a problem. I just had to root around on my (three-dimensional) bench for tubes containing male spiders preserved in alcohol. I located a male *Tegenaria saeva*, one of the family of large spiders sometimes seen running across the carpet, causing panic among arachnophobes, and also a male *Larinioides cornutus*, a large Araneid. I dissected out the pedipalps from these, and also removed the heads, complete with chelicerae and fangs, spinners, and a few legs. These I plunged into acetone at -20 Celsius and left them overnight in the freezer compartment of my fridge-freezer in the lab.

The following morning I transferred these items from the jar of acetone to fine metal gauze containers supplied with my CPD (Figure 3). I topped the boat of the CPD up with more acetone, at room temperature, inserted the boat into the dryer and sealed the cylinder. I then started the drying cycle. The first operation is to flush out the acetone and replace it with liquid $CO_2$. Next the dryer is filled with liquid $CO_2$ and left to stand to allow the $CO_2$ to penetrate the specimens and displace any remaining acetone. The dryer is flushed again and the liquid level replenished to the top of the boat, as viewed through a thick glass window in the front. Next, warm water is passed through the jacket surrounding the pressure vessel of the CPD, and as the temperature rises through about 32 Celsius the meniscus indicating the level of the liquid $CO_2$ disappears as the "critical point" is reached. The pressure at this stage has risen to around 1100 psi. The temperature (and hence the pressure) are allowed to rise slightly more, to ensure that the critical point really has been reached, before the vent valve is slightly opened to allow the $CO_2$ slowly to escape as gas.


Fig. 3: The Critical Point Dryer, on left, with its boat and mesh basket on the bench in front. The instrument on the right is for circulating warm water round the water jacket of the dryer.

The point behind this long and potentially dangerous operation is to avoid surface tension of either water or a dehydration liquid deforming the specimen as the liquid evaporates from its surface, as was mentioned in Diaries - 1. The overall result was a selection of bone dry and rather brittle body parts of the two spiders (plus a few *Varroa* mites I put through the

process at the same time). The legs and heads were mounted on conventional stubs using double sided sticky discs. The pedipalps were glued into holes drilled into the ends of the 3/8th inch rods for the special mounts - but not without difficulty.

The "only" remaining operation required prior to putting the mounted specimens in the chamber of the SEM was to coat them with a thin layer (up to 5 nm) of gold, which was carried out in the sputter coater. The palps were rotated 180˚ in their holders and re-coated to ensure that there was a gold coating in the direction of the electron beam whatever the eventual orientation of the palp in its holder.

Figure 4 is a top view of one of my prototype mounts with a pedipalp mounted in the rod. The two orthogonal holes used for rotating the specimen can be seen on the right. The hole through the top of the bracket was intended for a clamping screw, but was not found to be required. The pedipalp glued to the horizontal rod is of the *Tegenaria.* It was a challenge to mount the palp in the hole I drilled into the middle of that end of the horizontal rod. It will be considerably more of a challenge when I attempt to mount pedipalps from smaller species, such as the money spiders! Alternative methods are under consideration, and it may well be that I have at last found a use for a micro-manipulator I bought at a Penkridge meeting several years ago.

Fig. 4: Complete "pedipalp" stub, viewed from above

Figure 5 shows a selection of stub types, mounted onto the revolving stage within the specimen chamber of the SEM. These are standard ½ and 1 inch stubs with specimens mounted on carbon pads, and a pair of my special pedipalp stubs. All four stubs, and their specimens, have been sputter coated with gold.

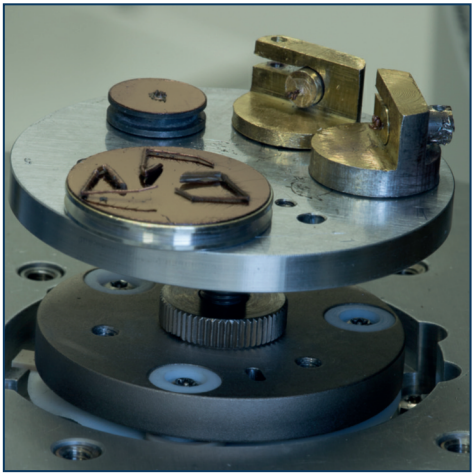


Fig. 5: Selection of stubs mounted on the revolving stage of the SEM

A few of the resulting micrographs from this exercise, are shown on the next page. Further micrographs, together with pictures of the interior of my laboratory can be found on a website I have created for this purpose [2].

## References


1. Roberts, M. J., Spiders of Britain & Northern Europe. Collins Field Guide
2. www.jeremypoolesem.org.uk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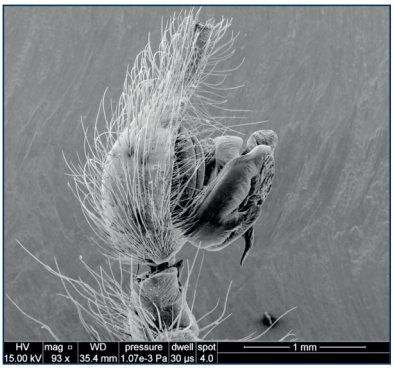
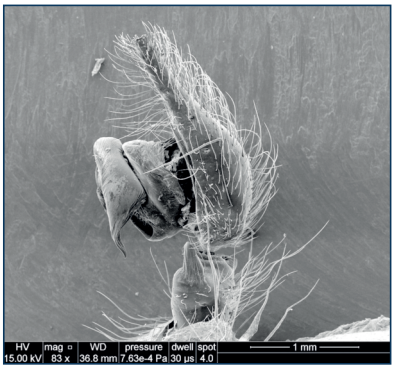
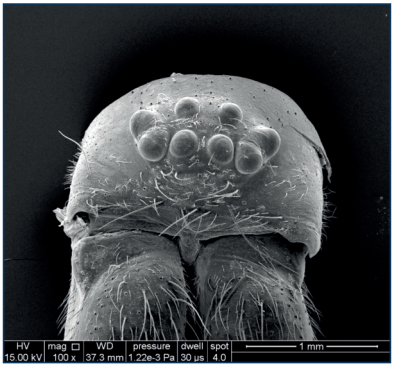


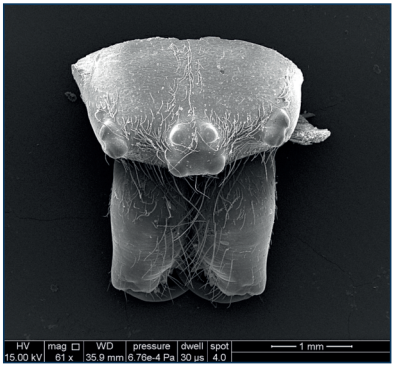

Lateral view of pedipalp of *Tegenaria saeva*



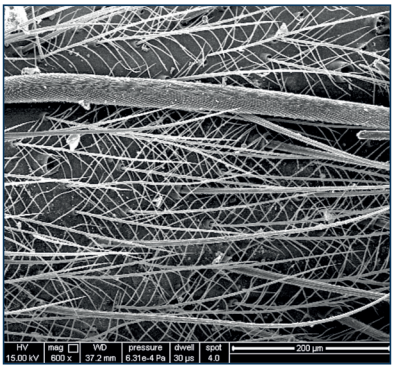

Opposing lateral view of same pedipalp of *Tegenaria saeva* (rotated in mount)



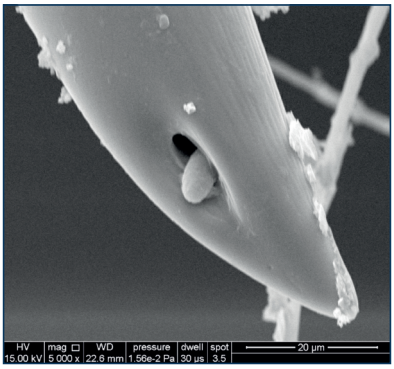

Head of *Larinioides cornutus,* showing how the eight eyes of the Araneidae are grouped




Head of *Tegenaria saeva,* showing the very different arrangement of the eyes in the Agelenidae.




Hairs on the leg of *Tegenaria saeva*, showing different types of hair for different purposes




Detail of fang of *Zygiella x-notata*, showing the opening through which the spider injects its venom