Supporting Information and Species Identifications

Key Identification Features of Some British Spiders, as seen using a Scanning Electron Microscope

Background

Since taking delivery of my SEM in January 2016 I have produced in excess of 3,500 electron micrographs of a wide variety of subjects, but predominantly of spiders. Although working alone, I have been fortunate to have received training in the use of the SEM from a number of sources. These include attendance at the Electron Microscopy Summer School, a week-long training course at Leeds University, run by the Royal Microscopical Society (RMS), and also through one-to-one tuition from a specialist from Bristol University and from the supplier and maintainer of my instrument. I have also attended an RMS course on enhancing SEM images. In addition to these sources I have received much encouragement from senior members of the RMS as well as from other suppliers of SEMs and preparation equipment. One of my electron micrographs was selected for the 2018 desk calendar of the RMS.

A wide angle view of my laboratory, identifying the key equipment used in specimen preparation and imaging. The SEM is in the centre. The sputter coater is on the bench on the left, above the gas cylinders. The cylinder with two dials, to the left of the sputter coater, is the critical point dryer. The stereo microscope for identifying specimens and laying out samples on stubs is on the bench to the right.

I publish a quarterly update on my SEM activities in Balsam Post, the Newsletter of the Postal Microscopical Society, which can also be read on my website [1]. The website also provides photographs and further information about my SEM and laboratory. In addition I have written about the experience of setting up my laboratory for the RMS [2].

Some years ago I attended a residential course on the identification of spiders at Flatford Mill Field Studies Centre. It was led by Paul Lee, who is the current President of the British Arachnological Society (BAS). Following this I shared my identifications with the Area Organiser of the BAS for Dorset, who was able to confirm or otherwise that these were correct. I then uploaded my records onto the national database, known as the Spider Recording Scheme, which is run by the BAS. Following this "apprenticeship" I am now sufficiently experienced to upload my identifications without formal confirmation, and have so far uploaded in excess of 450 records. In the few cases where I am unable to complete an identification I have access to various experts who can assist.

Chosen Identification Features

There are approximately 700 different species of spider in Britain, divided between 34 families. Most families are further divided between a number of separate genera.

The main identification features of spiders at species level are the sexual organs. References 3 and 5, between them, provide sketches of the male and female sexual organs of the vast majority of British spiders, grouped by family and genus. In order to determine to which family a particular specimen belongs, and thus find which section of the reference books to search in detail, a variety of other features of the spider are used. These include the number and layout of the eyes around the spider's head. Some families, such as the Salticidae (image 3) and Dysderidae (image 5) have a very characteristic eye layout. Many other families have groups of eight eyes in an arrangement that at first sight seems similar (images 1 and 2). For these, the relative size of the eyes (and of the spiders) and other details, such as the size of the chelicerae (jaws), help to narrow down the options to one or a small number of possible families.

In addition to the eyes and chelicerae a number of other features are used in the references to help key down to family or genus level. These include the relative lengths of the legs, the size and shape of the spinnerets and other similar features. The Linyphiidae provide a particular challenge in identification, requiring study of the location or presence of particular hairs on the various legs to identify many examples to genus level, before the sexual organs are examined to determine the species.

For the purposes of the current panel, and with a limit of 15 images, the three most important features have been selected, namely the heads and the male and female sexual organs.

Technical Details

All the submitted electron micrographs were taken on my FEI Inspect S50 Scanning Electron Microscope with a tungsten electron source. My instrument is fitted with three types of detector, each of which has advantages and disadvantages in use.

Everhart Thornley secondary electron detector (ETD). This was used for the majority of the micrographs and is characterised by giving images with a fair degree of "modelling", which provides a three-dimensional appearance. For the micrographs that use this the detector is located at the top of the image, which gives the impression that this is the direction from which the specimen is illuminated.

Secondary electrons are low energy electrons displaced from the surface of the specimen by the scanning electron beam. (In other words, these electrons do not originate in the electron beam but on the specimen.) The number of electrons displaced from a given location, and hence the brightness of the image at that point, is determined by the angle between the electron beam (vertical in my SEM) and the surface of the specimen. The smaller this angle (or the more vertical the point on the specimen) the brighter is the image. This can lead to a not unpleasant "edge effect" as is evident in image 13.

The accelerating voltage used with this detector was either 5 kV or 10 kV. The choice of voltage is a trade-off. The higher the voltage the better the signal-to-noise ratio, and hence the less grainy the image appears. Too high a voltage, however, can lead to specimen damage, or evidence of "charging". This is an effect where charge from the electron beam builds up on parts of the specimen and has the effect of interfering with the electron beam. This shows itself either as isolated white patches on parts of the image, or streaking, often emanating from the tip of a hair. All the specimens on the panel have been sputter coated with gold to help prevent the build-up of charge on the specimen.

Backscattered Electron Detector (BSED). This consists of a group of four photo-diode arrays, clustered round the electron gun. The location of the detector allows more uniform detection of electrons from all parts of the three dimensional specimen, at the expense of lower basic contrast.

Unlike secondary electrons, backscattered electrons originate in the electron beam. They penetrate the specimen to a depth dependent on the accelerating voltage and the characteristics of the specimen. A large number of internal reflections take place, at atomic level within the specimen, before some of these electrons re-emerge from the specimen and are detected by the BSED. The detector is mainly used for the study of materials, since the brightness of the image is dependent on the atomic mass of the material. However, it can be useful in other applications, such as here.

When imaging biological material with this detector it has the advantage that the effects of charging of parts of the specimen are much less obvious than with the ETD, since backscattered electrons emerge from the specimen at much higher energy levels than secondary electrons, and are thus less likely to be deflected by surface charge. The BSED is less sensitive that the ETD, and for this reason an accelerating voltage of 15 kV was used with this. Also, resolution is lower for a given beam spot size and accelerating voltage, since the backscattered electrons can exit the specimen over a wider area than that from which secondary electrons are emitted. This can lead to a somewhat softer appearance in the image.

Large Field Detector (LFD). This is used in conjunction with the Low Vacuum Mode of the SEM, which is designed for specimens that are susceptible to charging or are unable to be sputter coated with gold. Two of the micrographs in the panel were taken using this detector.

Resolution. All images in the panel were taken with a digital resolution of 2048 x 1887 pixels (including a data area, which has been cropped out in the prints). This provides a reasonable compromise between image quality and exposure duration. Images were cropped to a square format, resulting in files of between 1500 and 1800 pixels square. No interpolation has been carried out.

Specimen Preparation

All specimens were captured in the wild, narcotised with ethyl acetate and preserved in 70% Industrial Methylated Spirit (IMS) until required for preparation.

The required anatomical feature was dissected from the spider in a petri dish of alcohol under a stereo microscope, and dried. If the specimen were simply dried in air the surface tension produced as the moisture evaporates could lead to deformation of the required features. Thus the drying was carried out by one of two methods, depending on the specimen. One method used a Critical Point Dryer (CPD). The specimen is infused with pure acetone and placed in a pressure vessel. Liquid carbon dioxide is used to displace the acetone, which is drained off. The carbon dioxide is then heated to raise it to its "critical point" (approximately 31.5 Celsius and 1,015 p.s.i.). At this combination of temperature and pressure the characteristics of the liquid and gaseous phases of CO_2 are identical. The pressure is reduced to ambient while maintaining the specimen above 31.5 Celsius and the dried specimen is then able to be removed from the chamber.

A simpler drying method is to displace the IMS solution with increasing strengths of alcohol up to 100% to remove all water from the specimen. The material is then placed in one or more baths of hexamethyldisilazane (HMDS), which in turn displaces the alcohol. After a suitable time (from an hour to overnight) the material is removed from the HMDS and left to dry in air. HMDS has a low surface tension, and as such the chances of damage to the specimen as it evaporates are reduced compared to evaporation from alcohol or water. Given that the material imaged is relatively robust, this method was used for the majority of the micrographs on the panel.

Following drying, the specimen is mounted on a flat or angled aluminium "stub", designed to slot into the turntable (called a "stage") in the SEM. This is the most delicate part of the preparation, and is carried out under a stereo microscope. Handling of the material is by tweezers or small paintbrush, and the specimen is fixed to the stub by means of either a double-sided adhesive carbon disc or silver or carbon based conducting glue. The orientation of the specimen is made as close to that shown in the reference material [3, 5] as is possible. The stubs are put in a vacuum overnight to draw out any solvents from the glues.

The final stage in the preparation is to coat the specimen with a very thin (~12 nm) layer of gold. The purpose of this is to provide a conducting path from every part of the specimen to ground. If the specimen were left un-coated, a negative electric charge would build up and prevent the creation of quality images.

Coating was carried out using a Quorum Q150R sputter coater, fitted with a tilting stage. This particular stage continuously varies the angle of the stub face to the gold "target" and helps to ensure that there is a conducting path round "difficult" areas such as hairs. Coating is carried out in an atmosphere of Argon, at low pressure.

Imaging:

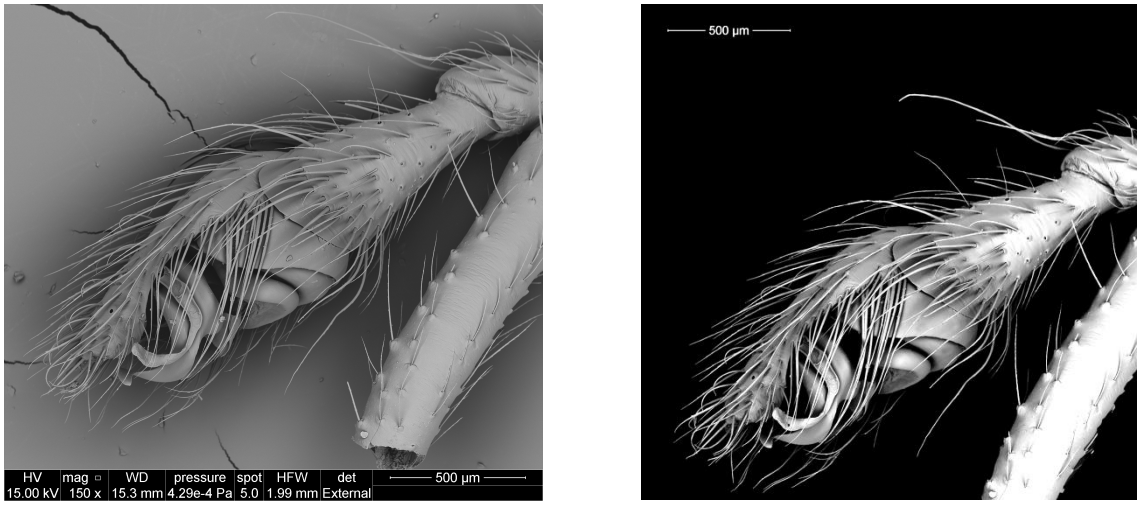
Up to seven sputter-coated specimens are loaded onto the stage in the specimen chamber of the SEM, which is then evacuated to around 10^{-4} Pa (10^{-9} atmospheres). The tungsten electron gun is activated and scanning commences. Specimen orientation, magnification and focus, along with other parameters, can all be altered using the computer interface. Brightness and contrast are also set to

provide the optimum image from the selected detector. Once the best possible image is displayed, this is captured at a resolution of 2048 x 1887 pixels, in tiff format, which takes around five minutes to complete.

Post-Processing:

Post-processing of images is normally necessary with scanning electron micrographs, even if this is simply to tweak the curves and levels. In addition to adjustments to these parameters, I have carried out the following, using Photoshop CC®, on some or all of the images in the panel.

- Masking of un-wanted backgrounds. This has been done on each of the images in the top two rows. The backgrounds often contain evidence of glue, manufacturing marks on the aluminium stubs, or crazing in sticky carbon pads. In addition, the heads, as imaged, include evidence of severed limbs. These are all masked out to highlight the "wanted" features.
- Removal of blemishes. In a few images I have used the clone tool to remove small cracks in the skin of the specimen, or dust spots. No attempt has been made completely to remove evidence of particles clinging to the skin of the spiders, however, as these would be present in the wild.
- Minimal sharpening. In a few images I have slightly sharpened the content. This is normally where the image was captured with the Backscattered Electron Detector, where resolution is less fine than with the ETD.
- Relocation of scale bar. The images from the SEM include a data area. The scale bar from this has been moved onto the body of the image in each case.
- Cropping to a square format: Images are cropped to achieve a pleasing framing. A square format has been chosen for consistency between all images.



Illustrating the appearance before and after post-processing of Image 6. Note the low contrast and the crazing on the carbon pad mount on the original micrograph (left).
(When viewed on a screen or on the prints, these images have a neutral grey hue)

Printing:

This was carried out by a commercial mass-production laboratory. Glossy paper was chosen, to provide the maximum contrast and sharpness.

Mounting:

At the suggestion of Andy Moore (RPS Distinctions Manager) the prints are supplied simply stiffened with card, rather than put in window mounts.

References:

1. http://www.jeremypoolesem.org.uk
2. Poole, Jeremy. *An SEM at Home. From Fantasy to Realisation over Half a Century*. InFocus, the Proceedings of the Royal Microscopical Society Issue 50, June 2018.
3. Roberts, M.J. 1985, 1987 *The Spiders of Great Britain and Ireland*. Harley Books, Colchester Volume 2, Linyphiidae
4. Roberts, M.J. 1985, 1987 *The Spiders of Great Britain and Ireland*. Harley Books, Colchester Volume 3, Colour Plates
5. Roberts, M.J. 1996 *Collins Field Guide Spiders of Britain and Northern Europe*. Harper Collins, London

Identifications:

The tables below provide an identification for each specimen included in the panel. This table also includes further details of the settings used on the SEM. The images of the sexual organs are orientated to match the sketches in the references.

The spiders represented on the panel are all common in my local area, and identifications can be easily verified by consulting the references and my notes in the table.

Image Number	Species and (Family)	Identification Justification	Reference	Detector	Acceleration Voltage	
Row 1: Heads						
1	*Steatoda bipunctata* (Theridiidae)	The eye arrangement of the Theridiidae is similar to that of some other families, so acts as much to eliminate a family as to identify the species as one of the Theridiidae. The chelicerae (jaws) do not have cheliceral teeth, and the rest position of the fangs is horizontal. (Compare this with the rest positions of the Tetragnathidae (image 2) or the Linyphiidae (image 4).) The genus *Steatoda* has a characteristic white horseshoe shaped band on its abdomen, which allows easy identification to genus. Examining the sexual organs under a microscope permits identification to species.	[5] Pages 265, 273-275	ETD	5 kV	
2	*Tetragnatha montana* (Tetragnathidae)	This very common spider is easily identified to family level by its slender abdomen and large chelicerae (jaws). Identification of males to species level, by examining its pedipalps, is straightforward, though determining the females is more difficult.	[5] Page 298	BSED	15 kV	
3	*Salticus scenicus* (Salticidae)	The eye arrangement is unique to the Salticidae, permitting identification to family level without difficulty. The specimen in this image was tilted forward to enable the rear four eyes to be seen.	[5] Page 45	BSED	15 kV	
4	*Erigone dentipalpis* (Linyphiidae)	Many of the Linyphiidae are small and not easy to identify. Identification of the genus *Erigone* is made easier by the teeth around the carapace, visible in the image. The large size of the clypeus (the area between the eyes and the anterior edge of the carapace), is characteristic of the Linyphiidae.	[4] Plate 203	ETD	5 kV	

5	*Harpactea hombergi* (Dysderidae)	A primitive spider. The fact that it only has six eyes permits easy keying down to genus. Identification to species level requires examination of the pedipalps (male) or spines on femur IV (female) under a stereo microscope.	[5] Pages 93, 95	BSED	15 kV	

Row 2: Pedipalps (male sexual organs)						
6	*Steatoda nobilis* (Theridiidae)	The genus *Steatoda* has a characteristic horseshoe shaped white band on its abdomen. The shape of the pedipalps allows easy identification to species level.	[5] Page 276	BSED	15 kV	
7	*Steatoda grossa* (Theridiidae)	Despite its name, this species is significantly smaller than *S. nobilis* (image 6). As for *S. nobilis* and also *S. bipunctata* (image 1), these spiders are easily identified to genus by the characteristic white horseshoe shape on the abdomen. As can be seen by comparing images 6 and 7, the shape of the pedipalps differs sufficiently between the species to permit easy determining.	[5] Pages 273-274	ETD	5kV	
8	*Araniella opisthographa* (Araneidae)	The six members of the genus Araniella are very similar in outward appearance, all having green abdomens, often with a red spot above the spinnerets. Determining to species level requires careful examination of the sexual organs under the light microscope, and is made more difficult by the small size of the pedipalps and the fact that these spiders are known to hybridise.	[5] Pages 327, 328	BSED	15 kV	
9	*Erigone atra* (Linyphiidae)	The eyes and carapace permit easy determining to genus level (see image 4). The pedipalps of this genus are distinctive, although require careful examination under a stereo microscope at high power to determine a specimen to species. The number and arrangement of the teeth on the palpal femur also assist in identification of males to species level.	[3] Text Fig. 45 Page 98	LFD	5 kV	

10	*Metellina mengei* (Tetragnathidae)	There are three members of the genus *Metellina,* two of which, *M. segmentata* and *M. mengei* are very common. Their pedipalps are very similar in appearance, even under a microscope, and a final identification is made easy by the arrangement of the hairs on the metatarsus and tarsus of the front legs.	[5] Pages 306-308	ETD	5 kV	

Row 3: Epigynes (female sexual organs)						
11	*Steatoda nobilis* (Theridiidae)	As noted above, the genus *Steatoda* has a characteristic horseshoe shaped white band on its abdomen. The shape of the epigyne allows easy identification to species level. (Compare this image with image 12, of the epigyne of *S. grossa*.) *Steatoda nobilis* is also known as the "noble false widow" spider, and has an unjustified reputation for being dangerous.	[5] Page 276	ETD	5 kV	
12	*Steatoda grossa* (Theridiidae)	Despite *S. grossa* being in the same genus as *S. nobilis* the shapes of the two epigynes differ significantly, making identification to species level straightforward.	[5] Page 273	ETD	5 kV	
13	*Pisaura mirabilis* (Pisauridae)	This spider, the only one in its genus, is colloquially known as the "nursery web spider", because of the way it nurtures its young. It is easy to identify in the field, especially while it is tending its brood. Its epigyne shape makes it very easy to determine with a hand lens or microscope. The halo effect around some of the edges is caused by the edge effect (mentioned above in describing the Everhart Thornley Detector), rather than by excessive sharpening in Photoshop.	[5] Pages 236, 237	ETD	5 kV	

14	*Labulla thoracica (Linyphiidae)*	The tunnel shape of the epigyne of *L. thoracica* is characteristic of *Labulla sp., Linyphia sp. and Neriene sp.* among the Linyphiidae, and is also present in some genera of the Theriidae. Distinguishing between Linyphiidae and Theriidae is easily achieved by observing the size of the clypeus (the area under the lower eyes).	[5] Pages 352, 353	LFD	5kV	
15	*Scotophaeus blackwalli* (Gnaphosidae)	This species is commonly seen indoors, on walls and ceilings. The epigyne is easily distinguished from other species in the genus, using a stereo microscope. Under the SEM its shape is seen extremely well.	[5] Page 108	ETD	10 kV	