Secondary Male Sex Characteristics of Hoplolaimus galeatus[1]

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Abstract: The secondary male sex characteristics of *Hoplolaimus galeatus* consisted of caudal alae, two independently retractable spicules and a gubernaculum with two bilobed titillae. The spicules were dimorphic, with the outer one possessing a velum. When both spicules were completely extruded, the only open orifice on the ventral surface of the posterior region was formed by the close association of these two appendages. In specimens where the inner spicule was slightly retracted, the velum almost completely surrounded the inner spicule. When the inner spicule was retracted further, the velum appeared to convolute, closing the orifice described above. *Key Words:* caudal alae, spicule, velum, gubernaculum, titillae.

Critical-point drying is a simple and rapid method for preparing samples for scanning electron microscopy (SEM). The technique was first described by Anderson (1), and has been modified for biological specimen preparation for SEM (2). Presently biological specimens are dehydrated in an alcohol series, transferred through an amyl acetate series and then infiltrated by a transitional fluid, such as liquid CO_2. When the transitional fluid containing the specimen is heated under pressure to its critical point (31 C for CO_2), the liquid phase is converted to the gaseous phase, leaving the specimens dried and nondistorted. Critical-point drying and freeze-drying techniques were used to depict the secondary male sex characteristics of *Hoplolaimus galeatus* Cobb (1913) Thorne, 1935.

MATERIALS AND METHODS

Specimens of *H. galeatus* were extracted from soil by a centrifugation-flotation technique (3), transferred individually to 5 ml of tap water in 20-ml vials, and killed by adding 5 ml of boiling water. The specimens were cleaned by adding 1 drop of Kodak Foto-Flo® to the vials and shaking for 1 min. After the nematodes settled, the supernatant was withdrawn and the nematodes were rinsed twice in tap water and fixed for at least 12 h in 2.5% formaldehyde. The nematodes and fixative were transferred to Bureau of Plant Industry (BPI) dishes and incisions were made with an eye knife into each specimen to facilitate the penetration of dehydration fluids and prevent osmotic pressure differences between the specimen and the medium.

Specimen dehydration was initiated by withdrawing the fixative, replacing it with 20% ethanol, and placing the BPI dishes on a rack stationed above absolute alcohol in a sealed desiccator. After 24 h at 42 C, absolute ethanol was added drop-by-drop to the nematode suspension. After 10 min the liquid was withdrawn and more absolute ethanol added. The procedure was repeated with a series of four concns of ethanol-amyl acetate (4:1, 2:1, 1:1, and 1:2). The last ethanol-amyl acetate solution was replaced with 100% amyl acetate, withdrawn after 10 min and replaced with more amyl acetate.

The nematodes were transferred in approximately 1 ml of amyl acetate to a SEM specimen carrier mounted in a 6-mm-deep well, and covered with 25 μm and 1-mm mesh screens. The well was placed in a pressure chamber, which was subsequently filled with liquid CO_2, and flushed with CO_2 until all of the amyl acetate had been replaced. The chamber was sealed and heated 42 C at 180 atmospheres. The CO_2 was removed slowly to prevent the temperature from dropping below 32 C. The nematodes on the specimen carrier were placed in a vacuum chamber and rotated while a thin layer of evaporated gold was deposited on the nematodes, to make them electrically conductive.

For comparison, specimens of *H. galeatus* were prepared by freeze-drying. They were washed as described for critical-point drying. Both living and Formalin-fixed specimens were quickly frozen in a small drop of deionized water in an aluminum boat floating on liquid nitrogen. The ice was sublimed for 12 h on cooled SEM specimen carriers on a brass block cold sink in a vacuum coater at 10^{-6} Torr. The dry specimens were coated with gold.

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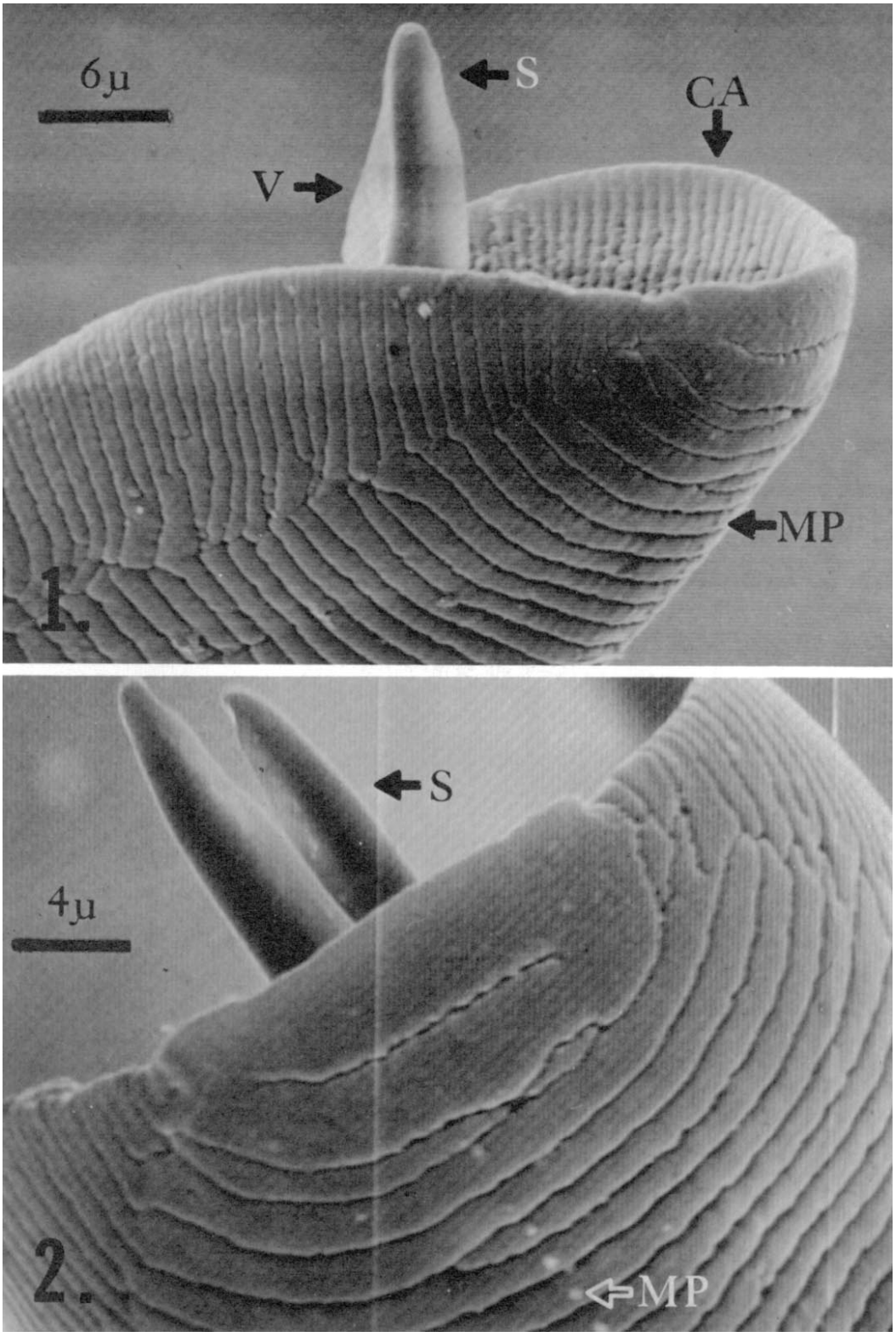

FIG. 1-2. **1.** Lateral view of the caudal region of *Hoplolaimus galeatus*. CA, Caudal ala; S, Spicule; V, Velum; MP, Microprojection. **2.** Posteriad view of the caudal region of *Hoplolaimus galeatus*. S, Spicules; MP, Microprojection.

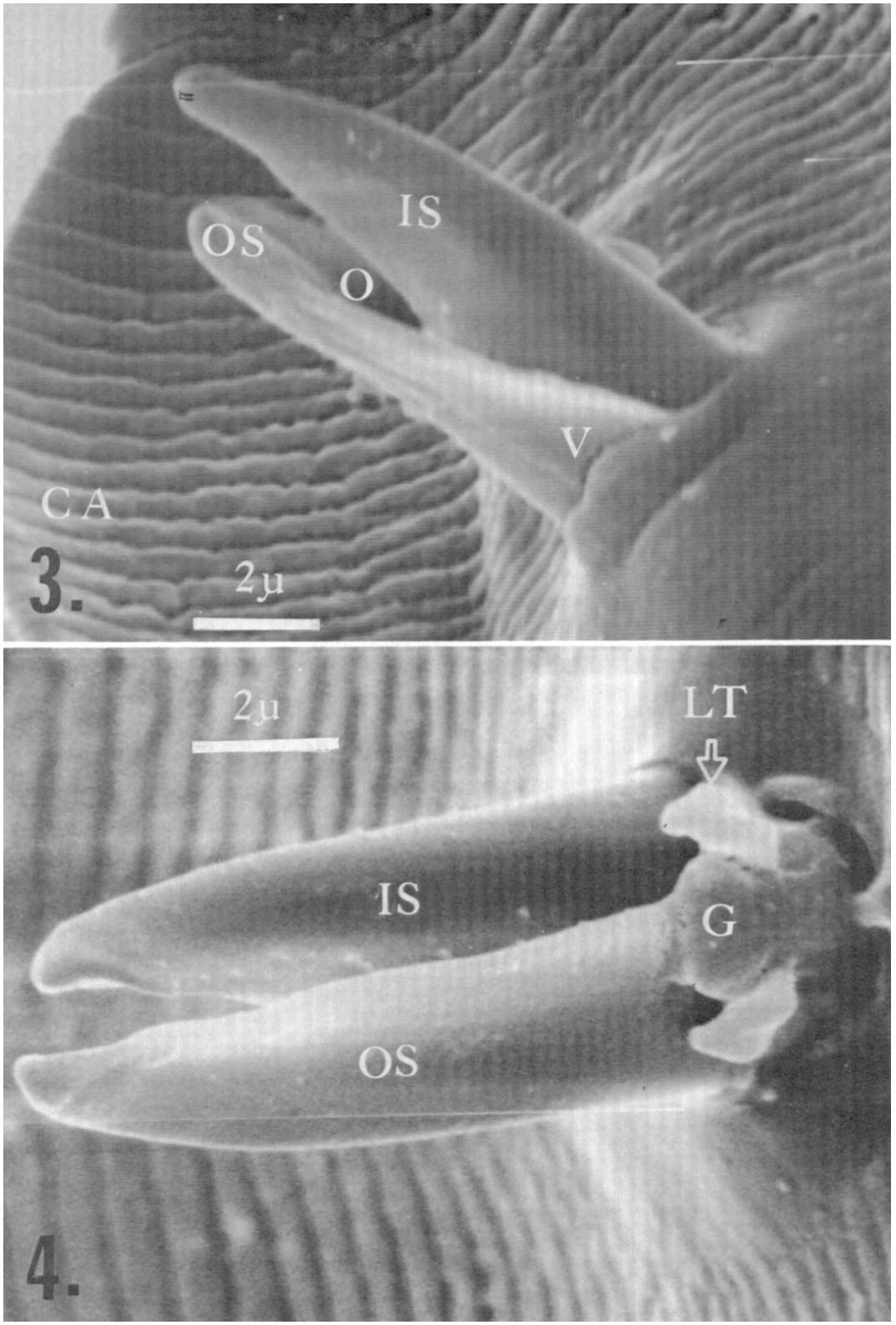

FIG. 3-4. 3. Anteriad ventral view of the ventral surface of the caudal region of *Hoplolaimus galeatus*. CA, Caudal ala; OS, Outer spicule; IS, Inner spicule; V, Velum; O, Orifice. 4. Posteriad ventral view of the ventral surface of the caudal region of *Hoplolaimus galeatus*. OS, Outer spicule; IS, Inner spicule; G, Gubernaculum; I T, Lateral titilla.

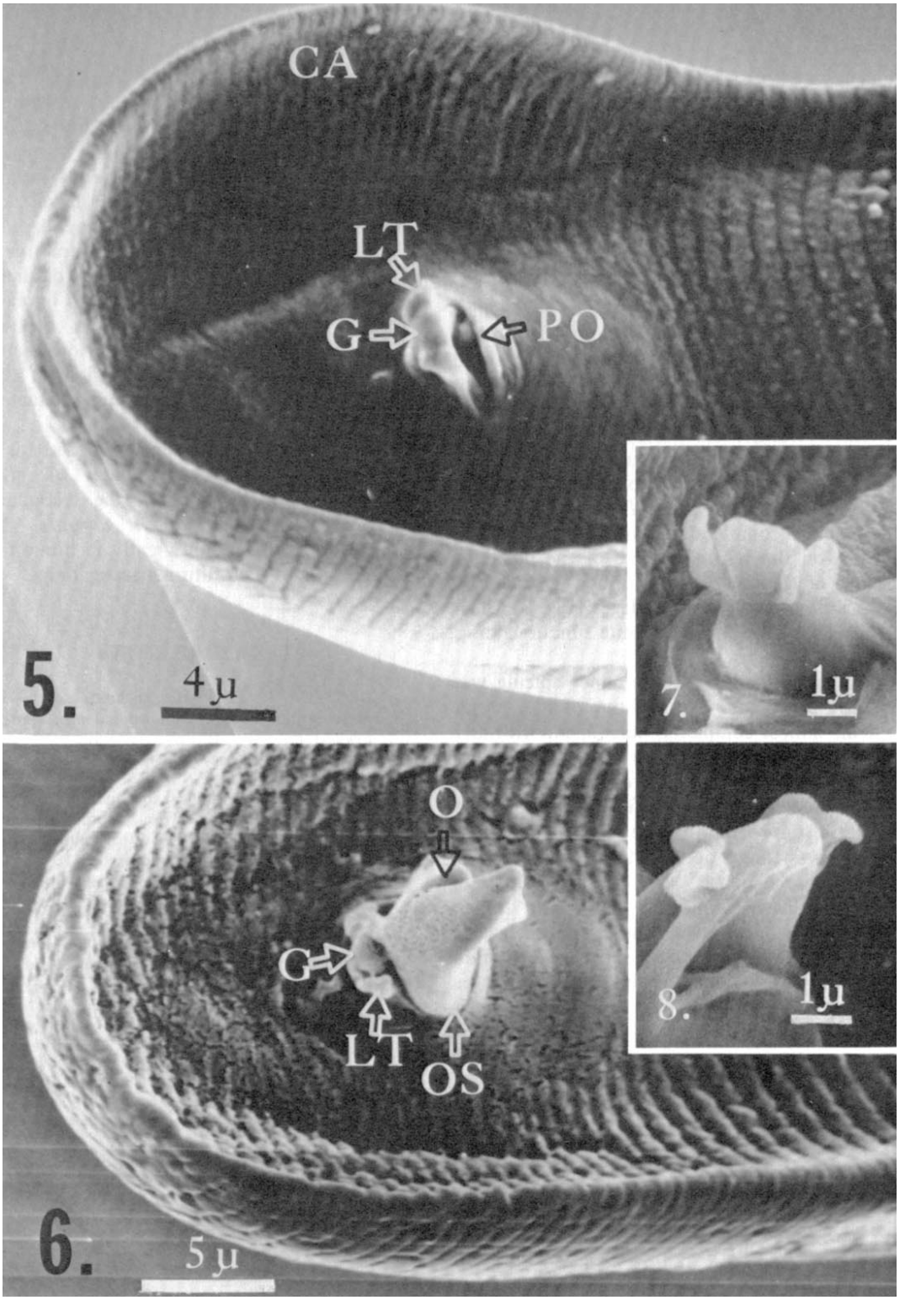

FIG. 5-8. **5.** Ventral view of caudal region of *Hoplolaimus galeatus* with spicules retracted. CA, Caudal ala; PO, Pouch orifice; G, Gubernaculum; LT, Lateral titilla. **6.** Ventral view of caudal region of *Hoplolaimus galeatus*, with velumated spicule protruding. OS, Outer spicule with velum; O, Closed orifice; G, Gubernaculum; LT, Lateral titilla. **7.** Posteriad view of gubernaculum. **8.** Anteriad view of gubernaculum.

The SEM micrographs of *H. galeatus* were taken with a Mark 2A Cambridge Stereoscan electron microscope operated at 15-20 KV. Light microscopy was employed whenever possible to substantiate our conclusions.

RESULTS AND DISCUSSION

A lateral view of the caudal region of *H. galeatus* indicated the presence of caudal alae and a spicule with a distinct velum (Fig. 1). From a posterior view there were two spicules (Fig. 2). Ventral views from the anterior end of the nematode, however, indicated that the spicules differed slightly in both morphology and orientation. The outer spicule had a distinct velum, while the inner spicule did not appear to possess this structure (Fig. 3).

The spicules seemed to be extruded and retracted independently. When both spicules were almost completely extruded, the only open orifice on the ventral surface of the posterior region of *H. galeatus* was formed by the close association of these appendages (Fig. 3). Such an arrangement could be quite functional as a conduit for sperm.

In specimens where the inner spicule was slightly retracted, the velum of the outer spicule almost completely surrounded the inner spicule (Fig. 3). When the inner spicule was retracted further, the velum of the outer spicule convoluted, closing the orifice described above (Fig. 6). It appeared that the velum of the outer spicule was forced open during the extrusion of the inner spicule.

The gubernaculum and two lateral titillae were clearly visible when the ventral surface of the nematode was observed from a posteriad direction (Fig. 4). All of the male appendages except the gubernaculum could be retracted into a common central pouch (Fig. 5). When this occured, the gubernaculum, two lateral titillae, and pouch orifice were readily visible.

The specimens depicted in Fig. 1-6 were prepared using the critical-point drying technique. Freeze-drying of living or formalin-fixed *H. galeatus* resulted in collapsed and distorted specimens. Sclerotized structures, however, were readily visible and well defined. Each titilla of the gubernaculum was bilobed (Fig. 7, 8). The proximal region of the gubernaculum was convex posteriorly and concave anteriorly. The distal region of the anterior surface of the gubernaculum contained three grooves.

Although histological work is needed for a more complete understanding of the morphology and function of the secondary male sex characteristics of *H. galeatus*, the present SEM study should greatly facilitate the interpretation of such future investigations.

LITERATURE CITED

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