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Description of the structure of different silk threads produced by the water spider *Argyroneta aquatica* (Clerck, 1757) (Araneae : Cybaeidae)

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ABSTRACT. The ultrastructure of the different silk threads (walking threads, diving bell, cocoon-sac, egg-sac, anchor threads) produced by the water spider *Argyroneta aquatica* (Clerck, 1757) has been analysed by means of Scanning Electron Microscopy (SEM). Possibilities for future research linking the observed structure with the particular way of life (life under water) are discussed.

KEY WORDS : *Argyroneta aquatica*, spider silk, microscopy, SEM

INTRODUCTION

Spiders are distributed in all kinds of environments and hence they have to adapt to different environmental conditions. However, most species prefer a particular climatic zone and occupy a specific strategic niche in consonance with the availability of prey. Because of their trophic level (predators) and their special way of prey capture (by means of web building, sit-and-wait predators and visual hunters), spiders have colonized almost every known terrestrial ecosystem and play a major role in regulating insect abundances (e.g. MOULDER & REICHLE, 1972; RIECHERT & LOCKLEY, 1984). Spiders have exploited even non-terrestrial ecosystems : *Argyroneta aquatica* (Clerck, 1757) is the only spider known to live most of its life under water (SHUNMUGAVELU & PALANICHAMY, 1992; SELDEN, 2002). Although a wide range of animals produce silk (e.g. KOVOOR, 1987; CRAIG et al., 1999), former studies have put emphasis on silk produced by the caterpillar *Bombyx mori* (Linnaeus, 1758) and by spiders. Recently, the structure of spider silk threads has been intensively analysed because of its unusual chemical and physical characteristics (high strength and high elasticity) (review in VOLLRATH, 2000). Most of these studies are restricted to the threads used in webs and draglines, while almost no data on cocoon fibres and structure is found. The available research on cocoon structure includes a detailed study of the cocoon within one genus : *Argiope bruennichi* (Scopoli, 1772) (BERGTHALER, 1995) and *A. aurantia* Lucas, 1833 (HIEBER, 1985 & 1992a,b), within one family : Uloboridae (OPELL, 1984) and within this family more specifically *Polenecia producta* (Simon, 1873) (PETERS & KOVOOR, 1989) and between different spider genera and species : *Diguetia canities* (McCook, 1889) (CAZIER & MORTENSON, 1968), *Nephila clavipes* (Linnaeus, 1767) (CHRISTENSON & WENZL, 1980) and *Cyrtophora citricola* (Forskal, 1775) (KULLMANN, 1961). The studies of BARGHOUT et al. (1999, 2001) only deal with the molecular (crystalline) structure of the cocoon thread (by means of Transmission Electron Microscopy) without describing the structure of the cocoon itself. A more formal structural description (in the framework of egg-cocoon parasites) has been done for three mimetid spiders (*Mimetus notius* Chamberlin, 1923, *M. puritanus* Chamberlin, 1923 and *M. epeiroides* Emerton, 1882) (GUARISCO & MOTT, 1990; GUARISCO, 2001a,b) and the linyphiid spider *Pityohyphantes costatus* (Hentz, 1850) (MANUEL, 1984). So until now, no study has been done on the structure of threads used by the water spider *Argyroneta aquatica*. With regard to the special way of life of this species, it is obvious that the structure of the different threads can yield interesting questions related to the adaptation to this specific environment. This work examines the types of threads produced by the water spider by means of Scanning Electron Microscopy. Furthermore, although substantial work is available on the biology of *Argyroneta aquatica*, no information on the morphology of silk threads of this species has been published.

Systematic position and distribution of the water spider Argyroneta aquatica *(Clerck, 1757).*

Argyroneta aquatica is a spider, which is hard to classify in spider taxonomy. Originally, it was placed in the family Agelenidae, afterwards it was classified in a separate monotypic family Argyronetidae (ROTH, 1967), which was approved and noted in the catalogue of BRIGNOLI (1983) mainly on the basis of the special way of life. Later, the species was returned to the Agelenidae (subfamily Cybaeinae) (GROTHENDIECK & KRAUS, 1994), but finally it was moved to the newly appointed family

Cybaeidae (due to an elevation of the former subfamily Cybaeinae into a new family), which is the current systematic position according to PLATNICK (2006). A recent comparison with fossil material states, that *Argyroneta aquatica* indeed belongs to Cybaeidae and that differences from other cybaeids are due to specializations for aquatic life or derived with respect to other cybaeids (SELDEN, 2002). Nevertheless many authors question this positioning because several studies on the karyotype of the *Argyroneta aquatica* sex chromosomes revealed it to be different from that of most members of the Cybaeidae (haploid versus diploid) (KRAL et al., *pers. comm.*). Despite all arguments, the systematic position of the species is still questionable.

Argyroneta aquatica is a palearctic species living even as far as in Siberia and in Central Asia (PLATNICK, 2006). In Europe, the occurrence of the species is restricted to Northern and Central Europe. The species is widespread in our country and especially in Flanders in the Campine and the coastal region (RANSY & BAERT, 1987). In all countries, it was observed that the species is preferably found in places with a fluctuating water level. In Belgium, the species is not found in areas with upwelling water (although not all areas have been investigated), but it is more abundant in marshes, lakes, moors and canals where it lives between water plants. The species can be found the whole year through, but there are no records of captures in January.

Biology of **Argyroneta aquatica** *(Clerck, 1757).*

In order to be able to live under water, *Argyroneta aquatica* has a number of adaptations to this unusual environment for spiders and numerous authors have dealt with the aquatic mode of life and associated morphological and physiological adaptations (e.g.; BRAUN, 1931, BRISTOWE, 1958; SCHMIDT, 1959; CROME, 1951, 1952; BROMHALL, 1987, 1988; IZUMI 1991; KAYASHIMA, 1991; GROTHENDIECK & KRAUS, 1994; KOTIAHO, 1998; MASUMOTO et al., 1998; TOSHIYA et al., 1998a,b; SCHÜTZ & TABORSKY, 2002).

MATERIALS AND METHODS

The spiders (adults and subadults, no juveniles) were caught in a small ditch in the nature reserve Bourgoyen-Ossemeersen near the city of Ghent. Each spider was isolated in separate plastic bottles filled with aerated water (this to eliminate chlorine in the water which is often a limiting factor for lots of invertebrates). A polystyrene cube was pierced with plastic straws and/or wooden sticks and placed in the water so that spiders had an attachment for building diving bells and 'walking' threads (Fig. 1). Apart from the difference in material for

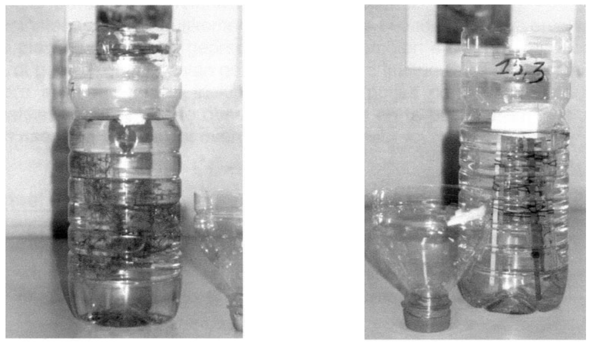

Fig. 1. – Construction for keeping the water spiders in lab conditions (digital photographs taken by Petra Willems).

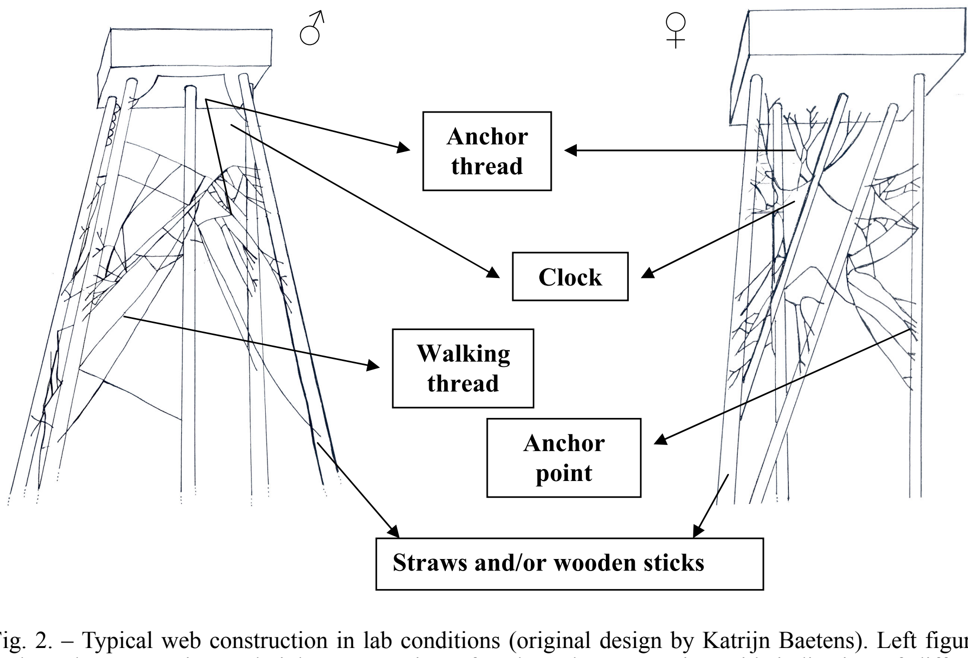

Fig. 2. – Typical web construction in lab conditions (original design by Katrijn Baetens). Left figure : male web construction and right construction : female web construction with indication of different thread types.

the sticks, all constructions were made as uniform as possible so that any difference between housing possibilities could be excluded. To avoid the spiders from escaping, the top of the bottle was cut off and placed in reverse on the rest of the bottle. Spiders were fed with 20 water isopods (*Asellus aquaticus*) each week found in ponds of Ghent University's botanical garden. When available, the diet was enriched with water fleas (*Daphnia* spec.), mosquito larvae *(Culex* spec. and *Chaoborus* spec.) and fairy shrimps *(Branchipus* spec.).Samples were taken from each type of threads and investigated under a Scanning Electron Microscope (SEM, JEOL JSM 840).To this end the samples were fixed in 70% isopropyl solution, dried and covered with a tiny layer of gold.

To have an idea of the diameter of the different threads, twenty-five observations were made on the selected pictures (the average value and standard deviation are mentioned).

RESULTS AND DISCUSSION

Within a few days, the spiders had constructed a network of silk threads together with a diving bell attached to the straws or wooden sticks in the water bottles (Fig. 2). The difference in material for the sticks has no significant influence on the web building behaviour of the spider for both males and females.

Argyroneta aquatica makes four kinds of threads under water : a diving bell (functions as an air reservoir), anchor threads : firm threads used to attach the diving bell to the substrate (mostly water plants), so-called 'walking' threads used for movement when a prey is near and threads produced when making the egg-cocoon and the cocoon threads themselves. Differences are observed between the web construction of males and females. Males tend to build a large diving bell that is suspended in the four corners of the polystyrene cube with only a few threads while females build a smaller, more rounded diving bell that is attached by anchor threads to the nearby straws/sticks. Males make larger diving bells probably because they are larger than females and need more space to live in. As to anchor threads, males apparently produce less of them than females. This confirms the assertion of CROME (1952) that males are less careful in building their diving bell. It is not clear why this difference between sexes occurs since these threads (diving bell and anchor threads) are necessary for everyday life. Conversely, males make more walking threads than females (although this cannot be generalized). It hasn't been investigated if this difference can be explained by the need for a higher mobility of males (in search for food and females), which is still doubtful.

Threads of the diving bell

The SEM images (Fig. 3) show that the diving bell threads consist of three different types in congruence with earlier findings (SCHOLLMEYER, 1913). We observe thick threads (1.05-1.15 µm); loose, thin, single-stranded threads (0.34 ± 0.04 µm) and thin threads that are aggregated, but visible as separated threads. Furthermore, the threads have no clear spatial ordination : they are all woven very chaotically without any clear direction. It is noticed that a kind of film was present, draped over and woven between the strands. It is not certain whether this film is produced by the spider (serving probably as a water repellent layer) or deposited by other aquatic organisms. In Fig. 3, a diatom and some globular objects (possible bacteria) can be seen, which are known to produce some kind of biofilm (BOSSELAERS, *pers. comm.*). However, future research is required to check whether these organisms are responsible for producing this film (see further). The presence of the film is certainly connected with the physical-chemical properties of the diving bell. The diving bell, when pulled out of the water, became shiny, lost its suppleness and became fragile. When replaced into the water, its original suppleness and roughness was regained. The real structure and clarification of the film has not been investigated and could be a subject of future research.

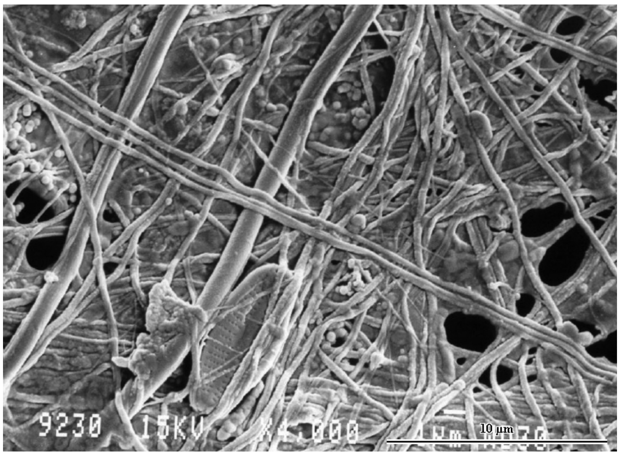

Fig. 3. – SEM picture of the three different types of thread used to produce the diving bell.

Anchor threads

Anchor threads seem to consist of a crowded mass (possible anomaly occurring with preparation), which is held together with threads (Fig. 4). The most probable function of the threads is tightening the film of the diving bell to the substrate. When viewed more in detail, the anchor threads consist of several small threads (Fig. 5). It seems that anchor threads are tightly connected to each other through smaller threads and that interstitial space is filled up with dirt. The smaller threads are similar to a cable, consisting of several smaller fibres covered by some kind of casing (Fig. 5). Whether this casing consists of the same material than that of the diving bell film is not clear.

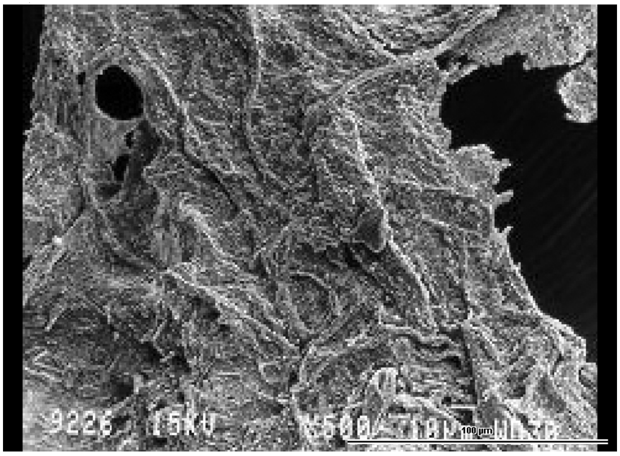

Fig. 4. – SEM picture of crowded mass with included anchor threads.

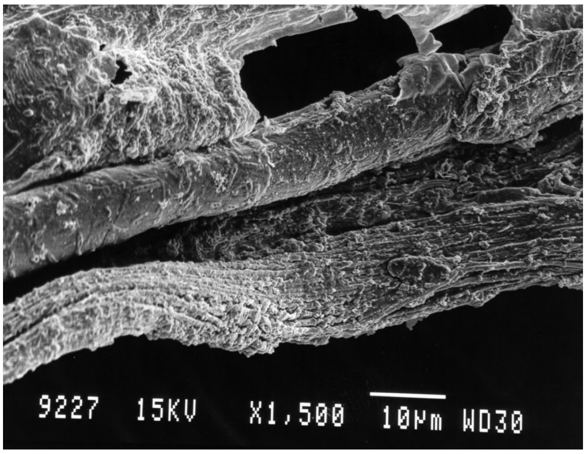

Fig. 5. – SEM picture of an anchor thread surrounded by a case (upper thread) and one consisting of several partial threads (lower thread). The latter is indicated with an arrow.

Walking threads

The walking threads were not analysed due to logistic reasons (difficulties when making a preparation due to the thinness and delicacy of the threads). However, we did observe that the walking threads are spun as one strand, but are branched off into several fibres as soon as the substrate is reached. These threads form a close network of fibres (comparable to the anchor threads, but smaller) along the substrate (downwards) and end somewhere where no further attachment is possible. A lot of 'dirt' is incorporated into the threads, probably because the spider itself actively incorporates parts onto the threads, as it also does in or around the diving bell (KAYASHIMA, 1991).

The egg-cocoon

The egg-cocoon is formed within the diving bell. The spider builds a vast structure in the upper part of the diving bell ('cocoon-sac') that follows the shape of the diving bell and is sometimes even, for a certain part, incorporated into the diving bell. Partial incorporation of the cocoon-sac in the diving bell is often observed with large cocoons. It is a separate structure that can be removed from the diving bell without destroying the latter. In this structure, a second sac is woven (consisting of fine threads) that is attached with several threads to the interior of the cocoon-sac, called the 'egg-sac'. The egg-sac does not make contact with the cocoon-sac. The two constructions are sealed off with a very thick and closely woven sheet. Our observations of the structure were somewhat different from those of WAGNER (1894) and HAMBURGER (1910) (Fig. 6). In our case, an egg-sac was built and in that of SCHOLLMEYER (1913) a third division was observed. According to our own observations, the shape of the egg-cocoon differed between individuals (intraspecific variability) although there were no environmental influences (all individuals received the same breeding regime in the lab). Since environmental influences were not discussed in other articles concerning the cocoon shape, no final conclusions of our observations with those of others can be made. It is not clear whether the observed differences depend on the specific microhabitat in which the species lives or on physiological limitations of the several individuals. Both structures (cocoon-sac and egg-sac) will be discussed separately.

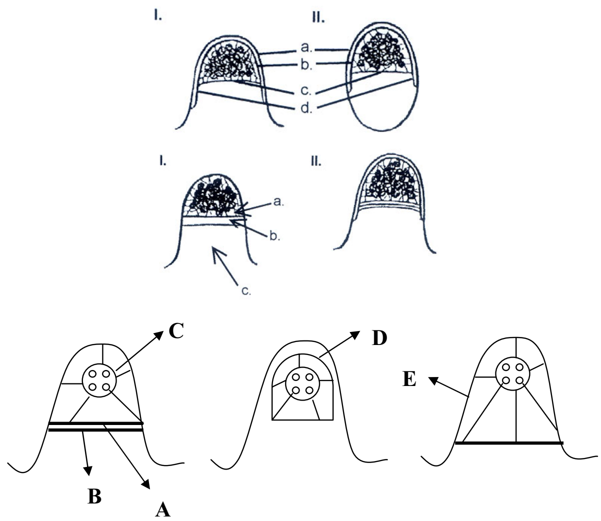

Fig. 6. – Structure of the cocoon sac according to WAGNER (1894)[upper figure : (I) the summer nest and (II) the winter nest, a= diving bell; b=casing of egg cocoon, c=lower casing, d=edge of casing] and HAMBURGER (1910)[second figure, (I) egg-diving bell with a=egg chamber, b=air chamber, c=residence of the female, (II) egg diving bell according to SCHOLLMEYER (1913)]. Own interpretation (lower figure) with a, b= structure which closes down the entrance, c= egg-sac, d=cocoon-sac, e= cocoon-sac incorporated into the diving bell.

The thread of the cocoon-sac consists of a single fibre type (there are no different types like those in the diving bell). Nevertheless, the fibres were observed to be different in thickness (0.37 ± 0.10 µm) and to lie randomly orientated (Fig. 7). Bundles of fibres are observed in which the fibres are wrapped around each other (like some kind of coiling). On the outer side, a film was observed although not as thick as in the case of the diving bell (Fig. 8). Due to the presence of the film, at first sight no differences were observed between the threads (diameter : 0.41 ± 0.04 µm) when investigated through light microscopy. The structure of the film that closes the whole ‘cocoon-sac’ comprises several layers of single thick and thin fibres put over each other combined with a film that does not cover the whole of the fibre-matrix (interstitial space is present in some parts of the sheet) (Fig. 10). Moreover, no differences were observed between fertilized and non-fertilized cocoons.

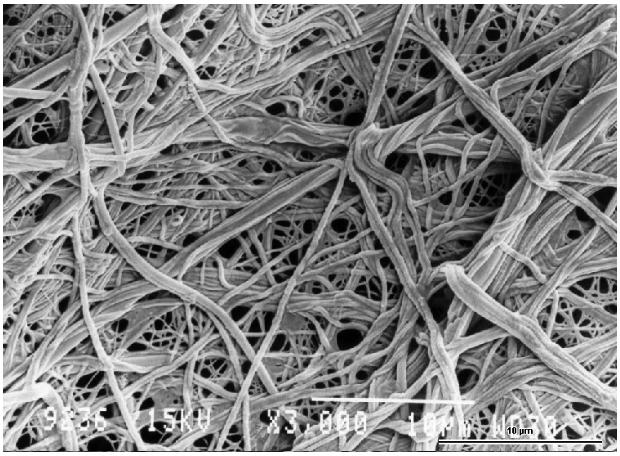

Fig. 7. – SEM picture of the film present on the outer side of the ‘cocoon-sac’.

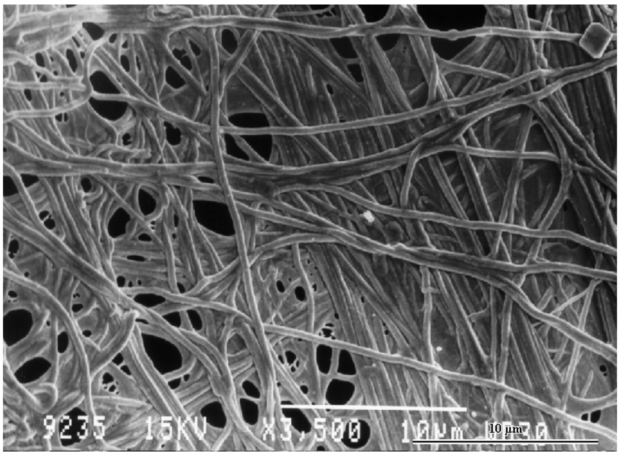

Fig. 8. – SEM picture of a part of the ‘cocoon-sac’ showing different kinds of threads.

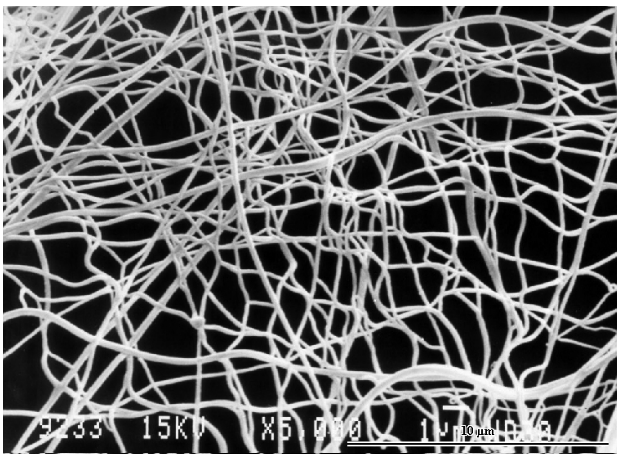

Fig. 9. – SEM picture of threads used for producing the ‘egg-sac’.

The threads of the ‘egg-sac’ consist of only one type (no big differences in thickness : 0.24 ± 0.06 µm). In comparison with the ‘cocoon-sac’, the ‘egg-sac’ threads are loosely woven (Fig. 9). This kind of pattern is also observed within a non-fertilized cocoon and a film was not present. Fig. 7 shows that the kind of thread is the same as the type used for building the ‘cocoon-sac’ (coiled threads).

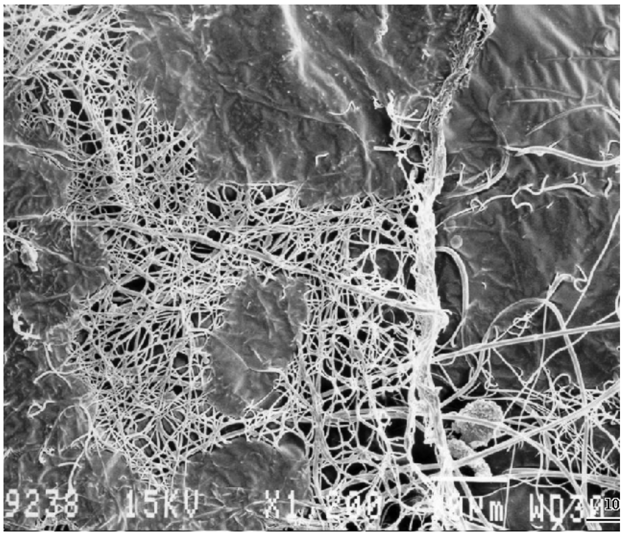

Fig. 10. – SEM picture showing the structure of the sheet that closes the whole of the ‘cocoon-sac’. Certain parts are covered with a film, while others aren’t.

Conclusion and remarks on future research

Although this work gives a first analysis of the different threads and structures made by *Argyroneta aquatica*, it is clear from our findings that a lot of questions remain unanswered. It is not clear how the threads and structures are produced in order to be adapted for life under water. Further investigation is necessary to look for the differences in physical-chemical properties between the different types of threads produced by this spider species in order to find in which way the spider alters the chemical-physical properties of its silk as an adaptation for life under water. Another possible experiment is to check whether diatoms and bacteria could be responsible for producing the film and/or if this film is made as an answer to this particular way of life. It would be possible by comparing spiders raised in sterilised containers (and initially sterile water) with spiders kept in ‘normal’ conditions after which threads could be plated in several agar-media. It is obvious that the special structures found with this species raise a lot more questions and that further research on the chemical-physical composition (in relation to an adaptation to life under water) is necessary.

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