

Microstructure of the Silk Spinning Nozzles in the Lynx Spider, *Oxyopes licenti* (Araneae: Oxyopidae)

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Abstract: The lynx spiders are free wandering spiders with long spines on their legs. They do not build web, but hunt small insects on plants. In spite of the facts that the wandering spiders do not produce webs for prey-catching, they also have silk apparatuses even though the functions are not fully defined. This paper describes the microstructural organization of the silk-spinning nozzles and its silk glands of the lynx spider, Oxyopes licenti, revealed by the field emission scanning electron microscope (FESEM). The silkspinning nozzles of this spider were identified as three groups: ampullate, pyriform and aciniform glands. Each group of silk gland feed silk into one of the three pairs of spinnerets. Two pairs of major ampullate glands send secretory ductules to the anterior spinnerets, and another two pair of minor ampullate glands supply the middle spinnerets. In addition, the pyriform glands feed silk into the anterior spinnerets (25-30 pairs in females and 24-40 pairs in males), and the aciniform glands send ductules to the middle (9-12 pairs in females and 7-11 pairs in males) and the posterior spinnerets (16-20 pairs in females and 16-17 pairs in males). Among these, the ampullate one is the most predominate gland in both sexes. However the flagelliform and the aggregate glands which had the functions of cocoon production or adhesive thread production in other webbuilding spiders were not observed at both sexes of this spider.

Key words: Microstructure, silk, nozzle, lynx spider, *Oxyopes licenti*

Although the araneid spiders produce various kinds of silk fibers, which are indifferently used for maintaining their life, the main function of spider silk is prey-catching. Recently, the silk glands and their functional spinning apparatuses are regarded as important characteristics of the modern spiders. Because it now seems that all spiders who

actively hunt their prey, or who use little or no silk in preycatching, are descendant of web building spiders (Shear 1994).

Previous reports have also demonstrated that the functional specialization of silk producing apparatus involves precise modifications of the shape of the spinnerets, the number and morphology of spigots, and anatomical characteristics of the silk glands (Peters, 1987; Shear, 1994; Moon and Tillinghast, 2004). Thus, the variations in the composition of the spinning apparatus of araneid spider have studied by many workers (Coddington, 1986; Kovoor, 1987; Nentwig and Heimer, 1987; Peters and Kovoor, 1991; Park and Moon, 2002; Moon and An, 2006).

The lynx spiders are members of the family Oxyopidae. Some members of the genus *Oxyopes* are abundant enough to be important in agricultural systems as biological control agents (Coddington and Levi, 1991). The lynx spider, *Oxyopes licenti*, makes no web to catch prey. They use their silk as a dragline and for protecting their eggs (Foelix, 1996). Although, they are the most abundant and conspicuous spiders in temperate areas, little is known about their spinning systems both of spigots and silk glands.

This paper describes the microstructural organization of the silk spinning system in the lynx spider, *Oxyopes licenti*, especially its functional morphology of the spinning nozzle revealed by scanning electron microscopic observations.

MATERIALS AND METHODS

Adult individuals of the lynx spider, *Oxyopes licenti* Schenkel, 1953 (Araneae: Oxyopidae) were collected in a local area near Cheonan campus of Dankook University, Cheonan, Korea. All spiders were maintained under ambient conditions with natural lighting in wooden frames with glass plates on the front and back, and fed insects and water daily. Specimens were anesthetized with CO₂ and dissected under a dissecting light microscope in a drop of spider

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Ringer's solution consisting of 160 mM NaCl, 7.5 mM KCl, 4 mM CaCl₂, 1 mM MgCl₂, 4 mM NaHCO₃, 20 mM glucose, pH 7.4 (Groome et al., 1991).

For scanning electron microscopic examination, the whole abdomen which containing a total set of spinnerets were gently removed and fixed in a mixture of 2% paraformaldehyde and 2.5% glutaraldehyde buffered with 0.1 M phosphate buffer at pH 7.4. Postfixation was performed with 1% osmium tetroxide in the same buffer and washed several times in 0.1 M phosphate buffer following fixation.

The specimens were then dehydrated in ascending concentrations of ethanol from 30 to 100% (1 hour at each concentration, with one repeat at 100% ethanol). The specimens were then transferred to hexamethyldisilazane (HMDS) and allowed to air dry. All samples were coated to a thickness of approximately 20 nm with gold-palladium alloy using a sputter coater and examined on a Hitachi S-4300 (Hitachi Co., Japan) field emission scanning electron microscope (FESEM) operated with accelerating voltage of 5-20 kV.

RESULTS

The silk-spinning apparatus of the lynx spider, Oxyopes licenti, is composed of three pairs of spinnerets: the

Table 1. Silk-spinning apparatuses of the lynx spider, Oxyopes licenti

Spinneret	Gland type -	No. of pairs	
		Female	Male
Anterior	Ampullate	2	2
	Pyriform	25-30	24-40
Middle	Ampullate	2	1-2
	Aciniform	9-12	7-11
Posterior	Aciniform	16-20	16-17

anterior, middle, and posterior spinnerets. Of the three pairs of spinnerets, the anterior pairs of both sexes are the most prominent. The anterior and posterior pairs of spinnerets consist of two segments each, and the middle pair comprises only a single segment (Fig. 1A, B). The silk glands are associated with a particular type of spigots on each spinnerets and the silk spigots of both sexes are associated with a particular type of spinning tubes on each silk glands, namely, ampullate, pyriform and aciniform glands (Table 1).

Even at low magnification, two types of spinning tubes could be distinguished; there were a few large sized spigots and numerous small hair-like spools. The major and minor ampullate glands are connected to the spigot-type spinning tubes, and the pyriform and aciniform glands are connected



Fig. 1. Scanning electron micrographs of the silk spinning apparatus, which is composed of three pairs of spinnerets in the lynx spider, *Oxyopes licenti*. A, Female spider. B, Male spider. The anterior pairs of spinnerets of both sexes are the most prominent. AS; anterior spinneret, MS; middle spinneret, PS; posterior spinneret. Scale bars = 100 μm.

to the spool-type tubes. Both of spigots and spools are composed of two functional segments; the basal and terminal segments (Fig. 1A, B).

The anterior spinnerets comprise two pairs (in both sexes) of ampullate glands and approximately 25-30 (female) or 24-40 pairs (male) of pyriform glands. Another two pairs of ampullate glands and 9-12 (female) or 7-11 pairs (male) of aciniform glands in both sexes are connected on the middle spinnerets, respectively. In addition, the posterior spinnerets comprise approximately 16-20 pairs (female) or 16-17 pairs (male) of aciniform glands, respectively (Table 1).

Ampullate glands

The spigots of the ampullate glands are located on both the anterior and posterior spinnerets. Two pairs of major ampullate glands send secretory ductules to the anterior spinnerets, and another two pairs of minor ampullate glands supplies the middle spinnerets. When viewed from above the spinnerets, two large spinning tubes of these ampullate spigots are the most prominent. The spigot sizes of these minor ampullate glands are somewhat smaller than those of the major ampullate glands on the anterior spinneret (Figs. 2A, 2D, 3A, 3D).

The spinning tubes of the ampullate glands are connected to so-called the bullet-type spigots, and spigots of the ampullate glands are also composed of two main segments; more flexible basal segments and elongated terminal segment commonly. Each silk fiber is spun through small opening of the terminal segment. The middle pairs of the ampullate glands are located in the most posterior position of the middle spinnerets along the body axis. There are no additional spigots corresponding to those of tubuliform glands on the middle spinneret (Figs. 2B, 2E, 3B, 3E).

Pyriform glands

In addition to the large spinning nozzles (spigots) of the major ampullate glands, numerous small spinning nozzles (spools) of the pyriform glands are also present at the anterior spinneret. Approximately 24 to 40 pairs of the pyriform glands were counted in adult spiders. These spooltype nozzles are quite different from those of the spigottype nozzles of the ampullate glands with respect to size and morphology. These spinning nozzles are eccentrically distributed on the whole spinning surfaces of the spinnerets along the long axis of the body. The cuticular nozzles of this pyriform glands also comprise basal and elongated terminal segments with fine tips (Figs. 2C, 2F, 3C, 3F).

Aciniform glands

There are two kinds of spinning nozzles on the middle pairs of spinnerets - two pairs of ampullate glands and several pairs of aciniform glands. The aciniform glands in both sexes of spiders send secretory ductules to the middle and to the posterior spinnerets, respectively (Figs. 3A, 3D, 4A, 4D).

The microstructure of the nozzles of the aciniform glands differ from those of the ampullate and pyriform glands that were observed on the anterior spinnerets. The cuticular nozzles of the aciniform glands are located in a more anterior position than those of the ampullate spigots. The spinning nozzles of these aciniform glands are connected to the cannon-type tubes instead of the bullet-type spigots of the ampullate glands (Figs. 3B, 3E, 4B, 4F).

Both sexes of this spiders have neither the tubuliform spigots for cocoon production nor a typical "triad" spigots which composed of a pair of flagelliform spigots and two pairs of aggregate spigots for capture thread production. So, the posterior spinnerets have only one type of spinning tubes on their spinning surfaces. The spools of the aciniform glands also comprise basal segments and elongated terminal segment that have fine tips. The terminal segments of the aciniform nozzles are spread radically toward its long axis of spinneret (Figs. 3C, 3F, 4C, 4F).

DISCUSSION

Spiders can be classified by their shape and number of components of their silk-spinning apparatus. Even though, the spinning apparatus often undergoes adaptative variations, some basic characteristics usually remain unchanged at the familial level (Peters, 1987; Shear, 1994). It has been known that the functional specialization of the silk-spinning apparatus involves precise modifications of the spinnerets, the number and morphology of spigots, and anatomical characteristics of the silk glands (Peters and Kovoor, 1991; Foelix, 1996).

Although, spiders produce various kinds of silks, which are used for the remarkably diverse silk constructs (Denny, 1976; Coddington, 1986), the main function of the spider silk is prey-catching (Nentwig and Heimer, 1987). According to their behavioral characteristics, the lynx spiders are classified as one of typical hunting spiders somewhat similar to the wolf spiders and the jumping spiders (Coddington and Levi, 1991). Thus, they do not spin webs for prey-catching and do not wrap their prey in silk after biting. Nevertheless, they also possess silk-spinning apparatus even though the exact functions are still not fully defined.

However, it has been revealed that the silk-spinning apparatuses of this lynx spider do not exactly correspond to the patterns found in those of wandering wolf spiders (Moon, 1998) or other wandering Salticid spiders (Kovoor, 1987). On the basis of fine structural analysis using scanning electron microscope, only three types of silk glands were identified in this lynx spider, *Oxyopes licenti*;

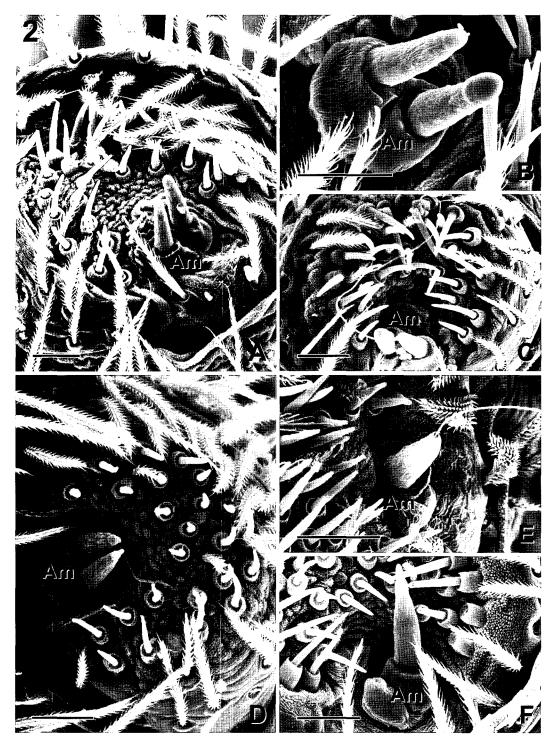


Fig. 2. Scanning electron micrographs of the anterior spinnerets of female *Oxyopes licenti* (A-C). The anterior spinneret has two types of spinning tubes, including two large spigots of the major ampullate gland (Am) and 25-30 small spinning tubes of pyriform glands (Py). All of these spigots are composed of two main segments; more flexible basal segment and slender apical segment. The anterior spinneret of the male spider also has two types of spinning tubes; two pairs of the major ampullate gland and 24-40 pairs of the pyriform glands (D-F). Scale bars = 20 μm.

the ampullate, the pyriform and the aciniform glands. The two pairs of ampullate glands and the pyriform glands were connected with the anterior spinnerets; another two pairs of the ampullates and the aciniforms were connected with the middle spinnerets, and the rest of aciniform glands were connected with the posterior spinnerets. Few isolated reports showed that wandering salticid spiders of both sexes also have the same three kinds of glands (Kovoor,

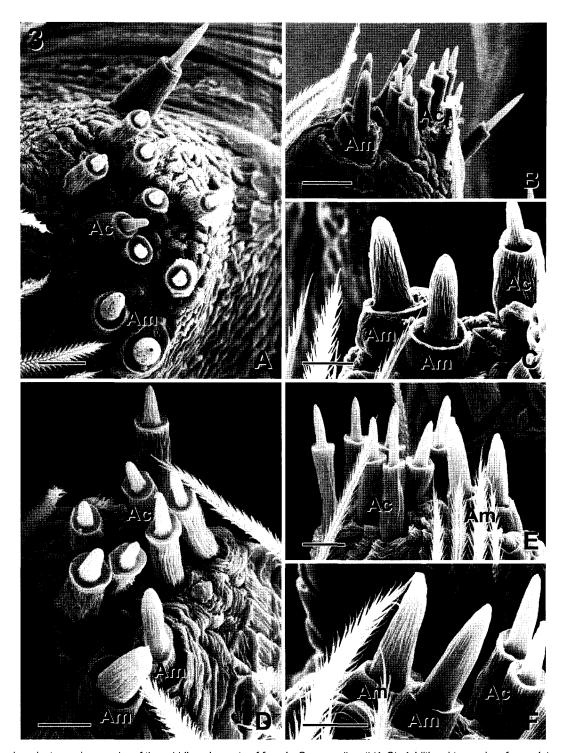


Fig. 3. Scanning electron micrographs of the middle spinnerets of female $Oxyopes\ licenti$ (A-C). Additional two pairs of ampulate spigots (Am) and 9-12 pairs of aciniform spigots (Ac) are distributed on this spinneret. The aciniform spigots are different from those of pyriforms that observed on the anterior spinneret. Male spiders also have two pairs of the ampullate spigots and 7-11 pairs of the aciniform spigots (D-F). The apical segments of the ampullate spigots are bigger than those of the aciniform spigots. Scale bars = 10 μ m (A,C-F) and 20 μ m (B).

1987; Peters and Kovoor, 1991), and ampullate glands are in four pairs (Moon, 1998; Park and Moon, 2002).

Comparing with other araneid spiders, the silk-spinning apparatus of this lynx spider is considered as the simplest

system among the araneid spiders with the exception of some exclusively primitive species (Kovoor, 1987; Peters and Kovoor, 1991). It has been revealed by the precise investigation with scanning electron microscope that even

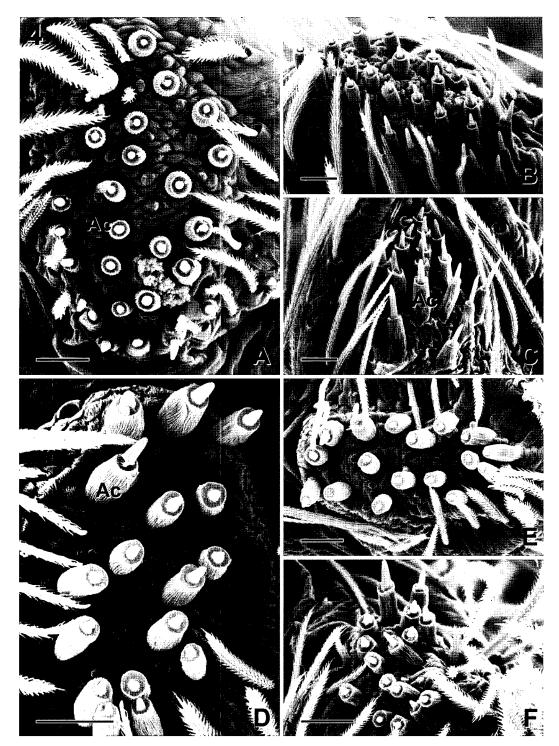


Fig. 4. Scanning electron micrographs of the posterior spinnerets of female *Oxyopes licenti* (A, B). On the posterior spinneret, only one type of the aciniform spigots (Ac) can be seen. Approximately, 16-20 pairs are counted in female spiders. Even in female spiders, the spigots of tubuliform glands for cocoon production as well as the triad spigots for capture thread production are not observed. The posterior spinneret of the male spider also has approximately 16-17 pairs of the aciniform spigots which looks like fine syringes (D-F). These aciniform spigots are also composed of a basal segment and an elongated terminal segment. Scale bars = 20 μm.

the female spiders possess none of the tubuliform glands for cocoon production and the so-called "triad" spigots for capture thread production. Recently, this type of simple spinning apparatus has been reported one of the crab spider, Misumenops tricuspidatus (Moon and An, 2005).

Previous research has demonstrated that most eccribellate orb-web spiders produce a gluey material from a complex structure of a flagelliform and two aggregate glands. This functional unit known as the "triad" spigots are connected to posterior spinneret and are used for spinning the sticky spirals of capture thread in a web (Peters, 1987; Peters and Kovoor, 1991). However, both sexes of *Oxyopes licenti* spider totally lack the "triad" spigots similarly to those of the other wandering spiders such as the wolf spider, *Pardosa astrigera* (Moon, 1998), the funnel-web spider, *Agelena limbata* (Park and Moon, 2002) and the crab spiders *Misumenops tricuspidatus* (Moon and An, 2005).

Most of Araneidae spiders use silk from the two kinds of ampullate glands for making draglines, frame threads and web building (Coddington, 1986). Like the other araneid spiders such as the wandering spiders (Moon, 1998; Moon and An, 2005), and other web-building spiders (Peters and Kovoor, 1991; Park and Moon, 2002; Moon and Tillinghast, 2004; Moon and An, 2006), the ampullate glands were the largest one in the lynx spider, *Oxyopes licenti*.

Previous reports have shown that there are two pairs of ampullate glands for the genus *Araneus* (Tillinghast and Townley, 1986; Townley et al., 1991; Moon and Tillinghast, 2004), genus *Nephila* (Tillinghast and Christenson, 1984) and most of Araneidae spiders (Peters and Kovoor, 1991). However, there are four pairs in this lynx spider similar to those of wandering Salticidae (Kovoor, 1987), Lycosidae (Moon, 1998), Agelenidae (Park and Moon, 2002), and Thomisidae (Moon and An, 2005) spiders. These ampullate spigots send secretory ductules to the both of anterior and middle spinnerets, respectively. This arrangement of the ampullate spigot were commonly reported in most araneid spiders except for a peculiar modification in Theridiidae spiders (Moon and An, 2006).

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