

SEM Diaries - 38

Some New Subjects

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Fig. 1: Harvestman *Lejobunum rotundum*, dorsal view. Note the ocularium (the black area in the middle of the head). This file is licensed under the Creative Commons Attribution-Share Alike 4.0 International license

Regular readers of this column may remember that I have recently been lacking “focus” in my imaging with the SEM. I do not mean that my images have been “out of focus”; more it has been a case of not having a particular project to pursue. Well, I have taken myself in hand, to some extent, and now have two projects of my own under way. These could keep me going for quite some time!

The first of these is to image as many species of harvestmen as I can lay my hands on. A “harvestman” is an arachnid of the order Opilliones. These have some similarities to spiders, with which they can be confused by the lay person, but

their differences are more marked than their similarities. In fact, just about the only feature harvestmen and spiders have in common is that they both have eight legs! Previously, I have regarded harvestmen as a “by-catch” when collecting spiders, and mostly I have released any caught back into the wild. From now on, any harvestman netted or vacuum sampled during a spider survey will be collected, determined, preserved and imaged!

Well, that is the idea. My early attempts at determining harvestmen (that is identifying their species), using the “WildID Guide to Harvestmen of the

British Isles” have failed, but in my defence I think this may be because the specimens I caught have been immature.

I started the project by looking at some specimens I had in my lab, under a stereo microscope. These had been preserved in alcohol since a spider weekend in 2023. Unfortunately, it did not appear that these specimens had been particularly well preserved, with evidence of air having penetrated under the outer layers of the extremities. In fact a similar effect had also been observed in preserved specimens of pseudoscorpions imaged in 2020. I might have “got away with” imaging these with the SEM, as the air bubbles would have been hidden from the electron beam by the outer layer, but in any event these specimens were immature, having been collected in June. Mature harvestmen normally “appear” from August onwards.

Collection and Preparation of Specimens

Having decided that my existing stock of harvestmen was not suitable, I decided to collect some fresh specimens from my garden using my vacuum sampler. This turned up three specimens. Given I was doing this in June, these, too, were immature, but this was not an issue when it came to testing my preparation technique.

Regular readers of SEM Diaries will have learnt that it is important that specimens to be placed in the SEM are very dry, and furthermore that the drying process used must not deform the specimen. The protocol I tend to use is similar to that used for slide making, at least to the final stage. Thus, I immerse the specimens in a bath of 70% denatured alcohol for a period (say 10 minutes) and then move it to a bath of 85% alcohol for a similar period. This is followed by immersion in baths of 90%, 95% 98% and 100% alcohol (x2). In reality, what I call 100% is actually just neat (from the container) denatured alcohol, which is 99%. So I actually do away with the bath at 98%. Each bath reduces the water content in the specimen, and the use of a series of baths helps reduce any “shock” to the specimen as it is immersed in a different concentration of alcohol.

The final stage is to dry the specimens and for this my normal protocol is to

transfer the specimens from the “100%” alcohol into two consecutive baths of a chemical called hexamethyldisilazane (HMDS), followed by drying in air. HMDS has a low surface tension (compared with water or alcohol) and this enables the specimen to dry without deformation (or at least that is the theory).

My first efforts at preparing my newly caught specimens were carried out using this protocol, and although the results looked “OK” if not brilliant, I thought I would try the alternative preparation technique, using a critical point dryer (CPD). This process is described in SEM Diaries - 1 (July 2015). In summary, it involves using two baths of acetone following the final alcohol bath and then replacing the acetone with liquid carbon dioxide. The liquid CO₂ is then raised to 1050 psi at 31.5 Celsius (the “critical point”) where the physical characteristics of the liquid and gaseous phases of CO₂ are identical. The pressure is then slowly reduced to atmospheric and, in theory, the surface of the specimen is preserved without deformation.

I decided to collect some more specimens and prepare them using the protocol involving the CPD. Subjectively the results appeared to be slightly better, but there was not a major difference between the two techniques. More experimentation is required.

Results

On the following pages I show the early results of preparing and imaging (immature) harvestmen. I also highlight the difference between harvestmen and spiders.

Now that adult harvestmen are out and about my plan is to collect mature specimens and determine and image these. I have also asked the Opillone specialist on the Council of the British Arachnological Society if she would provide me with specimens herself.

These images have been “processed” using Photoshop, to increase the contrast and mask out unwanted debris in the backgrounds.

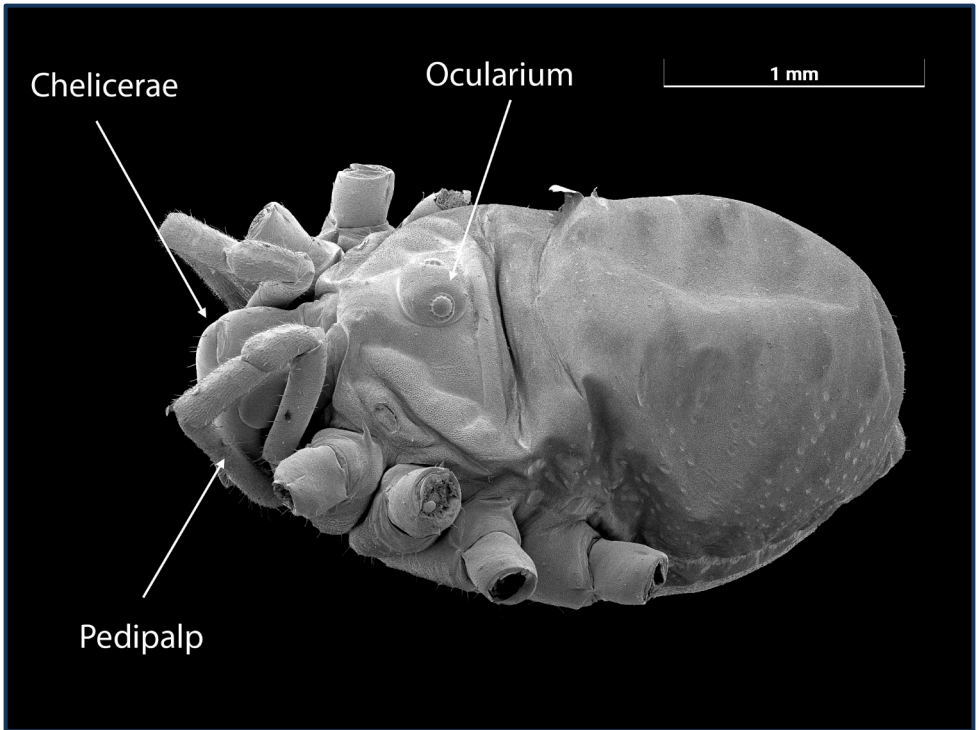


Fig. 2: Dorsal View of a small harvestman identifying three key features. Legs have been removed for clarity. Note that despite this sample having been dried using the Critical Point Dryer the abdomen (right hand part) shows significant deformation. This is usually also the case with spiders, where the abdomens shrink and deform, whichever drying method is employed.

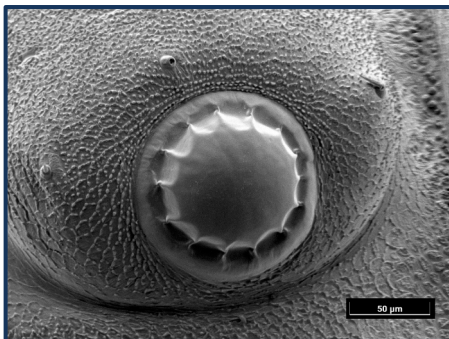


Fig. 3: Detail of left eye. The ocularium (Figure 2) is a raised area of the body of the harvestman that houses two simple eyes. Despite using the CPD, the drying process has not been perfect and the eye has shrunk. This has had the effect of emphasising the muscles or ligaments that connect the eye to the ocularium. The vision of harvestmen is poor, so as far as I am aware the eye does not rotate within the ocularium. For comparison, spiders normally have six or eight eyes.

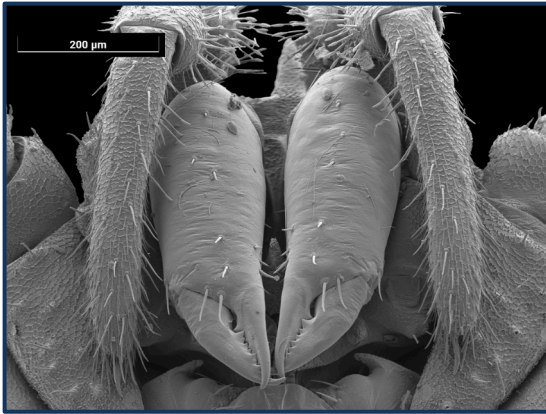


Fig. 4: Chelicerae and pedipalps. Whereas spiders feed by injecting fluids into the body of their prey using their fangs (on the chelicerae) then sucking out the digested “gloop”, the chelicerae of harvestmen are adapted to feeding on a variety of live and carrion prey, including (rarely) vegetable matter. Similarly, the pedipalps of male spiders are used in copulation, but sexual reproduction on harvestmen is by means of a penis (male) and genital operculum (female) located under a flap on their ventral side. Thus the pedipalps of harvestmen (shown here either side of the chelicerae) are simpler than those of spiders.

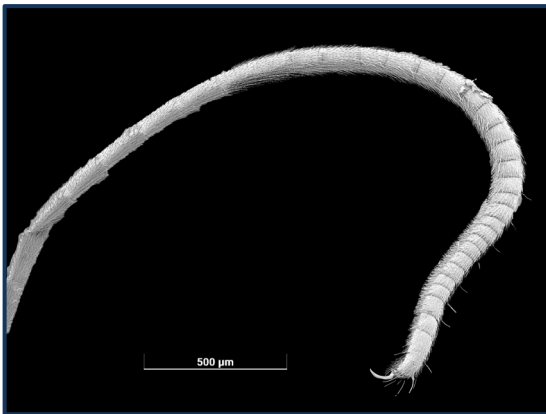


Fig. 5: Tarsus of Harvestman. This is made up of a large number of articulated segments. These enable the harvestman to use its legs rather like the prehensile tails of creatures such as monkeys, to support themselves on vegetation.

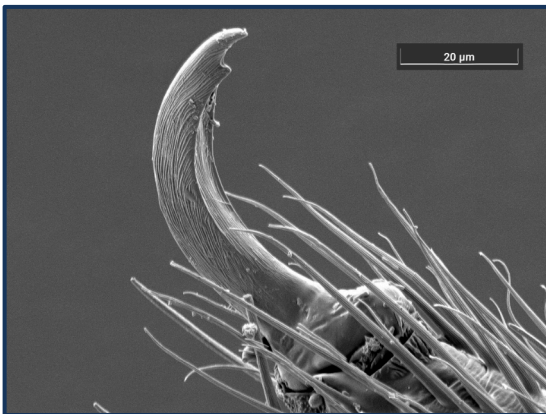


Fig. 6: Tarsal Claw of harvestman. Each tarsus of a harvestman terminates in a single tarsal claw. Spiders have two claws, normally including a comb-like structure, with web-dwellers having a third, hook-like, claw for grabbing hold of the silk of the web.

Rocks from Wales

I mentioned that I have two of my own projects under way at the moment. Well, the second project is to prepare, analyse and image rock samples I picked up during an FSC course on “The Geology of Snowdonia”. The fifteen students and their lecturer were based at the Rhyd-y-Creiau centre of the Field Studies Council and we were driven to various locations in the region to observe and be lectured on the features in view.

While most students on the course might have picked up the occasional pebble to take home and put in a display cabinet (or on the mantelpiece) I came home with about 10kg of samples collected over the five days of the course. My intention behind this is to add to my collection of different rock types, which includes embedded and polished samples, and also to carry out an elemental analysis using my EDS system.

I have got stuck into this project, and so far prepared and studied specimens of five different rocks. It is unfortunate that most of the rocks I have collected are very hard. This makes cutting and polishing somewhat more difficult than preparing the more workable sandstones, for example. My most “interesting” observation so far (and it is “early days”) is that the sample I have identified as Chlorite seems not to contain any chlorine! [I have since discovered that the formula for Chlorite does not include chlorine, despite its name!]



Fig. 7: My rock samples in their individual (and labelled!) bags.

Figs. 8-10 Top to bottom, opposite:
“Raw” Jasper stone; preparing the jasper for coring; the final embedded and polished specimen.

