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ABSTRACT: Staining techniques are relatively rare in the study of parasitic nematodes. A novel silver-staining method is described for elucidation of the synlophe (a system of longitudinal cuticular ridges), a character of great systematic importance among the trichostrongyloid nematodes. Ridges are stained optically black and appear in great contrast to the body of the nematode. This method augments current use of interference contrast for examination of the synlophe. Detailed studies of the configuration of the synlophe in entire specimens are possible with standard light microscopy for the first time.

The synlophe, a system of longitudinal cuticular ridges, is a characteristic structure among the trichostrongyloid nematodes (equivalent to the suborder Trichostrongylina of Durette-Desset and Chabaud [1993]). Application of the synlophe as a morphological character in systematics was introduced by Durette-Desset (1964) in research on heligmosome nematodes and later refined in exhaustive studies among families of the Trichostrongyloidea (see Durette-Desset, 1985). These studies were based principally on evaluation of transverse sections, concentrating on enumeration, structure, relative dimensions, height, and orientation of ridges in the midbody region (Durette-Desset, 1983). This basic approach was modified by Lichtenfels (1977) for species of *Cooperia* Ransom, 1907 and later other trichostrongylids. The synoptic method detailed traditional characters but also emphasized the specific patterns of ridges ventrally and laterally in the cervical zone (anterior to the base of the esophagus) , and their posteriad extent in males and females (Lichtenfels and Pilitt, 1983; Lichtenfels et al., 1988). Documentation of these characters has become standard practice in taxonomy and systematics of the Ostertagiinae and related nematodes of the family Trichostrongylidae (see Hoberg et al., 1993; Hoberg and Lichtenfels, 1994).

Although the configuration and pattern of the synlophe, particularly in the cervical region, could be studied in whole-mounted nematodes with standard transmitted light microscopy, fine details of structure were often obscure. Consequently, differential interference-contrast and scanning electron microscopy have commonly been used to elucidate the synlophe in entire specimens. However, these later methods often are not readily available in many laboratories. In the present report, we provide a simple, reliable, and rapid technique for staining of the synlophe, which augments and may replace the use of interference-contrast optics.

Stains have not been in general use for studies of nematodes (see Pritchard and Kruse, 1982), although there have been some specialized applications (Hooper, 1986). Where silver stains have been applied in comparative morphology, they have been used to differentiate internal and neural tissues (Croll and Maggenti, 1968) and some cuticular attributes in freeliving and plant-parasitic nematodes (Bedding, 1968; Rodríguez-Kabana and King, 1976). Additionally silver nitrate staining has been used to augment examination of sensory structures with scanning electron microscopy (Vanderburgh et al., 1987). In the present paper we describe a technique in which a silver stain is used specifically to enhance definition of the synlophe in entire specimens of trichostrongylid nematodes.

Intact specimens may be processed from solutions of buffered 10% formalin, 70% ethanol, alcohol–formalin–acetic acid, and other preservatives or fixatives. Specimens must first be hydrated, then are passed through a series of reagents. The process for a typical ostertagiine nematode, e.g., *Camelostrongylus mentulatus* (Railliet and Henry, 1909) is as follows at approximately 20 C: (1) wash in distilled H_2O; (2) incubate in 1.0% solution of NaCl for a minimum of 15 min; (3) stain in 0.2% solution of silver nitrate, e.g., 100 mg $AgNO_3$ in 50 ml H_2O for minimum of 5–30 sec; (4) wash in distilled water 30 sec; (5) place in photographic developer, e.g., Dektol diluted at 4 H_2O : 1 stock solution, for 5–20 sec; (6) wash in distilled water 1–2 sec; (7) differentiate in photographic fixative for 30 sec; and (8) wash in distilled water 10 sec prior to mounting. Commonly used mounting media include water, lactophenol, or phenol alcohol. Following rehydration, the stain can be removed by using an aqueous solution of 1% potassium cyanoferrate ($K_3Fe[CN]_6$), and the specimens can then be returned to fixative.

The timing of periods for washes and retention in each of the reagents are variable; fresh reagents should be prepared daily. It is requisite that specimens be retained in NaCl for a minimum of 15 min, because the presence of chloride ions is essential for reactions that result in the deposition of silver on the cuticle of the nematode. Small specimens, e.g., males among species of *Ostertagia* Ransom, 1907, may be passed through silver nitrate and fixative for a maximum of 5–10 sec, whereas some larger specimens, e.g., species of *Haemonchus* Cobb, 1898 and *Nematodirus* Ransom, 1907, may require a longer period for max-

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FIGURES 1–9. A comparison of the synlophe in specimens of *Camelostrongylus mentulatus* and *Ostertagia ostertagi,* as observed with light microscopy (LM; Figs. 1 and 4), interference-contrast microscopy (IC; Figs. 2 and 5), and silver staining (S; Figs. 3, 6–9). Scale bars = 25 μm for Figures 1–8 with same scale bars for Figures 1–6, 7, and 8; 50 μm for Figure 9. **1–3.** Cephalic region of *C. mentulatus* in lateral view showing termination of synlophe (pointers) in LM (Fig. 1), IC (Fig. 2), and with S (Fig 3). **4, 5.** Cervical zone of *C. mentulatus* in lateral view near level of cervical papilla in LM (Fig. 4), IC (Fig. 5), and S (Fig. 6). **7–9.** Synlophe in *O. ostertagi* in specimen prepared with silver stain, showing lateral view of posterior cervical region (Fig. 7); lateral view of region anterior to copulatory bursa in male (Fig. 8); and lateral view of copulatory bursa showing pattern of termination of ridges in the ventral field (Fig. 9).

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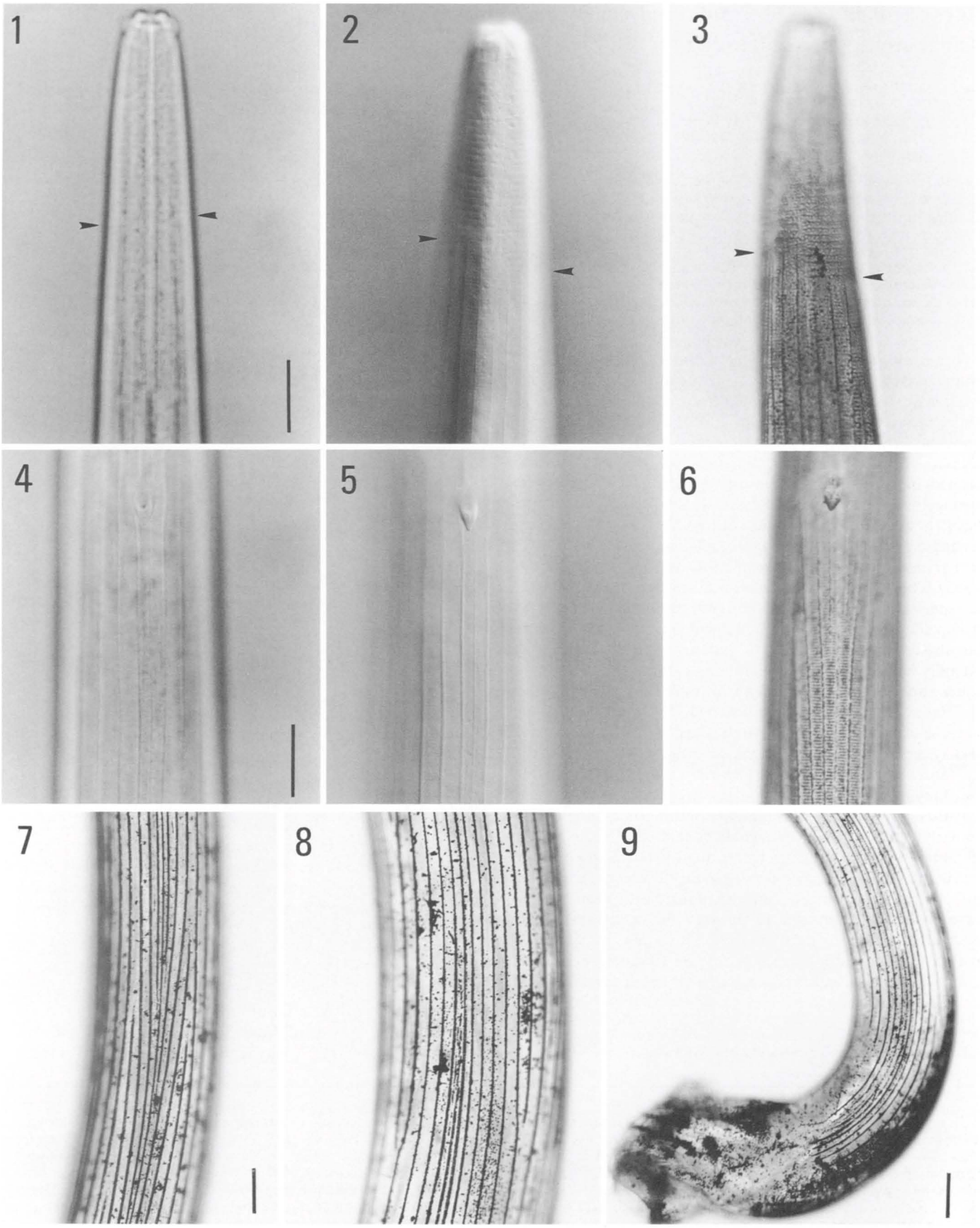

imum staining. Optimum results are achieved with careful attention to the concentration of NaCl and $AgNO_3$ and the duration of exposure in these reagents.

The ridges in specimens prepared in this manner are optically black and appear in great contrast to the body of the nematode as shown in specimens of *C. mentulatus* and *Ostertagia ostertagi* (Stiles, 1892) (Figs. 1–9). The transverse striations, typical of many trichostrongyles, are also strongly highlighted. This technique is particularly useful for examining ridges near their anterior termination at the base of the cephalic expansion (Figs. 1–3) and for patterns in the cervical zone (Figs. 4–7). The entire synlophe is stained, promoting detailed examination of the posteriad extent of the ridges in males and females (Figs. 8, 9). Characters may be revealed with this method that are otherwise difficult to observe using either standard or interference-contrast light microscopy and as such will substantially augment the study of the synlophe in entire specimens as currently practiced.

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