Some spider organs as seen by the Scanning Electron Microscope, with special reference to the Book-Lung

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Summary

A method is described for preparing whole organs and sections of spiders for scanning electron microscopy. The results are illustrated by a series of photographs of the heart, book-lungs, abdominal skin and part of the anterior gut lining together with notes on their structure.

Introduction

Entire organs and sections of spiders were prepared for viewing with the scanning electron microscope, in order to investigate the structure and the effectiveness of freeze-drying as a means of preservation. The organs were obtained from the following spiders:–

Tegenaria saeva Blackwall – book-lung longitudinal section.
Argyroneta aquatica (Clerck) – book-lung and tracheae, skin.
Argiope bruennichi (Scopoli) – book-lung, gut lining, skin, heart.
Araneus quadratus Clerck – book-lung, skin.
Micrommata virescens (Clerck) – skin.

Methods

Preparation of entire organs

The following method is successful for the preparation of organs for the scanning electron microscope.

1. Kill specimen with ethyl acetate vapour. (Avoid gravid females, the oily content of the eggs will not allow satisfactory freeze-drying.)
2. Pin out the specimen with the appendages as far from the body as possible so that they will not interfere with future dissection.
3. Cool the specimen in a refrigerator for up to one hour, then transfer to a deep freeze at −10 to −20°C. Direct transfer to a freezing temperature causes muscular contraction.
4. After several days, transfer to freeze-drying apparatus. The process of freeze-drying should be carried out at −5 to −10°C and at a vacuum decreasing to below 0.1 Torr. Freeze-dry the specimen until constant dried body weight is achieved.
5. If the specimen is still soft after apparent freeze-drying, this is probably due to oiliness within the specimen. Either it may be discarded or a few cm^3 of liquid nitrogen may be poured over the specimen while it is still cold. (The freeze-drying process is then repeated.)
6. Allow the specimen to warm up to room temperature either under vacuum or in the presence of a desiccant – this prevents moisture from condensing on cold objects such as pins, thus partially rehydrating the specimen.
7. Dissection is started by easing up and peeling away the abdominal skin with a scalpel blade. This reveals the organs which are then attached to the surface of a clean scanning e.m. stub using "Silver Dag"; double-sided sellotape is adequate if clear cement is applied around the base of larger organs.
8. The organs may now be coated with gold, or some other stable, non-tarnishing metal (aluminium or gold-palladium alloy). This will render the organs visible under a beam of electrons.

Preparation of sections of spiders for scanning

It is necessary to prepare thick sections, between 10 and 20μm, or there will be nothing gross enough to scatter the electron beam sufficiently to form a reasonable image.

1. Kill and fix the specimen by immersion in formol-acetic-alcohol for 3 to 24 hours, according to size of specimen.
2. Soften the chitinous exoskeleton in chlorine dioxide vapour (reacting potassium chlorate and concentrated hydrochloric acid). The specimen rests in the vapour on a wad of cotton wool soaked in fixative (a more detailed account of this is given in Moore, 1973). This process is continued until the specimen is bleached including the sclerotised areas. This may take from

10 minutes to several hours according to size and sclerotisation of specimen. This process must be carried out in a fume cupboard away from sunlight, since the vapour is toxic and explosive in sunlight.

3. Harden the specimen in original fixative, from 2 hours to overnight.
4. Transfer to 90% alcohol with a trace of eosin to give the specimen some colour so that it will be visible when embedded in paraffin wax – important for small specimens.
5. Transfer to absolute alcohol, two changes over 2 to 3 hours.
6. Clear in methyl benzoate celloidin.
7. Rinse in toluene, transfer directly to equal parts of toluene and molten paraffin wax at 60°C for one hour.
8. Transfer to pure wax at 60°C, using two changes over 3 hours.
9. Embed in wax and cut sections in desired plane between 10 and 20μm.
10. Mount the sections onto slides using water, warm the slides to flatten the sections.
11. With the sections still floating, view under microscope. Select those most suitable, isolate these and transfer them to a stub coated thinly with glycerin albumen..
12. Allow the stubs to dry overnight at 35°C.
13. Immerse the stubs in xylene for several minutes to remove the wax, then gradually hydrate them through absolute, 90%, and 80% alcohols to deionised water.
14. Transfer to fresh deionised water for several minutes, drain off excess moisture and freeze at −10 to −20°C.
15. Transfer to freeze-drying apparatus. Process as for entire organs – this usually takes overnight only as the loss in weight is fractional. Coat stubs as before.

Results

Book-lung and associated respiratory organs

In each of the spiders studied, there is one pair of book-lungs situated on the ventral surface of the anterior abdomen, one on each side. Externally there is a small skin flap covered with barbed hairs that prevent most foreign matter from entering the respiratory aperture that it covers (Plate 1). The aperture opens onto a neatly packed series of about one hundred whitish lamellae attached at the furthest point of the organ like the spine of a book (Plates 2, 3, 4). Air entering the organ first passes through a fine amorphous mesh (Plates 5, 6), which prevents the tips of the lamellae which are moistened, from sticking together. It also prevents any dirt which may have entered the air passage from entering the organ itself. Deeper inside the air passage is another dirt filter comprising a series of moist dendritic processes branching out from the opposite wall of the organ. These branches are fused at the proximal end to form a solid sticky surface; any dirt adhering to this is presumably dissolved away slowly by the moist surface (Plate 1).

The air passes through the mesh and amongst the lamellae of the organ (Plate 7). Each lamella contains nuclei which act as cellular organelles (Plate 9). The surface of each lamella is covered on the upper side by a large number of nail-headed spigots (Plates 8, 10, 11, 12). These prevent the lamellae from adhering to each other during respiration as the lamellae seem to possess some peristaltic movement – the surface undulates gently in a pumping action. This effect can be seen to have occurred in Plates 13 and 14 where the lamellae differ in thickness, especially one arrowed in Plate 14. The spigots are of amorphous structure, being of a similar nature to the mesh.

The water spider, *Argyroneta aquatica* (Clerck), possesses a rather different type of respiratory system from that of other spiders. The lamellae of the book-lung are reduced in number to about twenty and the spigots are less well sculptured (Plate 15). By comparison, a large and complex tracheal system is found that compensates for this more rudimentary organ (Plates 16, 17). Around the book-lung area, this system enlarges to fill most of the body cavity. Behind this area lies the tracheal spiracle from which two main trunks arise (Plate 18), which each split at the petiolar junction into the more complex system of finer tubes. These pass into the cephalothorax to the spider's limbs and right down to the posterior abdomen. The function of this system is presumably to keep air passing around the spider's body in the most efficient way, so that it can effectively use as much as possible of the limited oxygen supply that its

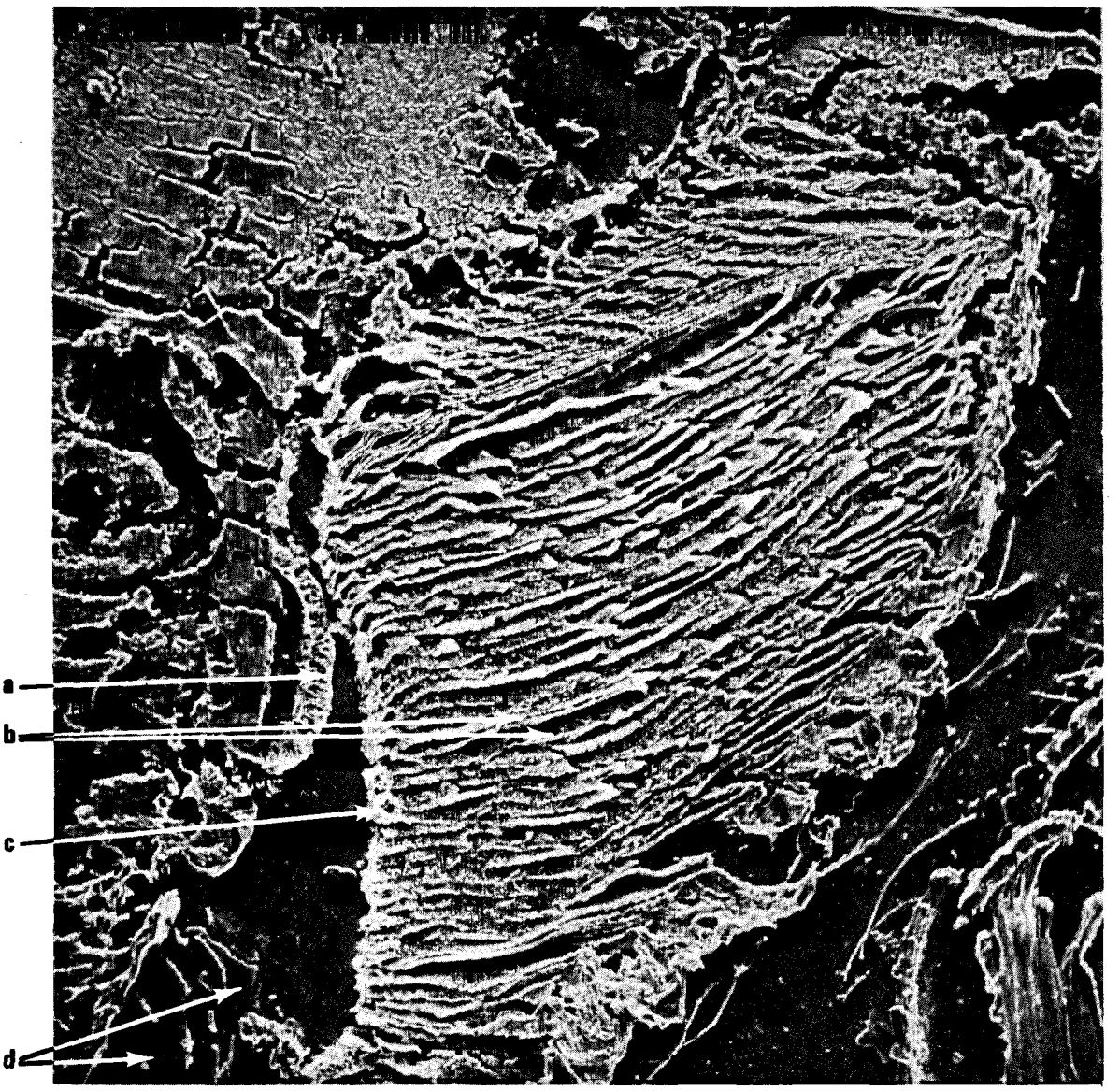
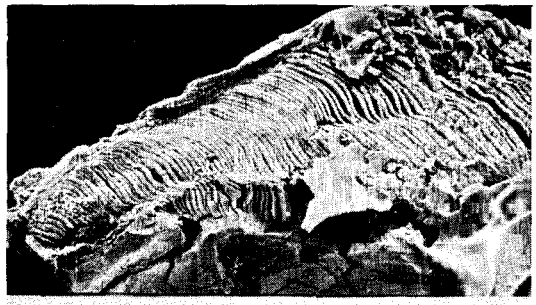


Plate 1: *Tegenaria saeva* Blackwall, female.
Longitudinal section of the book-lung, showing the arrangement of the lamellae, the lamellar supporting spigots, the mesh and air filtering passage (x 140).
a, air filter; **b**, lamellae; **c**, mesh surface filter; **d**, air aperture filter (barbed hairs)



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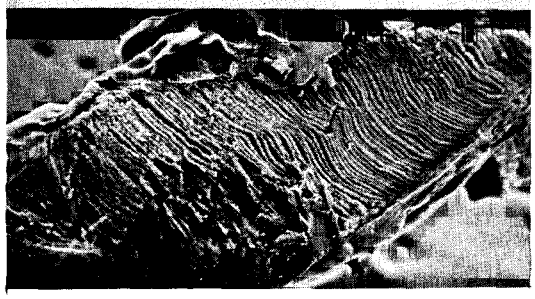

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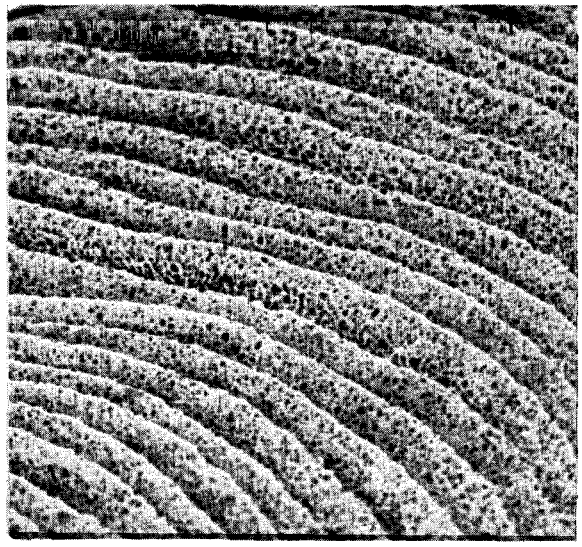

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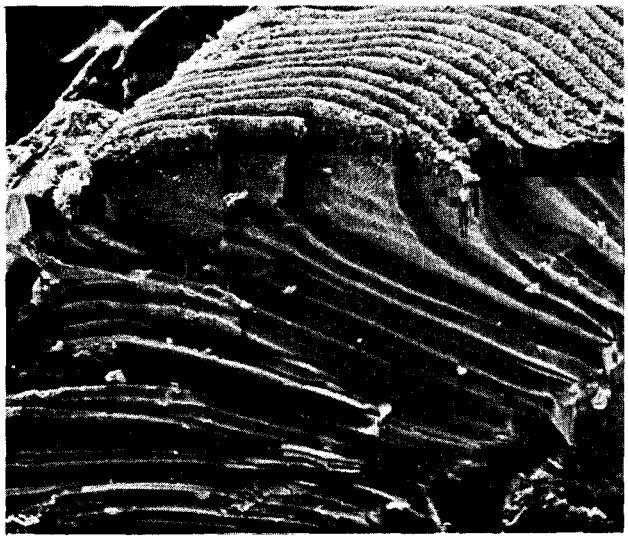

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Plates 2-6: *Araneus quadratus* Clerck, female.

2, Surface of book-lung, showing the entire mesh surface of the organ (x 50); **3**, View from the other side of the organ showing the mesh surface again and some of the lamellae where the organ has been damaged (x 50); **4**, Book-lung broken open showing the mesh surface and the layering of the remainder of each lamella underneath (x 200); **5**, Mesh surface of book-lung lamellae. The air passes down each furrow into the main body of the lung (x 500); **6**, Close-up of mesh surface showing complex random structure of this area (x 1,000).

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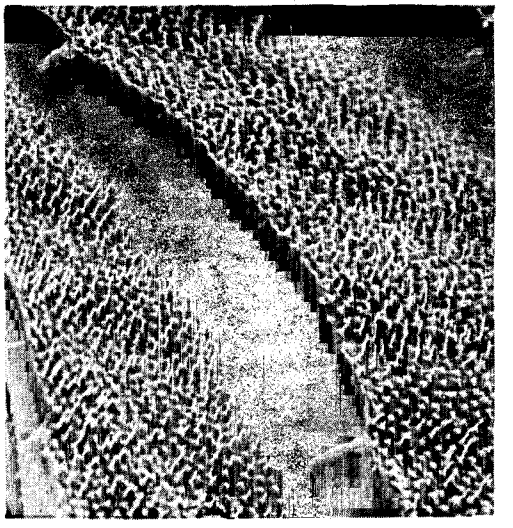

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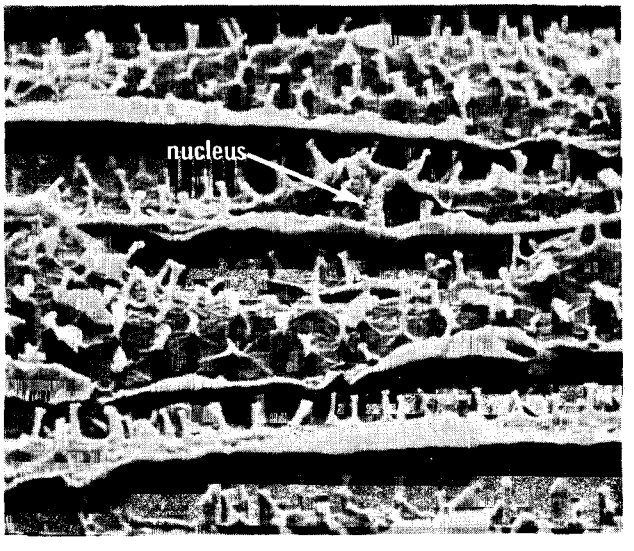

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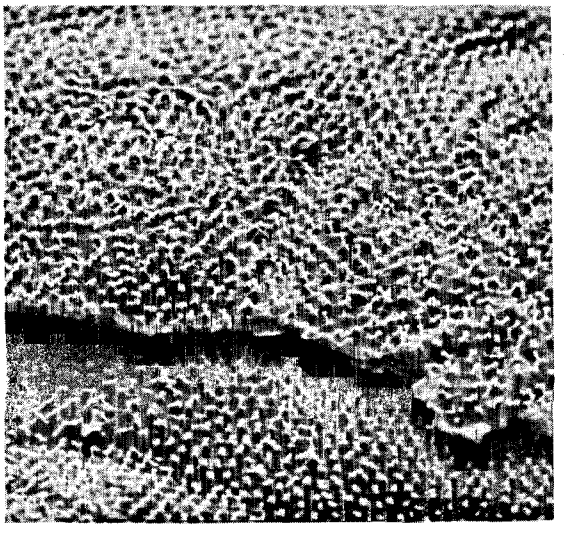

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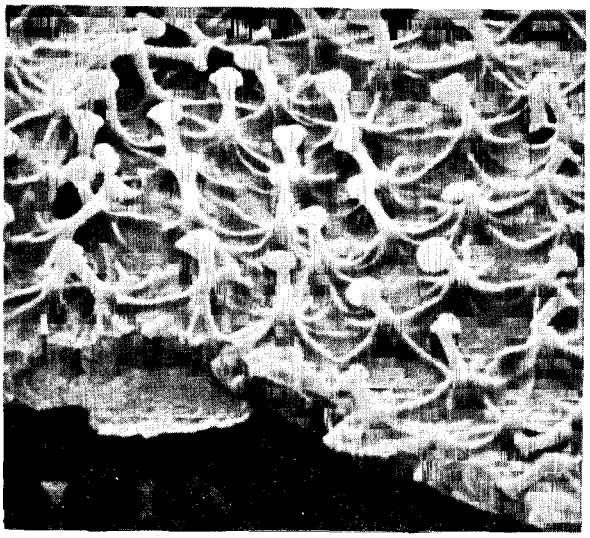
Plates 7-8: *Araneus quadratus* Clerck, female.
7, Book-lung broken open showing the surfaces of the lamellae (x 200); 8, Close-up of broken-off lamellae showing the surface spigots inside the lung, the thickness of the lamella at this point being about 20μm (x 1,000).

Plate 9: *Tegenaria saeva* Blackwall, female.
Close-up, longitudinal section of book-lung, showing spigots and a lamellar nucleus (x 2,000).

Plate 10: *Argiope bruennichi* (Scopoli), female.
Surface of lamella showing supporting spigots (x 1,000).



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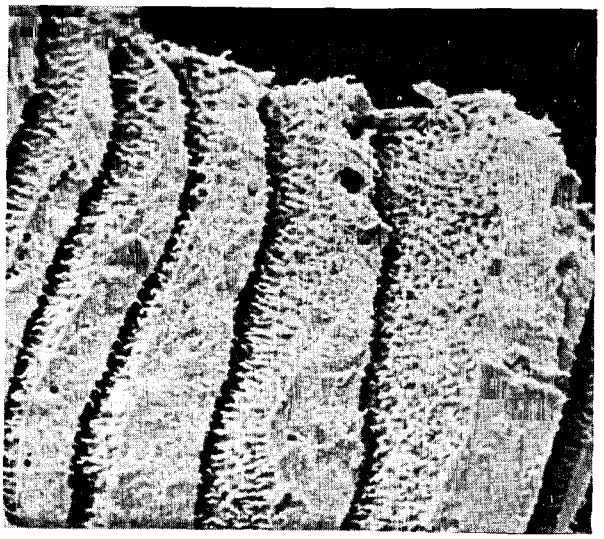

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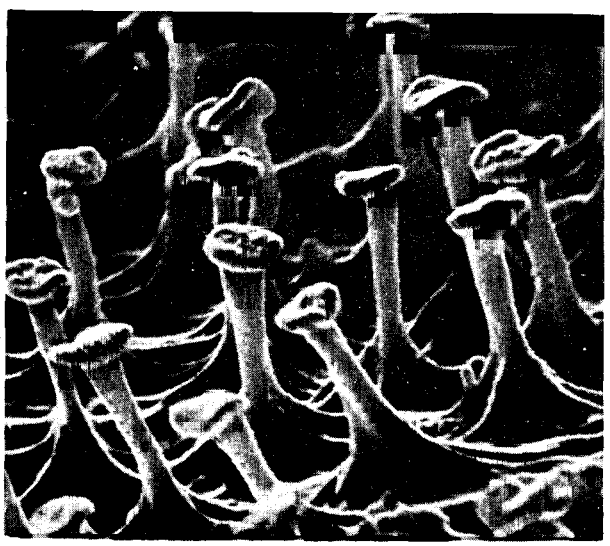

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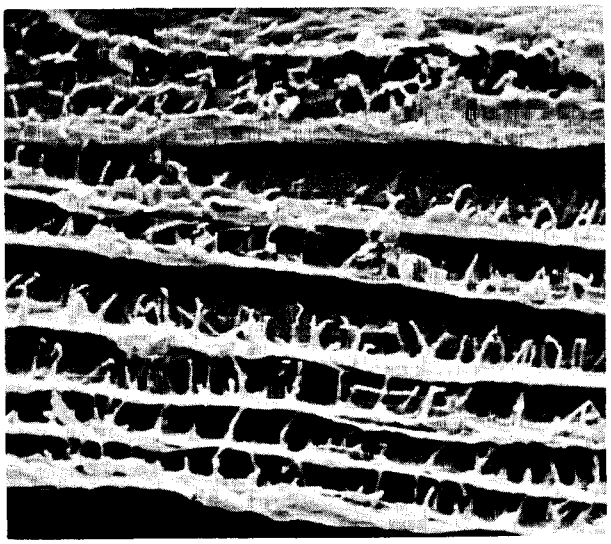
Plates 11-12: *Argiope bruennichi* (Scopoli), female.
11, Close-up of lamellar surface showing nail-shaped heads and star-shaped bases of spigots (x 5,000); **12**, Side view of spigots on lamellar surface (x 10,000).

Plates 13-14: *Araneus quadratus* Clerck, female.
13, Close-up, book-lung lamellae in section, showing how variable the width of each lamella can be due to the respiratory movement of the organ (x 1,000); **14**, Section of lamellae, showing relationship with mesh, and peristaltic movement (arrowed) (x 500).



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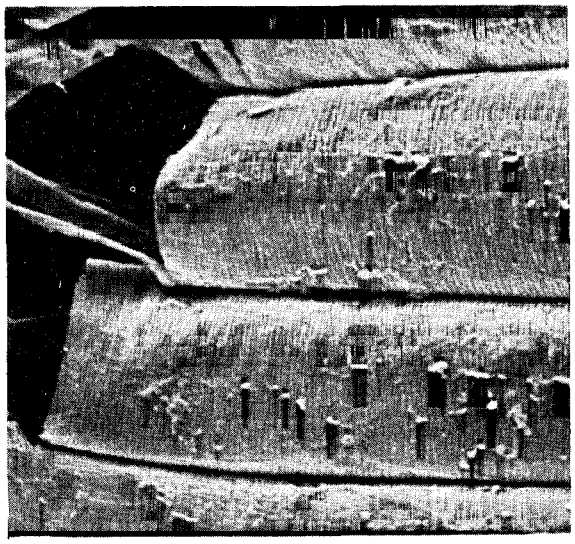

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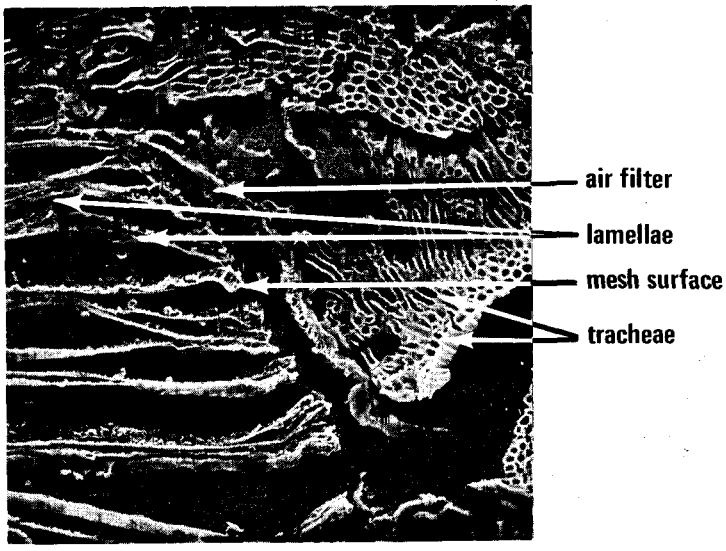

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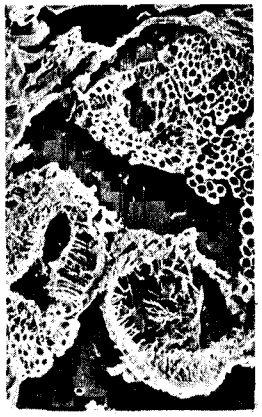

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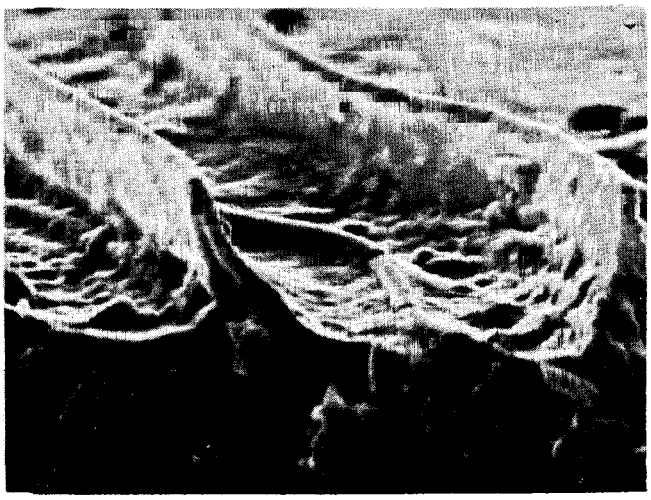
Plates 15-18: *Argyroneta aquatica* (Clerck), female.
15, Close-up, longitudinal section of book-lung, showing simpler structures than in other spiders (x 2,000); **16,** Tracheae of respiratory system, the average diameter of the tracheae shown here being about 10μm (x 5,000); **17,** Longitudinal section of book-lung showing mesh, air filtering device and some of the many tracheae which surround the organ in this spider (x 200); **18,** Main trunks and surrounding minor tracheae in section. The trunks contain tiny supports to prevent collapse (x 400).



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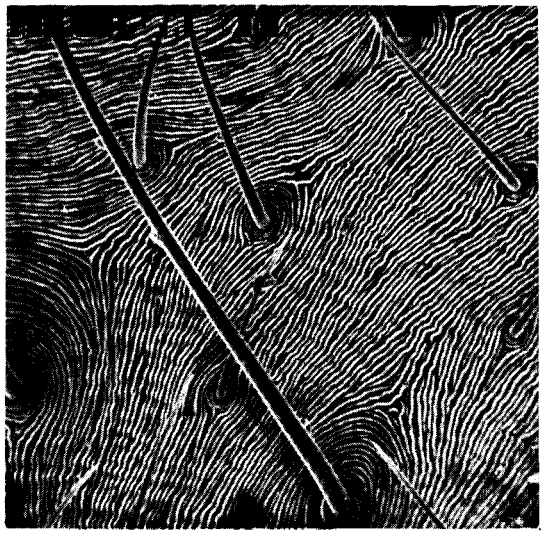

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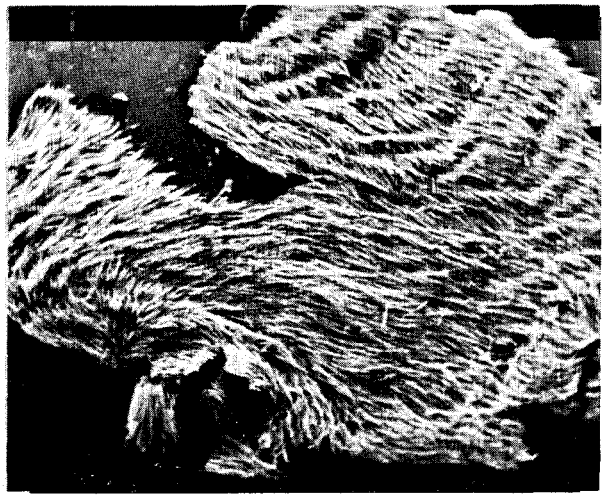

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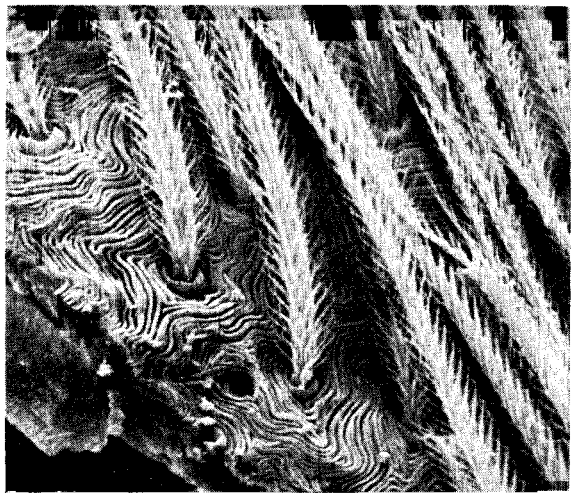

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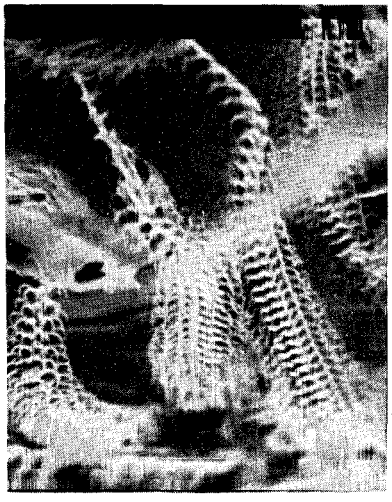
Plates 19-20: *Araneus quadratus* Clerck, female.
19, Close-up, edge-on view of skin whorl section showing the skin folding to form the whorl (x 10,000); **20,** Surface of skin of abdomen (x 200).

Plates 21-22: *Argyroneta aquatica* (Clerck), female.
21, Skin of abdomen, showing thick layer of air-trapping hairs emanating from the surface (x 20); **22,** Surface of abdominal skin, showing air-trapping hairs and different pattern of the skin whorl (x 1,300).



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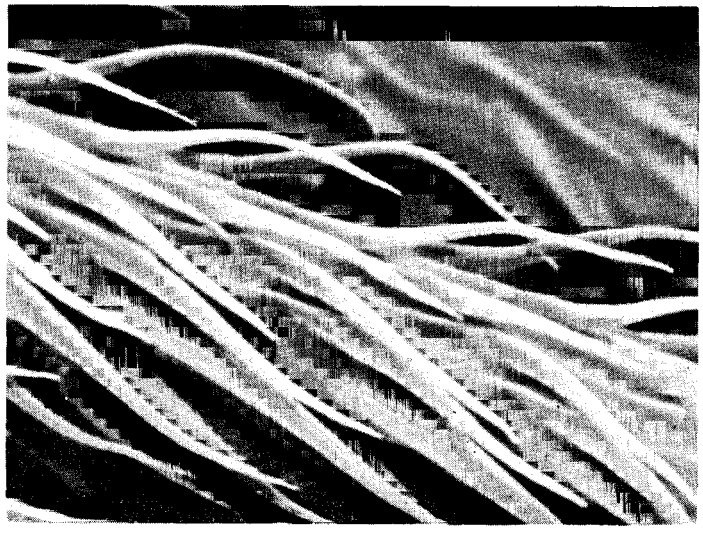

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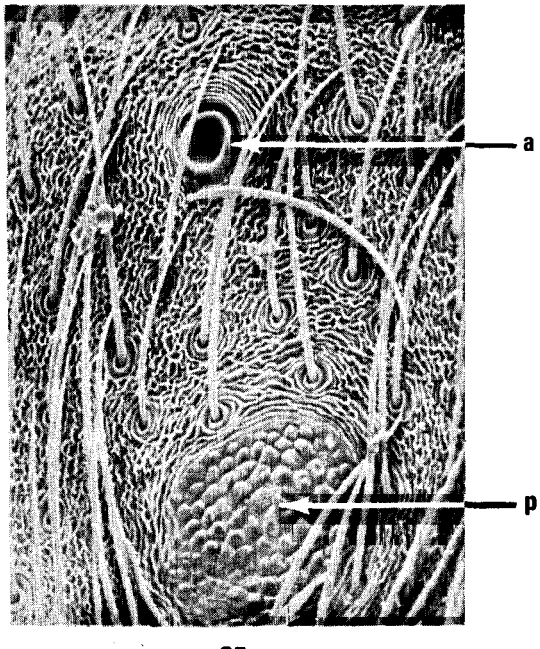

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Plates 23-24: *Argyroneta acquatica* (Clerck), female. **23**, Longitudinal view of air-trapping abdominal hairs (x 2,300); **24**, Close-up of air-trapping hair in side view, showing the barbules which enable the spider to hold the air-bubble over its abdomen while under water (x 9,300).

Plate 25: *Argiope bruennichi* (Scopoli), female. Surface of abdominal skin showing skin whorls, numerous hairs, aperture for a sensory hair **(a)**, and a pigment spot **(p)** (x 530).

Plate 26: *Micrommata virescens* (Clerck), female. Surface of abdominal skin, showing pore and base of sensory hair and the zig-zag skin whorls of this spider (x 2,000).

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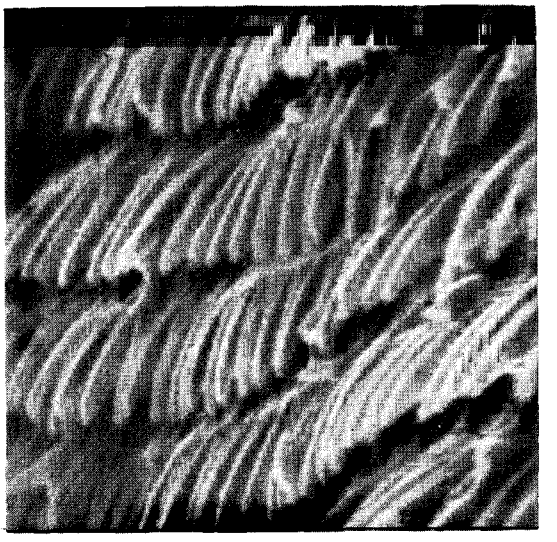

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Plates 27-28: *Argiope bruennichi* (Scopoli), male.
27, Longitudinal section of the anterior abdomen showing the heart in situ (x 100); 28, Lamellar lining of gut leading from mouth to sucking stomach (x 10,000).

air bubble holds. (Width of trunk at widest point: 120μm. Average width of tracheal tubule: 6μm.)

Abdominal skin

In each of the spiders examined, the skin of the abdomen is thrown up into a series of whorls that differ in pattern in each of the spiders so far examined (Plate 19). At fairly regular intervals, the whorls form a concentric pattern from whose centre emanates a hair (Plate 20). The smaller of these hairs are thought to prevent foreign matter from clogging the skin, while those of a larger size act as sensory appendages, although apart from size, no external features differentiate the two. The hairs on the abdomen of *Argyroneta* thickly cover the surface (Plate 21), and are composed of a central shaft from which radiate tiny barbules which are responsible for holding the air-bubble when the spider is under water (Plates 22, 23, 24). Also visible are the occasional pigment spots. On the skin of *Argiope bruennichi* (Scop.), these are found in rows and are responsible for the black stripes on the spider's abdomen (Plate 25). The knobbly non-reflecting properties of these areas would contribute to their dark appearance. A different pattern of skin whorls is seen in *Micrommata virescens* (Clerck) (Plate 26).

The Heart

This organ is situated centrally in the dorsal anterior end of the abdomen. It is a fairly long pear-shaped organ in which are found strut-like valves which permit the free flow of blood through the organ and prevent it from collapsing. (Plate 27).

The Gut

Plate 28 shows the area of the gut lying just beyond the mouth. It comprises rows of tiny finger-like processes similar to the villi of the mammalian ileum. The processes do not have any absorbing function, but may well be secretory since the salivary glands are associated with this region. The arrangement of the processes allows an efficient passage for the liquid food to the sucking stomach.

Acknowledgement

This paper is published with the aid of a grant from the Parliamentary Grant-in-aid for Scientific Publications administered by the Royal Society.

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Bull.Br.arachnol.Soc. (1976) 3 (7), 187-188

On the Evolution of Tracheae in Arachnids

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Tracheae, respiratory tubes that carry air directly to tissues, have evolved independently several times in arachnids. A similar structure has evolved in certain terrestrial crustaceans, the isopods (woodlice). Petrunkevitch (1933) considered that the absence of booklungs, the more usual arachnid respiratory organs, and their replacement by tracheae, was a significant character for grouping families of spiders, and on the basis of this character erected the suborder Apneumonomorphae. Dipneumonomorphae, two-lunged spiders, he considered a less homogeneous group, including spiders with varied development of the tracheal system. However, both Forster (1959) and Levi (1967) have shown that the replacement of booklungs by tracheae is related more to habits than to phylogeny. It is a characteristic of the more active arachnids. Furthermore, Anderson (1970) has shown that spiders with high metabolic rates possess tracheae. It would seem that tracheae are more efficient than booklungs in supplying oxygen. An interesting exception are wolf spiders (Lycosidae) which have high metabolic rates but lack efficient tracheae (Anderson, 1970). Lycosid spiders are, however, limited in their activity by being nocturnal or confined to relatively moist areas.

The most elaborate tracheal systems are found in active desert animals such as wind-scorpions (solifugids) and also in small arachnids with body surface areas proportionately greater than volume (Levi, 1967). Possibly tracheae are an adaptation for the reduction of water loss, a matter of major importance for terrestrial invertebrates. But then, why should the aquatic spider, *Argyroneta aquatica,* require an elaborate tracheal system (Crome, 1966)? A completely different explanation for the evolution and adaptive significance of tracheae must be devised.

It has been shown (Cloudsley-Thompson, 1957) that the rapid fatigue observable in some spiders is due to a lack of oxygen. Also Stewart and Martin (1974) and Kirber (1974) showed that due to the hydraulic system of leg extension, activity in spiders may be accompanied by a blood pressure differential between the leg-bearing prosoma and the opisthosoma, containing the localized booklungs and the heart. This pressure differential may be sufficient to effectively block circulation of oxygenated blood from the opisthosoma to the prosoma, resulting in fatigue (Wilson and Bullock, 1973; Kirber, 1974). It would be of selective advantage to a spider to have a direct supply of oxygen to its prosoma, independent of the circulation of hydraulic fluids. A tracheal system could provide this supply, while booklungs and blood carrying oxygen could not. Active wolf spiders lack an efficient tracheal system and as far as we know they run in short spurts. Measurements of running endurance in spiders with and without tracheae might constitute a good test for this hypothesis. But for many arachnids continuous activity may not be of survival value and booklungs remain.