SEM Diaries - 25

"It takes two to tango"

Jeremy Poole

Fig. 1: Tarsus of *Osornolobus sp. showing tarsal organ (mid left) and tarsal claw (right)*

Last time I was commenting on how working alone all the time has its drawbacks, from the point of view of exchanging information and learning new tricks (such as techniques or protocols for processing samples). Well, almost serendipitously, over the last period I have not only been working with two friends on different projects, but one of them has been teaching me new tricks!

Most readers of Balsam Post will realise that my "specialist subject" is arachnology, with an interest in collecting, identifying and imaging key identification features of British spiders. You will also know that I am trying to build up a database of these images and making them available via a website [1]. As a member of the British Arachnological Society (BAS) I have brought the website to the attention of other members at meetings and also through an article in their Newsletter.

I was still surprised, but also glad, when I was approached by a committee member of the BAS who asked for my help to produce electron micrographs of some interesting features of a (non-British) spider species he was working on. Specimens of this species were in short

supply, and the spider itself is quite small, so when the single male and female specimens arrived I had to ramp up my processes a bit to take account of their size and scarcity, and also determined that I should take extra care in identification of images.

In particular, my normal procedure when dissecting spiders is to chop off all their legs and put them in a single "basket" for taking them through different strengths of alcohol to dehydrate them. At the end of that process I would mount a number of legs on a single SEM stub and search out the best example to image and use for my website. Keeping track of each individual leg was always too much effort for, normally, little benefit.

With these spiders I determined that I should treat each leg individually. So, rather than one stub for a collection of legs I should use individual stubs for each leg and each sex. That way there would be no doubt as to which leg was which. As it happens, I was not entirely successful in this ambition!

I mention legs, in particular, because one of the features of this type of spider is a "tarsal organ". This feature is unique to this genus (*Osornolobus sp.*). Furthermore, the organ is so small that it is difficult to resolve with a stereo light microscope. In fact, when the specimens first arrived I had a good look using my stereo, and could not see an organ on any of the legs. I fell to wondering if indeed these specimens were actually equipped with any.

Having taken each individual leg (or as many as I could dissect successfully and not lose) through the various stages of dehydration, ending up with two baths of HMDS, I laid some legs out on conventional stubs with sticky tabs. I also mounted a few on my "rotating" stubs, as described most recently in SEM Diaries - 22 (October 2020). It did not take me long to find tarsal organs under the SEM and by tilting the stage, or rotating the special stubs, I was able to obtain some good top and side views of these, unobscured by setae.

A tarsal organ is shown in Figure 1. It is the "squid-like" feature seen at the left edge of the image, about half way up. It is approximately 20 µm in length, and its diameter is similar to that of the surrounding hairs. I was delighted to capture such a clear image, and I was equally interested in the configuration of the tarsal claws, to the right of the image, which was again quite new to me.

Other features of interest, as for all spiders, are the male and female sexual organs. Unfortunately, on this genus of spider the interesting part of the epigyne (female sex organ) is internal. To image this would have involved my taking a scalpel to the female abdomen and performing a very delicate operation. I do know my limitations (well, some of them at least) and I decided not to attempt this. Even with the male organ (pedipalp), where all the interest is external, I managed to damage the right palp while dissecting out the left one. Fortunately, I was able to capture some key features, which enabled my BAS colleague, from a combination of the shape of the appendages on the pedipalp and the appearance of the tarsal organ, to determine that this spider was a previously unrecorded species.

Last year, in my role as Editor of Balsam Post, I received a potential contribution from a PMS member I know well. It was on the subject of pollen, and included a number of excellent light micrographs. Anyone who has attempted to image pollen using a high magnification light microscope will know how difficult it is to obtain clear images of small but important details of a single grain, and her images did not always show the feature being described as clearly as one would have liked. I suggested that I could have a go at capturing the features using the SEM. She agreed that the SEM can provide much higher resolution than even the best light microscopes and was happy to collaborate. By that time the season for collecting pollen was come and gone, so her article has been delayed by a year and will now appear in BP133 (October 2021).

As it happens, I mentioned pollen in SEM Diaries - 24 in connection with protocols with which I was unfamiliar. "It's quite easy", she said. "All you need to do is put the pollen in dilute alcohol, centrifuge gently to ensure the pollen grains migrate to the bottom of the tube, then suck out the alcohol with a Pasteur pipette and replace it with a higher strength alcohol. Repeat a few times. I can send you pollen in Eppendorf tubes." (I paraphrase a bit.)

Fig. 2: My cheap centrifuge. Dehydrating alcohols on the left, standard tube and Eppendorf adapter bush on the right.

I do have a centrifuge, but since it only cost me £60 new, from an eBay store, you can imagine it is not that good. (In fact, it can be somewhat dangerous, leaping into action without provocation if I am not careful!) It came with six tubes 20 mm in diameter and 100 mm long. These seemed somewhat over the top for small pollen samples. In fact, when it arrived I rather assumed the tubes were holders for smaller devices and contacted the suppliers for clarification. The tubes are mounted at a fixed angle and the speed and duration of operation can be set with dials (Figure 2).

I decided that this current pollen interest was a good reason to finally make some adaptors to enable Eppendorf tubes to be used with the centrifuge, so I designed a simple bush and turned up three of these in aluminium on my lathe. Meanwhile, I had a look at the pollen that my friend had sent me, along with other samples I had gathered myself, prepared using the quick and dirty way - by sprinkling grains on a sticky carbon pad on an SEM stub, sputter coating this and putting it in the chamber of the SEM.

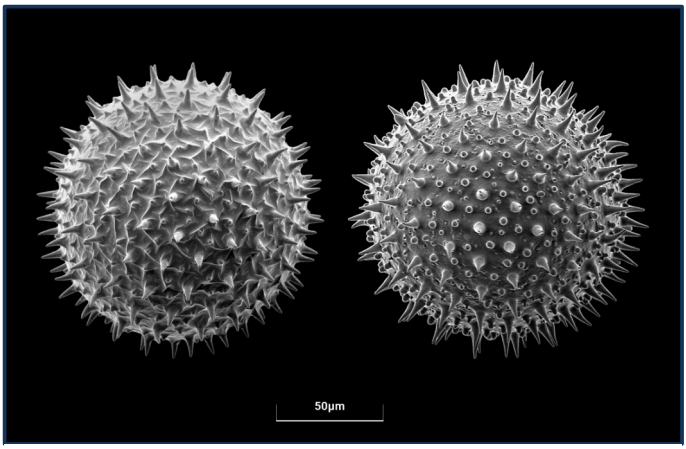

Fig. 3: Hollyhock pollen. Left: Pollen sputter coated before imaging. Note the wrinkly appearance of the outer skin. Right: This grain was imaged at a low accelerating Voltage without sputter coating.

Sometimes that works well, but at other times not so well. A case in point is the pollen of the hollyhock flower. This is a very attractive pollen but, as can been seen in

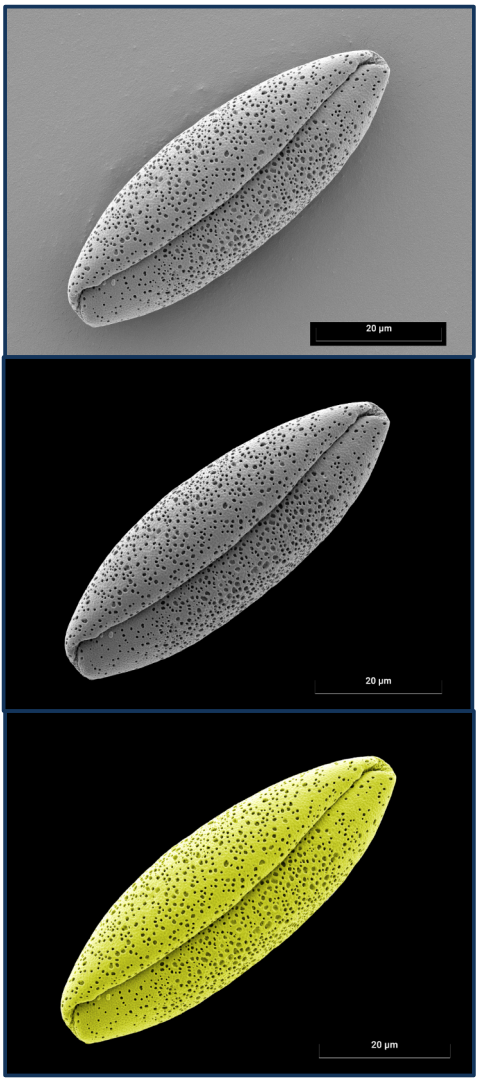

Fig. 4: Different ways of presenting micrographs of pollen

Figure 3, the process of sputter coating the grains has a deleterious effect on the outer skin.

This collaboration on pollen has taught me a great deal, to the extent that I have been collecting as many species as possible to build up a "library" of images.

Before the two collaborations mentioned above came along I was methodically putting together a "photobook" of my favourite electron micrographs taken on my new SEM to date. This would be for use as Christmas presents for family members and one or two others rather than for selling on Amazon, I hasten to add. In fact, even this has been a collaboration of sorts, as I have approached several PMS members for help with identifying specimens.

Figure 4 shows three different ways of presenting the same image. The top frame is how it came out of the SEM, other than the moved scale bar and a bit of adjustment to contrast and brightness. I like this look, but I was lucky that the background was clean and tidy. Much too often the background is cluttered with dirt, or the sticky tab is crazed.

The middle frame shows the effect of replacing the background with a black layer. This seems to be my default option these days. Of course, I could make the background any colour, even grey as in the top frame. I tend to use black backgrounds but having seen the top image, I am considering experimenting with grey in the future, for suitable subjects.

I include the bottom frame as a bit of fun. I have coloured the pollen grain and used a black background. All a bit over the top, I think. As I said in SEM Diaries - 24, colouring can improve an image, but a carefully presented monochrome image can also be quite spectacular.

Progress with the book has been slow, but at the beginning of June I received a proof copy of the book from Blurb, and the quality of reproduction certainly looks good. I have produced the book using Affinity Publisher rather than Blurb's own publishing software so I was a bit apprehensive as to how it might turn out. Now I need to make minor adjustments to the density of some of the images, replace a few pictures and refine the text a bit.

Reference

1. www.spiders.jeremypoolesem.org.uk