OBSERVATION OF THE SILK-GLAND SYSTEM IN *ARGIOPE MODESTA*: A POTENTIAL SOURCE FOR BIOMATERIAL SCAFFOLD

Anggraini Barlian*, Rizka Musdalifah Amsar, Candrani Khoirinaya, Meidiana Ebtayani Lingga, Willy Septian Anggrayana and Ahmad Riwan

School of Life Sciences and Technology, Institut Teknologi Bandung, Indonesia

*aang@sith.itb.ac.id

Abstract. Spider silk, which contains spidroin, is a biomaterial widely used in regenerative medicine. *Argiope modesta*, an orb-web spider found in Indonesia, produces silk via its glands. However, the structure of the gland has not yet been explored. The purpose of this study was to examine the silk gland system, including the morphology of the spider fiber of *Argiope modesta*. The ultrastructure of the silk gland system surface was observed using scanning electron microscopy. The histological structure of the seven glands in the spider was observed using hematoxylin-eosin staining. Scanning electron microscopy revealed the structure and morphology of the spider fiber, spinneret, and spinning tubes. The diameter of the spider fibers was 850 µm. The minor ampullate, major ampullate, aggregate, flagelliform, tubuliform, pyriform, and aciniform spinning tubes were observed on the anterior, median, and posterior spinnerets of *Argiope modesta* via scanning electron micrographs. Histological analysis revealed the structure of the spider silk glands. The aggregate, flagelliform, aciniform, and tubuliform glands were clearly visible. This study revealed that the silk-gland system of *Argiope modesta*, which is responsible for producing spider silk, has distinct properties. Further studies are needed to analyze the chemical components of spider silk produced in different glands of *Argiope modesta*.

Keywords: *Argiope*, spinneret, spinning tubes, spider silk

Article Info

Received 4th October 2022
Accepted 15th April 2023
Published 20th December 2023

ISSN: 1823-7010, eISSN: 2600-7444

Introduction

In tissue engineering, biomaterial scaffolds play an important role as niche providers for cell growth. Spider webs, especially spidroin, are commonly used in regenerative medicine because they are nontoxic and compatible with cell growth. Many studies have shown that spider silk from the web can be a good source of biomaterial that supports for cell growth [1-2]. Our previous study used spider silk as a scaffold material, and bio-ink showed that spider silk supported cell growth and facilitated stem cell differentiation [3-4]. Due to the significant role of spider silk, the study of the glands that produce various types of spider silk is important.

The family *Araneidae* is highly diverse, and one of its species is the *Argiope modesta*. The *Argiope modesta*, or garden spider, is an orb-web spider that produces spiral wheel-shaped webs. Webs produced by the genus *Argiope* are frequently used as an alternative foraging tactic [5]. Silk fiber, as a web spider material, has gained interest as a biomaterial in biomedical applications. A wide range of silk produced by spiders has a variety of functions [6]. The diversity of silk varieties is due to the several glands in adult spiders [7].

The spider spins silk fibers through the spinneret. The spinneret is a part of the spider's spinning machinery and is situated in the lower abdomen of the spider. In order to understand the functional specialization of silk fiber manufacturing, it is necessary to examine the structure and organization of the spinneret.

Adult spiders have seven types of silk glands, each with distinctive characteristics. The number and type of glands were different for each species. Each gland produces silk with different compositions and mechanical properties [6-7]. The *A. modesta* silk gland comprises seven basic types: major and minor ampullates, tubuliforms, aggregates, flagelliforms, pyriforms, and aciniforms [8]. Each gland generates silk with different chemical and physical characteristics. The silk gland of *A. modesta* consists of seven fundamental types: minor and major ampullates, aggregates, flagelliforms, tubuliforms, pyriforms, and aciniforms [8]. In our previous research, silk spidroin from *Argiope* sp. web was fabricated into a scaffold and showed the ability to promote human Wharton's Jelly derived Mesenchymal Stem Cells (hWJ-MSCs) differentiation into chondrocytes [4]. Therefore, it is important to study the structure of *Argiope modesta* glands, which have been proven to play an important role in tissue engineering.

Materials and Methods

Adult female *Argiope modesta* spiders were collected from Begonia Park in Lembang, West Java, Indonesia. In the laboratory, the spiders were maintained in a paper box under natural daylight. They were given insect larvae once every two days and water daily. Five spider samples were used in each experiment. The spiders were anesthetized and dissected under a dissecting stereomicroscope prior to the ultrastructural investigation. The spider fibers and spinnerets were removed, fixed in 2.5% glutaraldehyde in cacodylate buffer, and dehydrated in a concentration gradient of 30–100% ethanol. Hexamethyldisilazane (HMDS) solution was added to the samples and incubated overnight for air drying in a fume hood. The samples were then covered in gold and analyzed at a low-vacuum scanning electron microscope (SEM SU3500, Hitachi High-Tech Corporation, Tokyo, Japan) with a 5 kV accelerating voltage.

For histological analysis, the samples were anesthetized and dissected. The spinneret and silk glands of the spiders were carefully removed. The samples were fixed in Bouin's solution and dehydrated in an alcohol series ranging from 30 to 100%. The samples were transferred to xylene and embedded in paraffin. The tissue was sectioned and stained with hematoxylin and eosin (H&E).

Results and Discussion

Spider webs are a source of biomaterials in regenerative medicine, particularly in tissue engineering. Many glands in spiders can produce spider silk, and the structure and output of each gland are different. Thus, it is essential to study the silk gland system in spiders that produce spider silk. This study observes the structure of silk gland system in *A. modesta. Argiope modesta* is an orb-weaving spider because it forms a circular spider web. The web of the *Argiope* has a unique structure called stabilimentum. The stabilimentum is a zigzag silk in the center of the web. It has been hypothesized that the stabilimentum contributes to protection against predators and attractants to its prey [9]. Spiders make webs from silk, a natural fiber made of protein. The main proteins in spider silk are called spidroin, which are good biomaterials owing to their biocompatibility. The morphology of a spider fiber from the *A. modesta* with a diameter of 518 µm is shown in Figure 1. The body of the *Argiope modesta* has two segments: the cephalothorax and abdomen (Figure 2(a)). A spinneret was located at the posterior end of the spider's abdomen (Figure 2(b)). The spinneret is the organ responsible for spinning silk. It was then placed at the posterior end of the ventral surface of the abdomen. The spinneret secretes silk, and the composition of silk depends on the gland that supplies the spinneret.

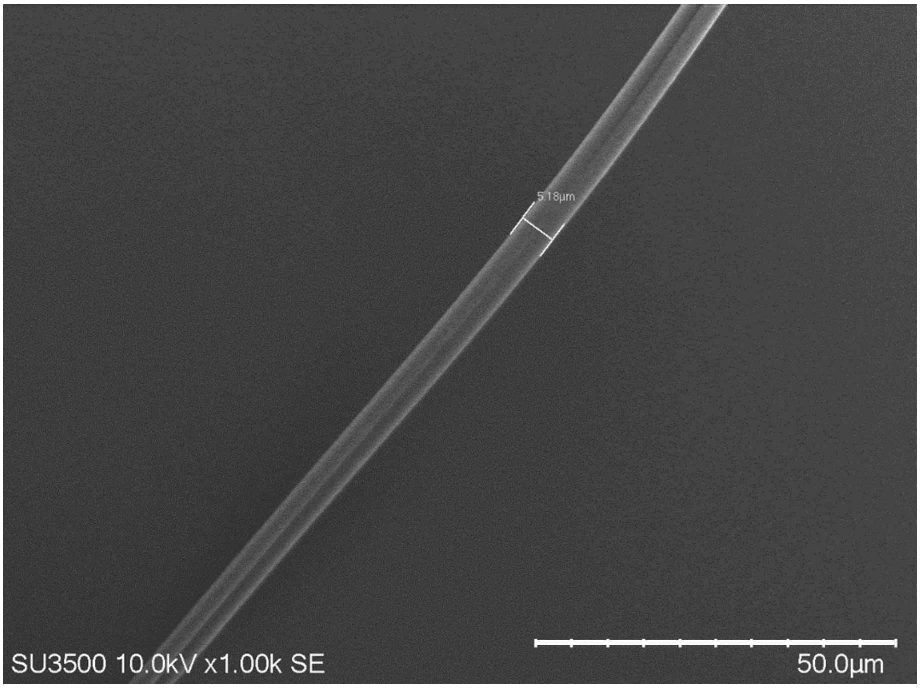

Figure 1: Morphology of *A. modesta* web fiber as viewed by scanning electron microscope.

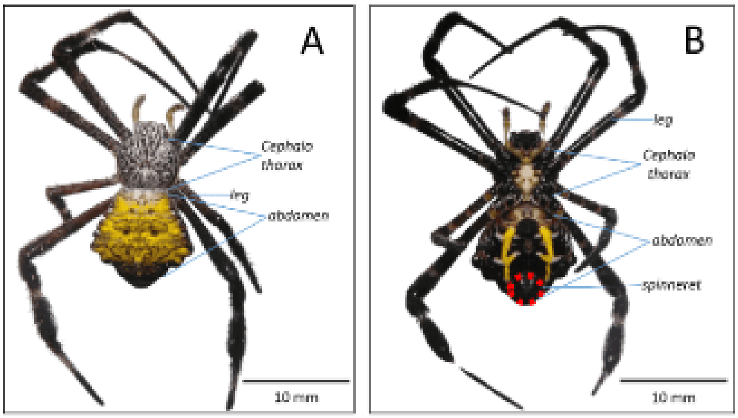

Figure 2: *Argiope modesta* dorsal side (a) and ventral side (b). The spinnerets are a complex spinning device found at the posterior end of the abdomen's ventral surface (red circle).

Argiope modesta typically have three types of spinnerets: the anterior spinneret, median spinneret, and posterior spinneret (Figure 3). The anterior spinneret is the largest of the three, while the middle spinneret is the smallest. The anterior and posterior spinnerets consist of two segments, but the middle spinneret has only one segment.

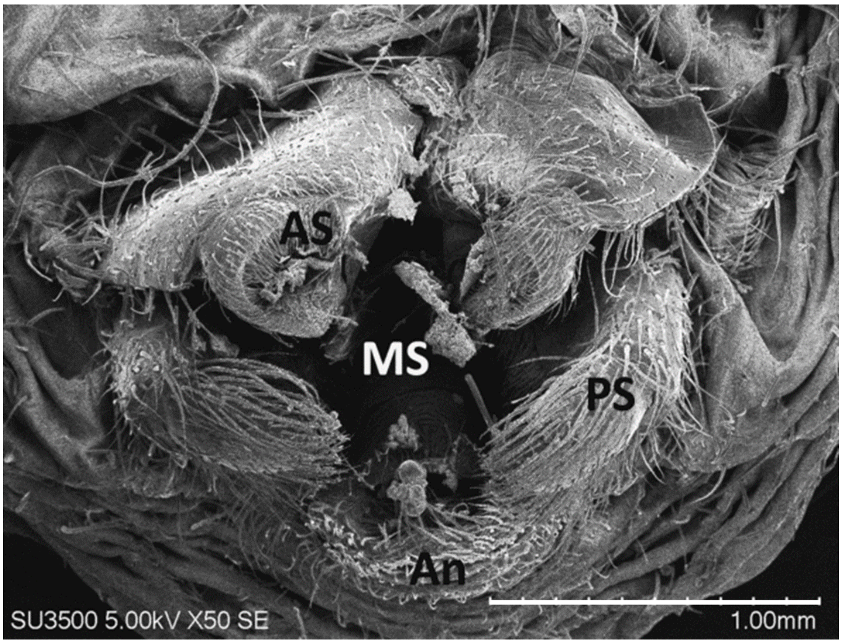

Figure 3: The visualization of scanning electron microscopy of the female *Argiope modesta* spinnerets. There are three pairs of spinnerets: anterior (AS), median (MS), and posterior (PS). The anterior spinnerets are the biggest, while the median are the smallest.

Figure 4 shows one pair of *Argiope modesta* ampullate spigots and more than 200 pairs of pyriform spools scattered over the surface of the anterior spinneret. The morphologies and sizes of the ampullate spigots and pyriform spools were significantly different. The ampullate spigots were attached separately from the pyriform spools, whereas the pyriform spools were structured around the ampullate spigots.

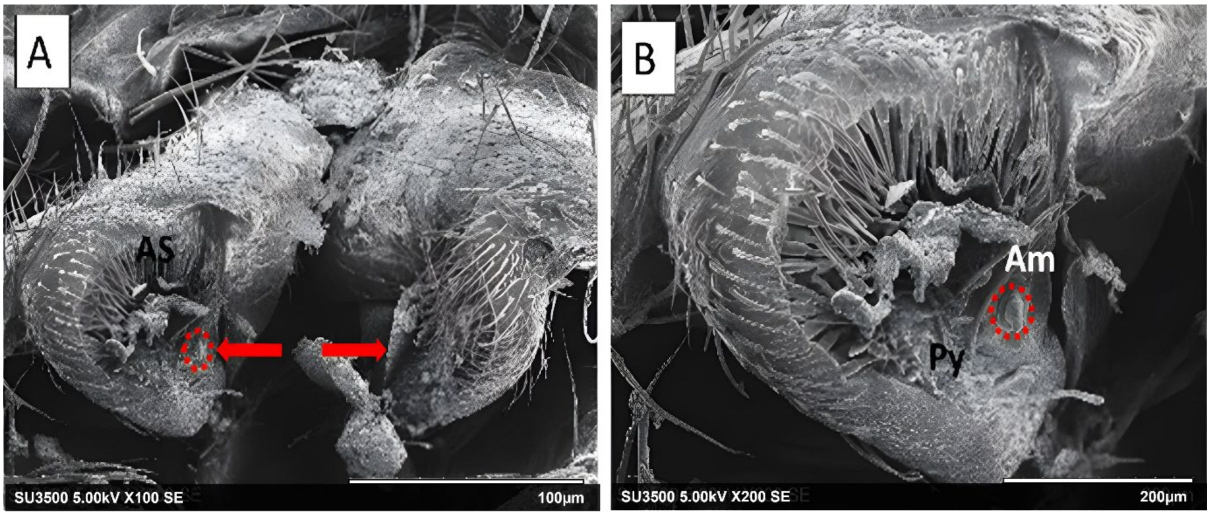

Figure 4: SEM images revealed magnification of one of the anterior spinneret (AS) regions (a) in the female *Argiope modesta* and magnification of AS regions (b). There is an ampullate spigot (Am) designated with arrows and a red circle, and pyriform spools (Py) structured around the ampullate spigot. The ampullate spigot is seen most prominently in the middle of anterior spinneret, apart from the pyriform spools.

In the spinneret area around the spigot in the anterior spinneret, there are other structures, namely, nubbin and tartipore (Figure 5 and 6). Nubbin and tartipore are non-functional cuticle remnants common in the *Araneidae* family [8] and are formed from modified spigot structures. Their presence is important in phylogenetic [10] . These are the distinguishing characteristics of spider species. This study demonstrated that nubbin and tartipore are also present in the anterior spinneret of *Argiope modesta* (Figure 5 and 6) surrounding the ampullate spigot. For each of the anterior spinnerets of an adult female, there is a major ampullate spigot, a major ampullate tartipore, and a major ampullate nubbin, all of which are clearly visible in this image.

Observations conducted using an electron microscope have identified two distinct types of spinning tubes: spigots and spools. The ampullate tube was a spigot, whereas the pyriform tube was a spool. Both spinning tube forms are composed of two segments, the basal segment (Bs) and the terminal segment (Ts), despite their different morphologies and sizes (Figure 6). The basal and terminal segments were joined by a flexible structure, facilitating the spider's ability to manufacture silk. The basal segments of the pyriform spinning tubes were shorter, with elongated and smooth segments. Tartipore (Ta) cuticle structure of the pyriform between the pyriform spinning tube (Figure 6(b)).

Three types of spinning tubes are present in the median spinneret: minor ampullate, tubuliform, and aciniform. There was one pair of tubuliform ampullate spigots located in the median spinneret, and more than 250 pairs of aciniform spigots (Figure 7). The ampullate spigot is also found in the median spinneret (Figure 7(b)) and is smaller than the same ampullate spigot in the anterior spinneret. In the median spinneret, only one ampullate spigot is functional, whereas the rest are converted into a structure called a nubbin.

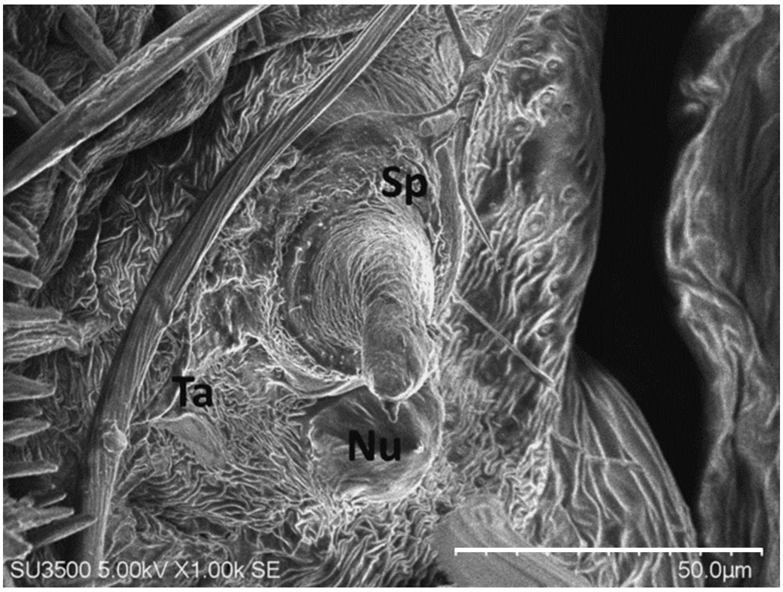

Figure 5: An ampullate spigot on the anterior spinneret of a female *Argiope modesta*. In addition to functional spigots, there remain some non-functional cuticles. The anterior spinnerets show the major ampullate spigots (Sp), nubbin (Nu) and tartipore (Ta)

There are four types of spinning tubes in the posterior spinneret of the female argiope modesta: flagelliform, aggregate, tubuliform, and aciniform. Flagelliform, aggregate, and tubuliform are spigot-type tubes, whereas the aciniform is a spool-type tube (Figure 7). In the spinning region, a spigot and a large number of spools were clearly visible. Both the spigot and spool are composed of terminal and basal segments. There are two pairs of tubuliform glands and more than 200 pairs of aciniform glands on the posterior spinneret of an adult Argiope modesta. Flagelliform spigots and two aggregate spigots comprise the triad spigot complex.

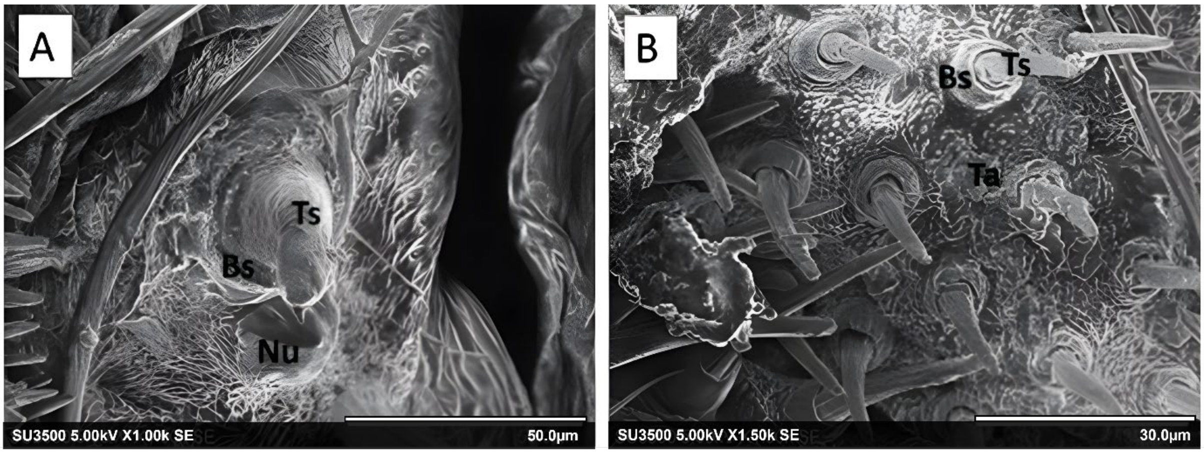

Figure 6: Spinning tubes of the female *Argiope modesta*. A variety of spinning tubes are found on the surface of the spinnerets. (a) The ampullate gland's spinning tube is attached to a bullet-shaped tube with a basal segment (Bs) and a terminal segment (Ts) and (b) The pyriform gland's spinning tube is attached to the cannon-shaped tube. Among the pyriform tubes are cuticle remnants termed tartipore (Ta).

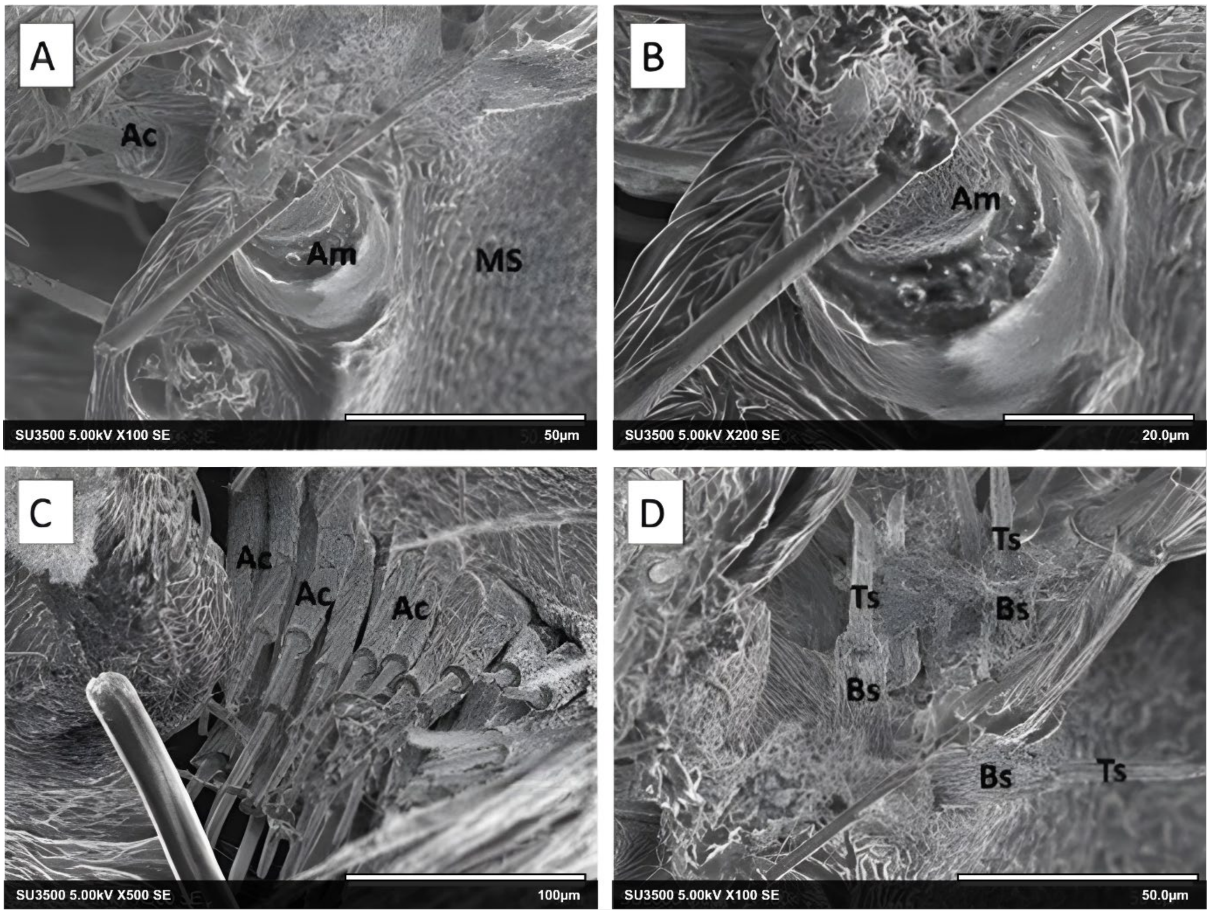

Figure 7: Morphology of the posterior spinneret of an *Argiope modesta*. (a) Aciniform glands (Ac) are attached to the spool-type spinning tube, (b) Ampullate gland's spinning tube is attached to a bullet-shaped tube with a basal segment (Bs) and a terminal segment (Ts), (c) Magnification of aciniform glands and (d) Magnification of ampullate glands.

Each spinneret contained glands that produced silk. Typically, each gland is attached to a spinning tube via which silk is expelled. However, different species of spiders may be differentiated based on spinneret morphology, which can be determined by spinneret modification, number of spinning tubes, spinning tube shape, and silk gland anatomical features [8]. One unique feature is that it belongs to the family Araneidae, also known as the orb-web spider. This type of spider can produce seven types of silk [8] because of its seven silk glands, each of which is connected to a spinning tube with a variety of spinning systems and structures, as revealed in this group of spiders in previous studies [11].

Seven varieties of spinning tubes have been identified in *Argiope modesta*, including the major ampullate, minor ampullate, tubuliform, aggregate, flagelliform, pyriform, and aciniform. These seven tubes are present in each of the three pairs of spinnerets of the *Argiope modesta*. The anterior spinneret of the orb-weaver spider contains large ampullate spigots and pyriform spigots. The structures of the minor ampullate, aciniform, and tubuliform spigots constitute the median spinneret. Finally, its posterior spinneret contains the structures of the aciniform, tubuliform spigots, aggregates, and flagelliform spigots [9], as shown in Figure 8.

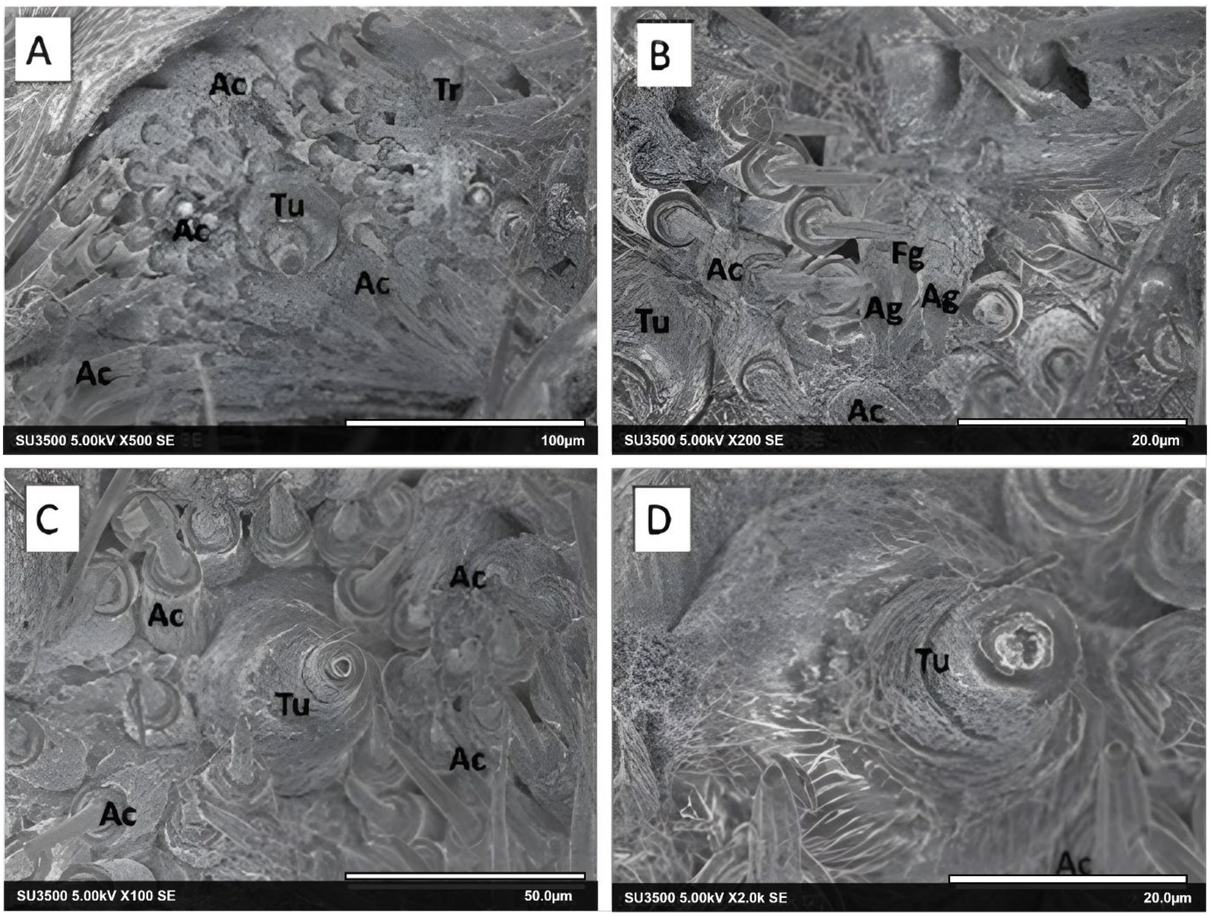

Figure 8: The visualization of the posterior spinneret of the orb-web spider *Argiope modesta*. (a) The female spider's posterior spinning tubes: The tubuliform (Tu) glands are attached to spigot-type spinning tubes, while aciniform (Ac) glands are connected to spool-type tubes, (b) A flagelliform gland (Fg) and two aggregate (Ag) spigots arranged as a complicated spinning device known as the triad spigot (Tr), (c) The tubuliform spigots in close proximity to the triad spigots at the lateral margin of the posterior spinneret and (d) The magnification of the tubuliform spigots.

Based on their form, spinning tubes can be divided into two distinct categories. Spigots are shaped like bullets and are larger and smaller in quantity than bullets. Spigots comprise the major ampullate, minor ampullate, tubuliform, flagelliform, and aggregate in the *Argiope* sp. [8]. Spools resemble cannons, and are smaller and more abundant than spigots. Pyriform and aciniform in *Argiope* sp. are classified as spools [8]. Despite their distinct shapes, the two types of spinning tubes had identical base and tip segments.

It is genewrally accepted that the ampullate glands that are connected to the anterior spinneret are the "major" ampullate glands, while the ampullate glands that are connected to the posterior spinneret are the "small" ampullate glands [8].The same rule applies to the spinning tubes. The ampullate spinning tubes in the anterior spinneret are called major ampullate spigots, while the ampullate spinning tubes in the median spinneret are called minor ampullate spigots.

Before making histological sections, scanning electron micrographs were also taken of the spider's abdomen from front to back. However, because to the disintegration of the abdominal contents, only a few anterior-posterior sections were successful. Paraffin sectioning made the structures of the glands more obvious. Histological analysis using hematoxylin-eosin staining revealed the structure of spider silk glands.

Figure 9 depicts the aciniform gland, aggregate gland, and flagelliform gland located nearby. All three glands are found in the posterior-median part. The aciniform gland generates aciniform silk, which is used for wrapping prey and lining the internal soft layer of egg cases [7]. The aggregate gland can be seen clearly in hematoxylin-eosin staining. The aggregate gland produces aggregate silk that the spider secretes as droplets on specific structures. Aggregate silk has a glue-like characteristic because the main component is glycoprotein. The aggregate silk protein is glycosylated and involved in the stickiness of the glue [7].

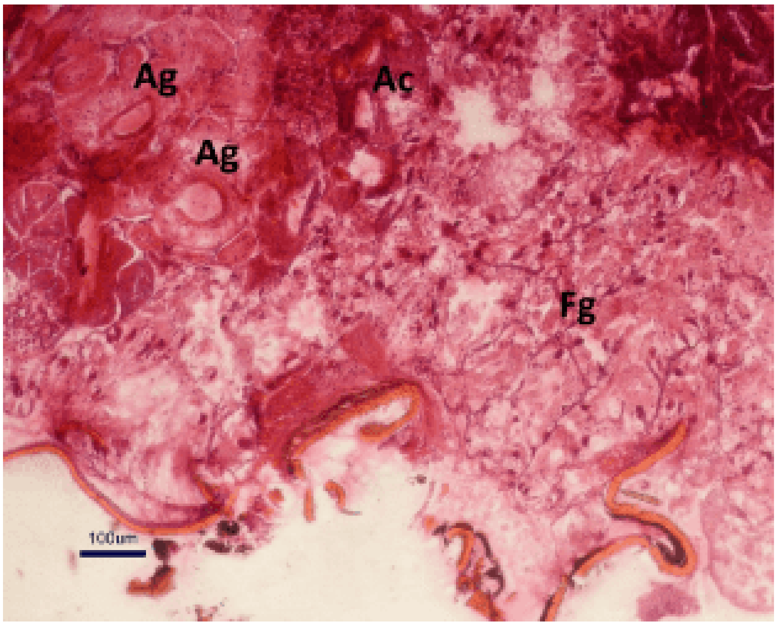

Figure 9: The spinning apparatus in the *Argiope modesta*. Ag: aggregate gland; Ac: aciniform gland; Fg: flagelliform gland. (Scale bar = 100 µm)

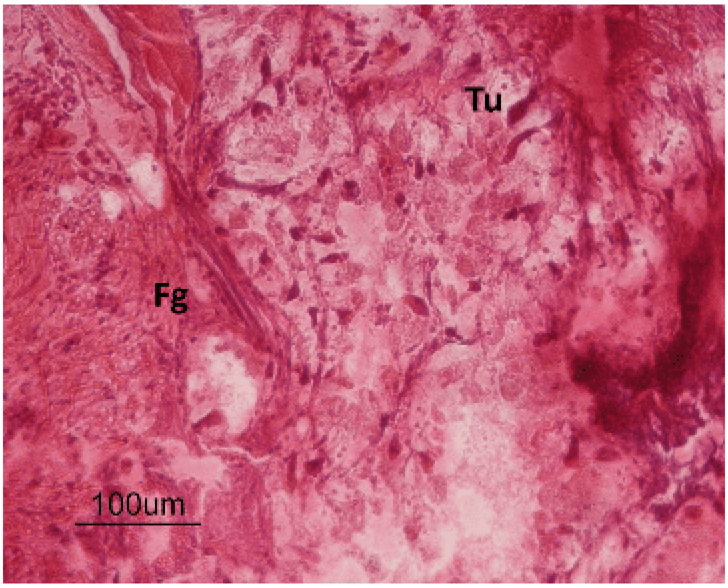

Figure 10: Histological structure of the tubulliform gland (Tu). (Scale bar = 100 µm)

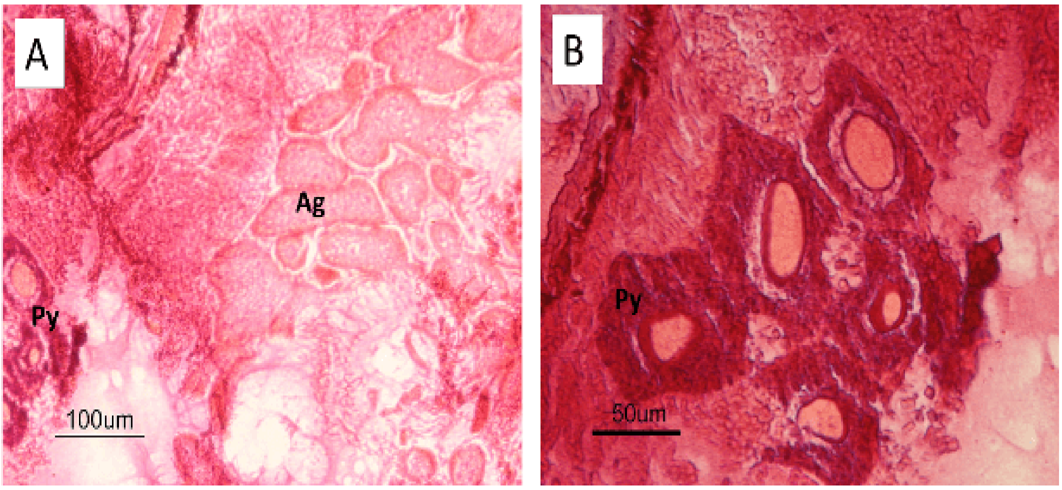

Figure 11: Histological structure of (a) pyriform (Py) located near the aggregate gland (Ag) and (b) magnification of the pyriform/ spinning tube region.

The tubuliform gland can be seen in Figure 10. Only female spiders have the tubuliform gland, which is responsible for producing tubuliform silk [7]. Tubuliform silk has a role in ovary development and also in the protection of spider eggs in their egg cases [7]. In Figure 11, a cluster of pyriform glands may be observed. The lumen of pyriform glands is pear-shaped, and a small duct leads to the spigot [12]. The pyriform gland or spinning tube secretes pyriform silk, which is utilized to generate disks for attaching silk to different surfaces [7]. The adhesiveness of pyriform silk is its defining property.

Conclusions

The structures of the major ampullate, minor ampullate, aggregate, tubuliform, flagelliform, pyriform, and aciniform spinning tubes on the anterior, middle, and posterior spinnerets of *Argiope modesta* are shown by scanning electron microscopy. The aggregate gland, flagelliform gland, aciniform gland, and tubuliform gland can all be seen clearly. The silk gland system of *A. modesta* has unique characteristics. Due to the fact that spiders have multiple silk glands and the chemical properties of the spider silk produced by each gland are unknown, additional research is required to study the chemical properties of the silk produced by each gland.

Acknowledgements

The authors thank the Research Center of Nanoscience and Nanotechnology (Institut Teknologi Bandung) for the help with the electron microscope. We would also like to thank Safira Meidina Nursatya for the discussion.

Author Contributions

All authors contributed toward data analysis, drafting, and critically revising the paper and agree to be accountable for all aspects of the work.

Disclosure of Conflict of Interest

The authors have no disclosures to declare

Compliance with Ethical Standards

The work is compliant with ethical standards

References

[1] Salehi, S., Koeck, K. & Scheibel, T. (2020). Spider Silk for Tissue Engineering Applications. *Molecules*. 25(3), 737.

[2] Hardy, J. G., Torres-Rendon, J. G., Leal-Egaña, A., Walther, A., Schlaad, H., Cölfen, H. & Scheibel, T. R. (2016). Biomineralization of Engineered Spider Silk Protein-Based Composite Materials for Bone Tissue Engineering. *Materials*. 9(7), 560.

[3] Barlian, A., Saputri, D. H. A., Hernando, A., Khoirinaya, C., Prajatelistia, E. & Tanoto, H. (2022). Spidroin Striped Micropattern Promotes Chondrogenic Differentiation of Human Wharton's Jelly Mesenchymal Stem Cells. *Scientific Reports.* 12(4837), 1-15.

[4] Barlian, A., Judawisastra, H., Ridwan, A., Wahyuni, A. R. & Lingga M. E. (2020). Chondrogenic Differentiation of Wharton's Jelly Mesenchymal Stem Cells on Silk Spidroin-Fibroin Mix Scaffold Supplemented with L-Ascorbic Acid and Platelet Rich Plasma. *Scientific Reports.* 10(19449), 1-18.

[5] Hauber, M. E. (1998). Web Decorations and Alternative Foraging Tactics of The Spider Argiope appensa. *Ethology Ecology & Evolution*. 10(1), 47-54.

[6] Vollrath, F. (1999). Biology of spider silk. *International Journal of Biological Macromolecules.* 24(2-3), 81–88.

[7] Humenik, M., Scheibel, T. & Smith, A. (2011). Spider Silk: Understanding the Structure-Function Relationship of a Natural Fiber. In *Molecular Assembly in Natural and Engineered Systems*, vol. 103. Ed. Howorka, S. (Progress in Molecular Biology and Translational Science, London), pp. 131-185.

[8] Moon, M. J. (2012). Organization of The Spinnerets and Spigots in The Orb Web Spider Argiope bruennichi (Araneae: Araneidae). *Entomological Research*. 42, 85-93.

[9] Blackledge, T. A. (1998). Stabilimentum Variation and Foraging Success in Argiope aurantia and Argiope trifasciata (Araneae: Araneidae). *Journal of Zoology.* 246, 21-27.

[10] Scharff, N., Coddington, J. A., Blackledge, T. A., Agnarsson, I., Framenau, V. W., Szűts, T., Hayashi, C. Y. & Dimitrov, D. (2020). Phylogeny of the orb-weaving spider family Araneidae (Araneae: Araneoidea). *Cladistics.* 36(1), 1-21.

[11] Eberhard, W. G. (2010). Possible Functional Significance of Spigot Placement on The Spinnerets of Spiders. *Journal of Arachnology.* 38(3), 407-414.

[12] Wolff, J. O., Grawe, I., Wirth, M., Karstedt, A. & Gorb, S. N. (2015). Spider's Super-Glue: Thread Anchors are Composite Adhesives with Synergistic Hierarchical Organization. *Soft Matter*. 11, 2394-2403.