Silk Glands and Capture Nets of *Hydropsyche kozhantschikovi* Martynov (Trichoptera: Hydropsychidae) Larvae[1]

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Abstract Histological studies of the silk glands of the caddisfly larvae of *Hydropsyche kozhantschikovi* Martynov demonstrated that these structure fold twice into a 'Z' shape with the volume in the center comparatively thicker than in other sections. The glands are more slender towards the mouth and unite with the labium. A pair of muscles, an apodeum between the muscles and the body wall, and a tendon between the muscles and the glands were observed within the thorax. We assumed that silk is spewed through these structures. Examination of the material from the inside of the silk glands showed varying composition. The center was loose and heterogeneous, while the surrounding area was homogeneous. These differences were verified through immunohistochemistry. Scanning electron microscopy show that capture nets of *H. kortizantschikovi* larvae have randomly arranged silk strands.

Key Words *Hydropsyche kozhantschikovi*, Trichoptera, silk gland, capture nets

Caddisflies (Order Trichoptera) are one of the most diverse and abundant groups of insects. They live in many kinds of running water and use silken nets as sieves to capture food. Most filter-feeding trichopterans belong to the Family Hydropsychidae (Wallace and Merritt 1980). Hydropsychid larvae construct and inhabit either an elongated or a sac-like, fixed nest or retreat (Noyes 1914, Ross 1967). Generally, they feed on particles that are filtered on the capture net constructed at the entrance to the nest (Edington 1968, Wallace 1976a, Alstad 1982). Hydropsychid larvae, like larvae in other insect orders, secrete silk from the labium (Wallace and Malas 1976b, Cianficconi et al. 1993). Silk that hydropsychid larvae secrete from silk glands can be one of the following three types: (1) retreat-forming silk to which organic or mineral debris usually are attached by mixing pebbles; (2) capture-net silk woven to entrap drifting organic matter in front of the entrance to the nest; or (3) inner-nest silk that is secreted inside the nest just prior to the pupal stage (Rudall and Kenchington 1971, MacKay and Wiggins 1979, Fuller and MacKay 1980, Wallace and Merritt 1980).

Mouth parts of trichopteran larvae have a series of paired glands associated with

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the mandibles. Secretions of mandibular glands are correlated with silk-forming organs. The role of nets in food gathering has been reported (Silvester 1983). Yet, there is a paucity of biochemical studies on the structure of the silk glands of the Hydropsychidae (Park et al. 2003). In this study, we describe the locations, opening structure, and internal secretions of the silk glands, as well as, the nets of *H. kortizantschikovi larvae* using light and scanning electron microscopy.

Materials and Methods

Hydropsyche kozhantschikovi larvae used in these studies were collected from Wangsuk Creek in Gyeong-gi Province, Korea. Silk glands and the capture nets of 20 individuals were examined by light and scanning electron microscopy. The labium and silk glands were dissected in a phosphate buffered saline (PBS) solution and examined with a dissection microscope (Zeiss, Axioskop 2, Bad Wildbad, *Germany*). To provide net-spinning sites, larvae were reared in a glass container (base dimensions 20 × 30 cm) containing 20 g of fine sand and several glass slides propped on their sides. Water within the container was aerated (7.9 mg/L dissolved oxygen) and maintained at 7.0 ± 0.3 pH and 26.5 ± 1°C.

Dissected larvae and capture nets were fixed in 10% formalin in PBS (pH 7.0) for 1 h at room temperature. Following a brief rinse with PBS, the larval fragments were dehydrated in a graded series of ethanol, substituted with isoamyl acetate, dried directly from hexamethyldisilazane, and sputter-coated with gold-palladium before examination by scanning electron microscopy (SEM) (Philips, SEM 515, Eindhoven, Netherlands).

Additional larvae were fixed immediately in 10% formalin. After fixation for 24 h at 4°C, the larvae were washed with PBS (pH 7.0) for 24 h. Fixed specimens were dehydrated in an alcohol series and embedded in paraffin. Embedded sections were cut to 8 µm thickness and stained with hematoxylin-eosin (Spector et al. 1997).

Larvae frozen in liquid nitrogen were embedded in an Tissue-Tek® optimal cutting temperature (OCT) compound. Embedded specimens were sectioned at 30 µm thickness on a cryostat (Zeiss, Bad Wildbad, *Germany*) and fixed in chilled absolute methanol at –20°C. Endogenous peroxidase activity was halted by immersion in 0.6% H_2O_2 for 15 min. After rinsing in PBS, sections were placed in a 1:100 dilution of primary antibodies, HkSG-01 and 03, overnight at 4°C. After washing four times with PBS, the sections were stained with the appropriate biotynylated secondary antibody, visualized using the horseradish peroxydase-linked system that employs diaminobenzoate as a substrate (DAKO, LSAB II kit) (Naish 1989), and microscopically examined with a dissection microscope.

The primary antibodies (HkSG-01 and 03), which are polyclonal antibodies to the silk gland proteins of *H. kozhantschikovi* larvae, were prepared in mice and partially purified by polyacrylamide gel electrophoresis using methods of Park et al. (2003). Silk gland proteins were injected intraperitoneally three times over a 2-wk period, serum was obtained at one month after injection (Smith et al. 1992).

Results and Discussion

Hydropsychid larvae construct capture-nets to trap organic debris suspended in water flowing through the net (Tachet et al. 1992, Gallardo-Mayenco 1998, Greenwood et al. 2003). A well-developed mouth brush and other mouthpart structures are

used in this process (Wallace and Malas 1976c). The labium of *H. kozhantschikovi* larvae has an aperture through which silk is emitted (Fig. 1). This aperture and the associated silk gland are responsible for secretion of silk in running water (MacKay and Wiggins 1979, Wallace and Merritt 1980, Sehnal and Akai 1990). Scanning electron microscopy shows the labium located among the labrum, maxilla and maxillary palps (Fig. 1A). Under higher magnification, the silk gland aperture appears as an elongated, elliptical slit on the ventral surface of the distal end of the labium between the labial palps (Fig. 1B, C).

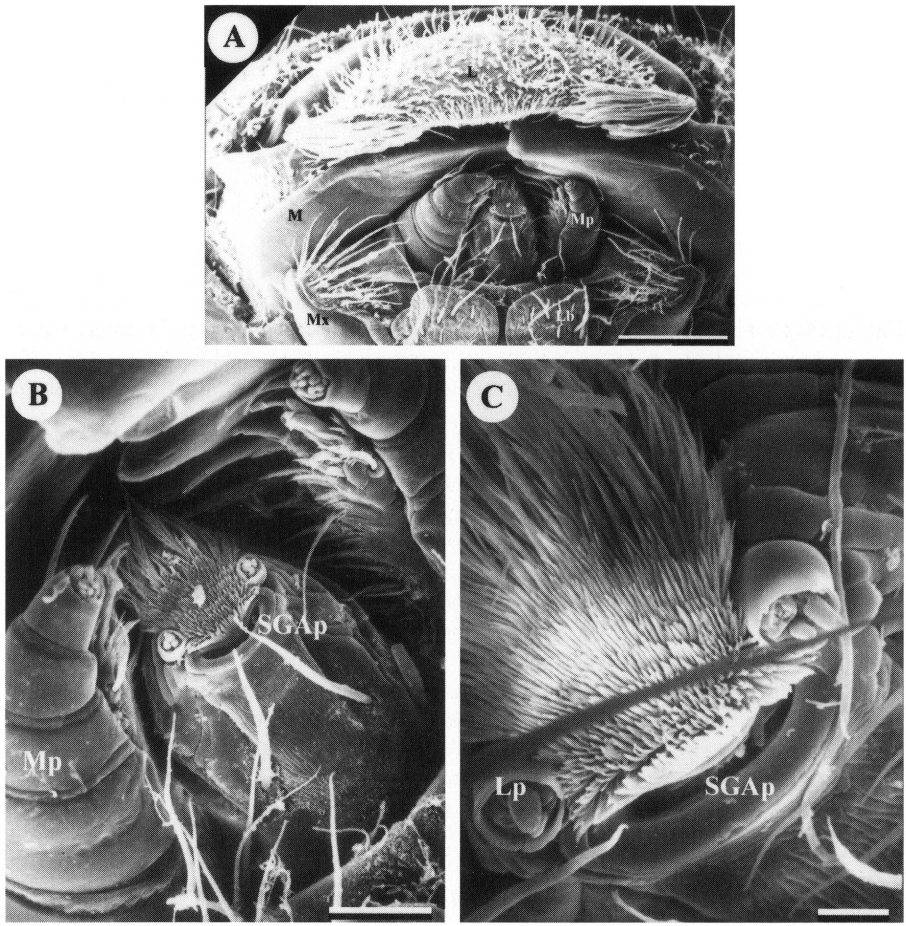

Fig. 1. Photomicrographs of silk gland external aperture on the labium of *H. kozhant schikovi* larvae including an angled front view of head (A), a front view of the mouthpart (B) and magnification of the silk gland external aperture (C). (L, labrum; Lb, labium; Lp, labial palp; M, mandible; Mp, maxillary palp; Mx, maxilla; SGs, silk glands; SGAp, silk gland external aperture). Scale bars = 500 μm (A), 25 μm (B), and 10 μm (C).

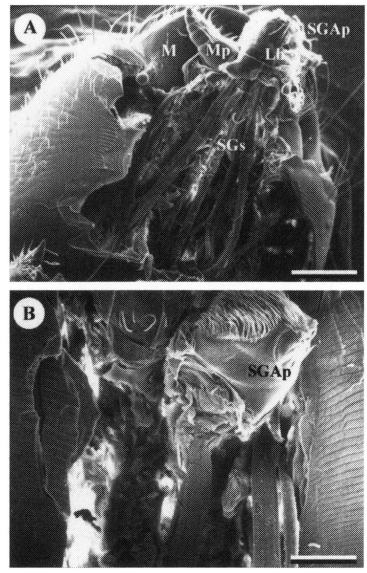

Fig. 2. Photomicrographs of the ventral view of the dissected head of *H. kozhantschikovi* larvae showing the labium (Lb), labium (L), labial palp (Lp), mandible (M), maxillary palp (Mp), silk glands (SGs) and silk gland external aperture (SGAp). Scale bars = 500 μm (A), and 25 μm (B).

Examination of the dissected labium shows a pair of silk glands within the labium (Fig. 2A). Each gland narrows distally towards the external aperture on the labium (Fig. 2). A pair of muscles and a tendon are attached to the dorsal and ventral surfaces of the glands and presumably controls the silk secretory process. These muscles are attached to the body wall and the apodeme. Rast and Braunig (2001) discussed control of the mouthpart and the silk glands.

Serial cross sections of the labium of *H. kozhantschikovi* larvae further show these structures and their interrelationships (Fig. 3). Moving proximally from the distal end of the labium, various structures can be examined and traced. The external aperture is a common opening for the two internal silk glands (Fig. 3A, B). More proximally, cross sections show attachment of the dorsal muscle, tendon, and the ventral muscle to the internal surface of the chitinous exoskeleton and the internal apodeme (Fig. 3C, D). These skeleto-muscular components are presumably involved in extrusion of silk through the aperture.

The silk glands are clearly seen moving posteriorly (Fig. 3E). Each gland was separate and unattached with diameters that increase in size moving posteriorly (Fig. 3F). Examination of cross sections of thoracic and abdominal areas of the larvae showed that the silk glands extended through the thorax into the sixth abdominal segment. They were largest in diameter (470 μm) within the third and fourth thoracic segments. Thoracic cross sections further showed that the glands are convoluted within the third and fourth thoracic segments. The posterior ends of the glands were attached to the external surface of the highest in the sixth abdominal segment.

Cross sections of silk glands stained with acid fuchin displayed two distinct regions

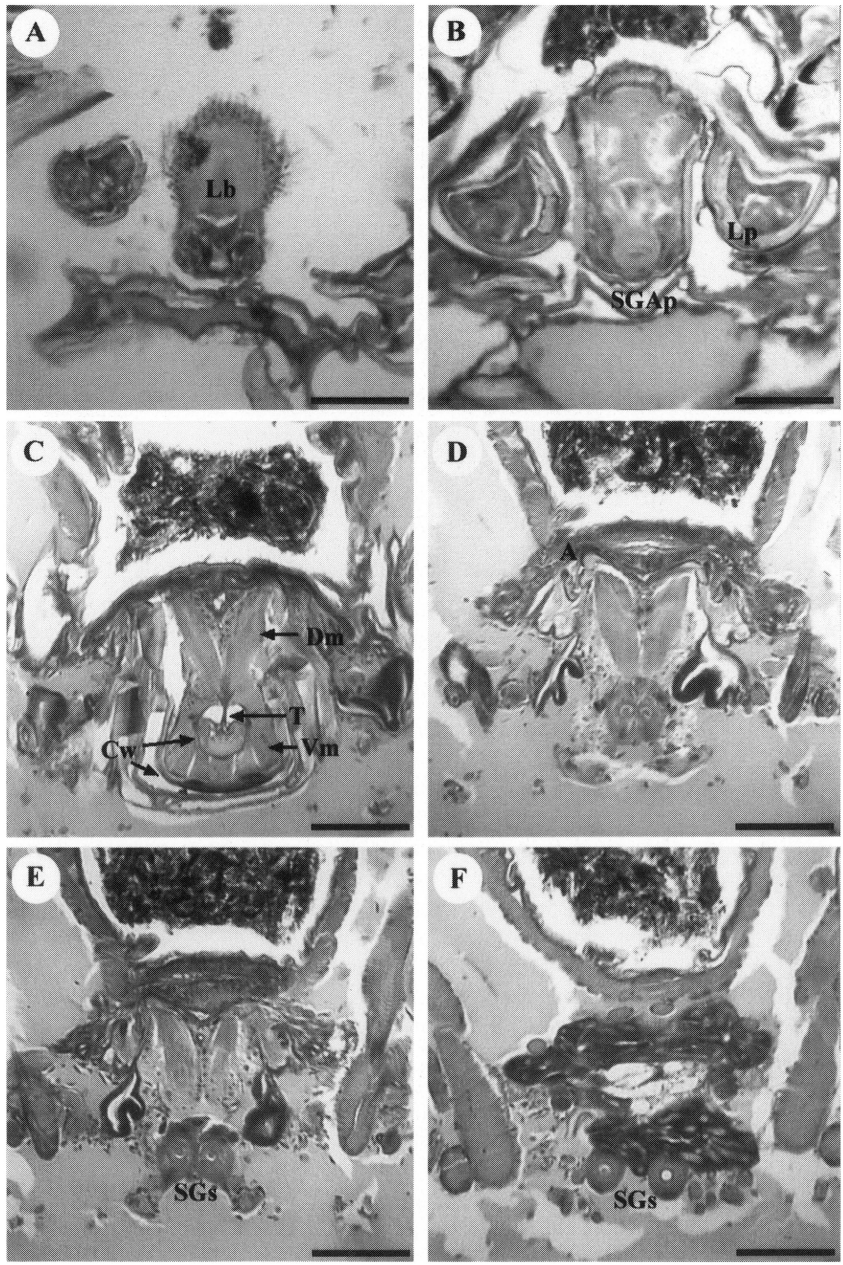

Fig. 3. Photomicrographs of serial sections of *H. kozhantschikovi* larvae. Section numbers are #3 (A), #5 (B), #11 (C), #15 (D), #17 (E), and #22 (F) with A, apodeum; Cw, chitin walls; Dm, dorsal muscle; Lb, labium; Lp, labial palp; M, mandible; Mp, maxillary palp; SGAp, silk gland external aperture; T, tendon; Vm, ventral muscle; SGs, silk glands. (100×). Scale bars = 100 μm.

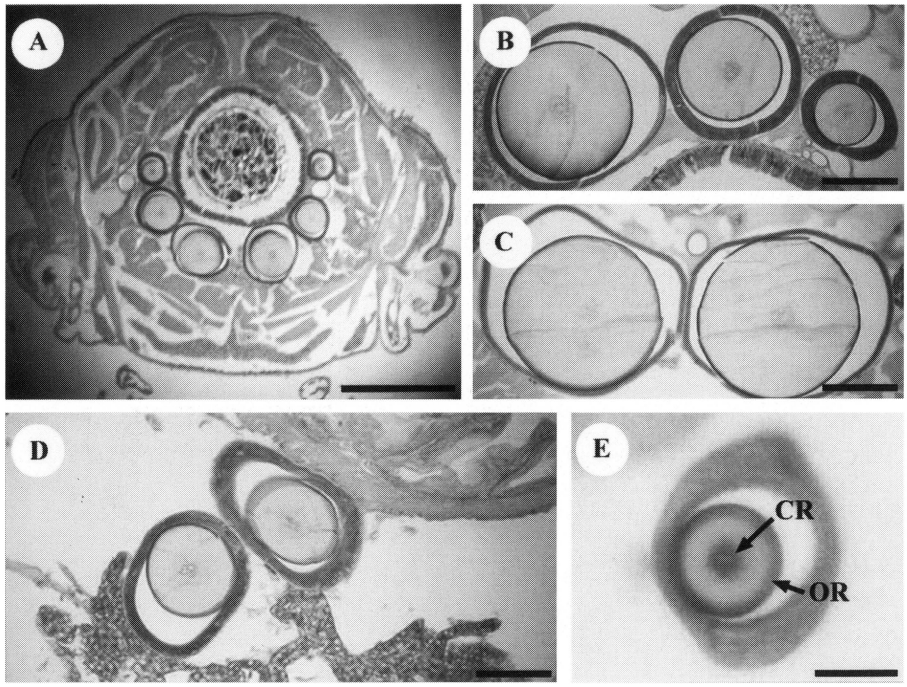

Fig. 4. Photomicrographs of cross sections of *H. kozhantschikovi* larval silk glands showing the third thoracic segment (A, B) a middle of the abdomen (C), a posterior segment of the abdomen (D), and junction of the head with the thorax (E). (CR, central region of silk; OR, outer region of silk). Scale bars = 1 mm (A), 250 μm (B, C), 200 μm (D), and 100 μm.

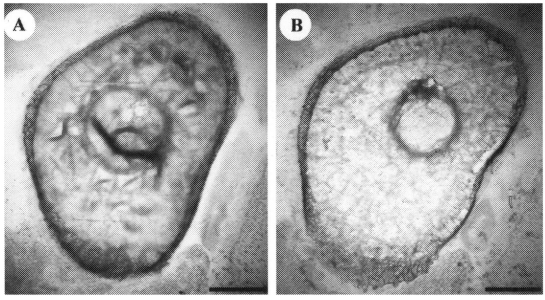

Fig. 5. Differentially stained regions resulting from polyclonal antibodies (HkSGP-1 and 3) showing the central heterogenous matrix (A) and the outer homogenous tissue (B). Scale bar = 250 μm.

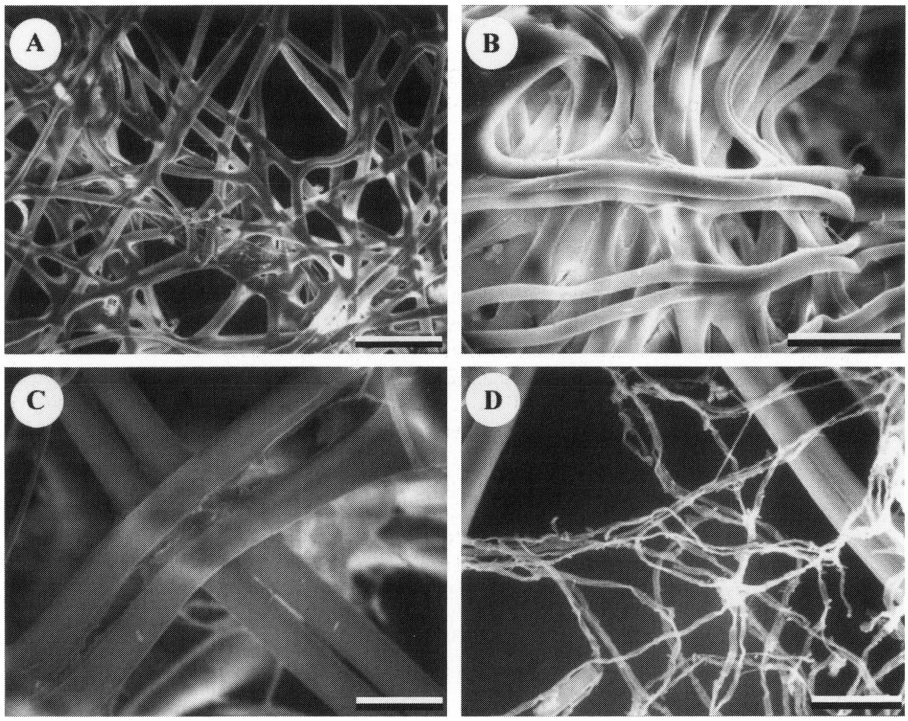

Fig. 6. Photomicrographs of the capture-nets of a *H. kozhantschikovi* mature larva at 250×. (A, bar = 100 μm), 500× (B, bar = 50 μm), and 1000× (C, D, bar = 20 μm).

in the glands (Fig. 4). The center of the glands was characterized by a heterogenous matrix that was not attached to surrounding tissues. A homogenous structure encircled the central heterogenous matrix (Fig. 4B). This same structure pattern was observed throughout the thorax (Fig. 4A, B) and abdomen (Fig. 4C, D). The sizes of these two regions relative to each other remained the same throughout the silk gland, except in the more anterior area of the thorax. Here, the structural integrity of the outer, homogenous tissue encircling the central matrix was less developed and prominent as observed in more posterior areas of the glands (Fig. 4E).

The internal structure of the silk glands was further characterized with immunostaining methods. Using the antibodies HKSG-1 and HKSG-3, produced from distinct fraction with different molecular weights from silk gland secretions, the two aforementioned regions were differentially stained (Fig. 5), thus, revealing the entire structure of silk gland secretions in *H. kozhatschikovi* larvae.

Scanning electron micrographs of the capture nets of mature *H. kozhatschikovi* larvae showed silk strands of two different but distinct textures (Fig. 6). The capture nets of caddisflies consist of silk strands that cross each other to produce a mesh structure, and two different strands can be identified. These strands were distributed at irregular intervals throughout the net (Fig. 6A, B). The thicker strands (20 μm diam) are composed of double parts (Fig. 6C) while the smaller strands (2 μm diam) are attached to the thicker strand (Fig. 6D).

Acknowledgements

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