

# Scanning Electron Microscopy of *Xiphinema*, *Longidorus*, and *Californidorus* Stylet Morphology<sup>1</sup>

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**Abstract:** Stylet ultrastructure of five *Xiphinema*, four *Longidorus*, and three *Californidorus* species was compared by scanning electron microscopy. Morphological differences were seen in the odontophores and odontostyle bases between the genera and some of the species. All *Xiphinema* studied had well-developed odontophore flanges; the *Longidorus* species lacked flanges, except for weakly developed ones in *L. diadecturus*; and none of the *Californidorus* had flanges. Three sinuses were present in the odontophores of all species. The sinuses varied in length depending upon species. In *Xiphinema* and *Californidorus* the odontostyle bases had distinct overlapping collars, but in *Longidorus* the collars were absent except for *L. diadecturus*. The odontostyle-odontophore junction from a lateral view appeared as a slanted transverse line in all the species, but in a dorsal view of *Xiphinema* and *Californidorus* it was V-shaped. Dorsal longitudinal seams of the odontostyle and odontophore were observed in all the species. The dorsally located odontostyle aperture was ca. 1  $\mu$ m from the anterior end in all species, except in one *Longidorus* sp. it was ca. 4  $\mu$ m from the end.

**Key words:** *Californidorus clavicaudatus*, *C. cylindricaudatus*, *C. pinguicaudatus*, dorsal seam, Dorylaimida, Longidoridae, *Longidorus diadecturus*, Nordiidae, odontostyle, odontostyle-odontophore junction, odontophore, scanning electron microscopy (SEM), sinus, stylet morphology, *Xiphinema americanum*, *X. bakeri*, *X. chambersi*, *X. coxi*, *X. rivesi*.

Scanning electron microscopy (SEM) has been used successfully to study the stylet morphology of various nematode genera. Ellenby and Wilson (8) were the first to observe stylets with SEM in studies of *Heterodera rostochiensis* second-stage juveniles. Differences in stylet morphology of males were helpful in Eisenback and Hirschmann's differentiation of selected species of *Meloidogyne* (6). Jepson (10) demonstrated that the stylet morphology of the *Meloidogyne* female is species specific, and together with other characters may be used as an aid to identification. Eisenback and Rammah (7) developed a stylet dissection technique for *Meloidogyne* and have since adapted it to study other genera of plant-parasitic nematodes.

Recent SEM studies on the stylet morphology of several longidorids have revealed details of stylets not seen by light microscopy (2,9,12). Swart and Heyns (12) proposed that differences in the odontostyle base and its odontophore junction,

the three odontophore sinuses, and the basal flange development are three features of excised longidorid stylets that yield useful information for the separation of taxa.

The objective of this study was to attain a better understanding of the interrelationships between Dorylaimida with long odontostyles and odontophores belonging to the families Longidoridae and Nordiidae.

## MATERIALS AND METHODS

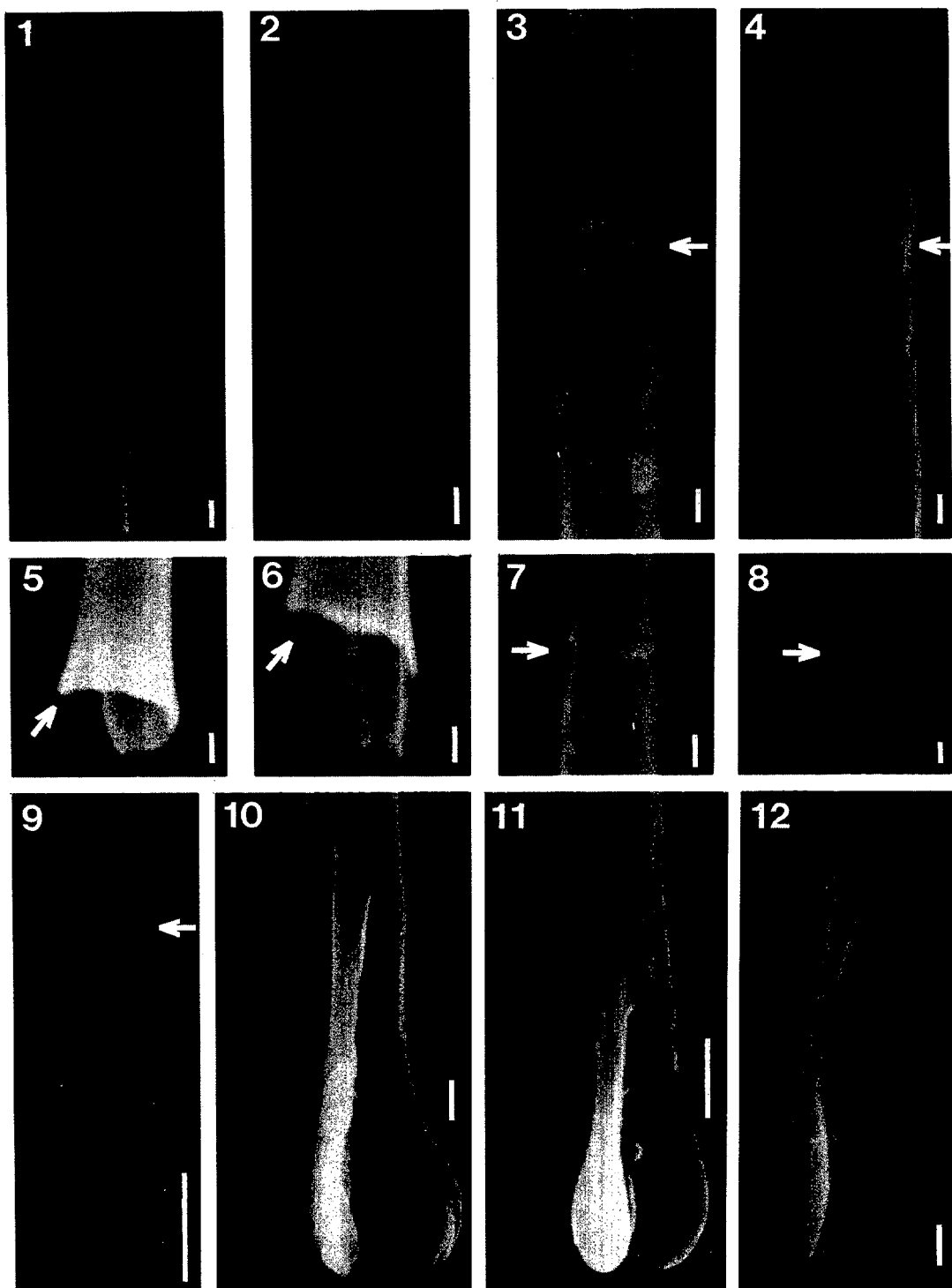
Five species of *Xiphinema* (*X. americanum* Cobb, *X. bakeri* Williams, *X. chambersi* Thorne, *X. coxi* Tarjan, and *X. rivesi* Dalmasso), four *Longidorus* spp. (*L. diadecturus* Eveleigh and Allen and three undescribed Arkansas populations from Beaver Dam, War Eagle, and White River), and three *Californidorus* spp. (*C. cylindricaudatus* Robbins, *C. clavicaudatus* Robbins, and *C. pinguicaudatus* Robbins and Weiner) were used in the study. Except for *X. coxi* from Gainesville, Florida, and *C. pinguicaudatus* from California, all the species were collected in Arkansas. Nematodes were extracted from soil by the combined roiling-sieving and Baermann technique (3) and then refrigerated before dissection.

The stylet extraction technique was

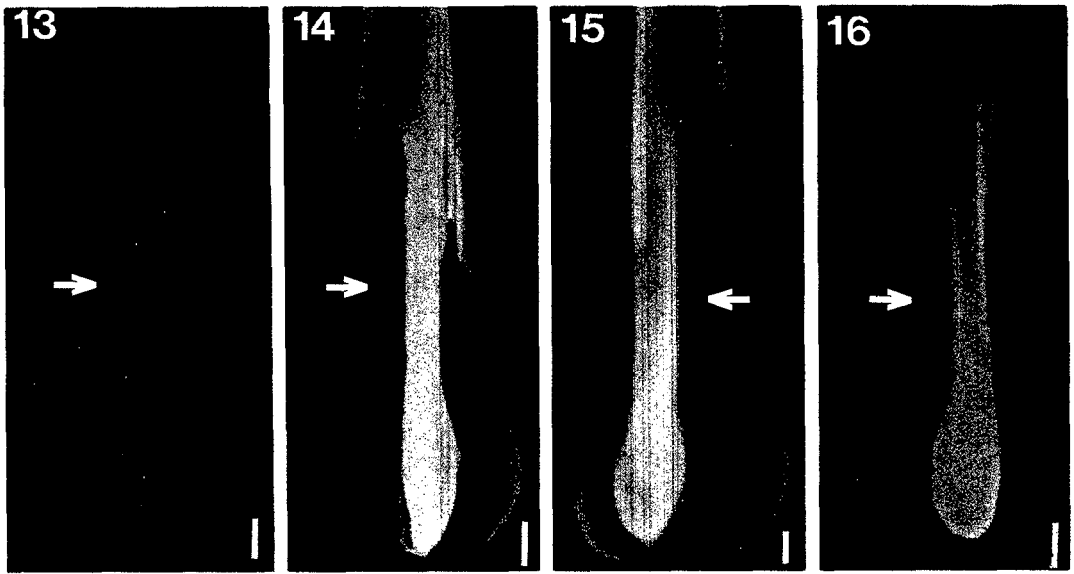
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Figs. 1-12. SEM photographs of excised stylets of *Xiphinema* species. 1) stylet, *X. chambersi*. 2) Odontostyle, *X. coxi*. 3) Odontostyle-odontophore junction (O-OJ) (arrow), dorsal view, *X. rivesi*. 4) O-OJ (arrow), dorsal view, *X. coxi*. 5) Odontostyle base collar (arrow) and internal extension, *X. coxi*. 6) Odontostyle base collar (arrow) and internal extension, *X. chambersi*. 7) O-OJ (arrow), ventral view, *X. rivesi*. 8) O-OJ (arrow), ventral view, *X. americanum*. 9) Odontostyle aperture (arrow), *X. americanum*. 10) Odontophore, view of subdorsal sinuses, *X. coxi*. 11) Odontophore, view of subdorsal sinuses, *X. rivesi*. 12) Odontophore, view of ventral sinus, *X. americanum*. Scale bars = 1  $\mu\text{m}$  (3-10), 2  $\mu\text{m}$  (11,12), and 10  $\mu\text{m}$  (1,2).



Figs. 13–16. SEM photographs of *Xiphinema* odontophores showing ventral sinuses (arrows). 13) *X. bakeri*. 14) *X. chambersi*. 15) *X. coxi*. 16) *X. rivesi*. Scale bars = 2  $\mu$ m.

modified slightly from the method of Eisenback (5). A nematode was placed in a drop of 0.525% NaOCl solution on a clean glass slide. Some *Longidorus* species were done in 0.26% NaOCl to prevent excessive odontophore dissolution. The anterior portion of the nematode was cut off just posterior to the nerve ring with a dissecting knife, then the stylet was pushed out of the cuticle and agitated using a very fine micropipette to aid in the dissolution of attached tissues. The stylets were rinsed in a drop of distilled water and placed on a 13-mm-d round cover glass. Stylet position on the cover glass was marked with a Liquid Paper circle. The cover glass was attached to a SEM stub and dried overnight in a desiccator. Stylets were sputter-coated with 200 Å of gold and then viewed and photographed with an ISI-60 scanning electron microscope operating at 30 KV. At least 10 stylets of each species were examined.

#### RESULTS

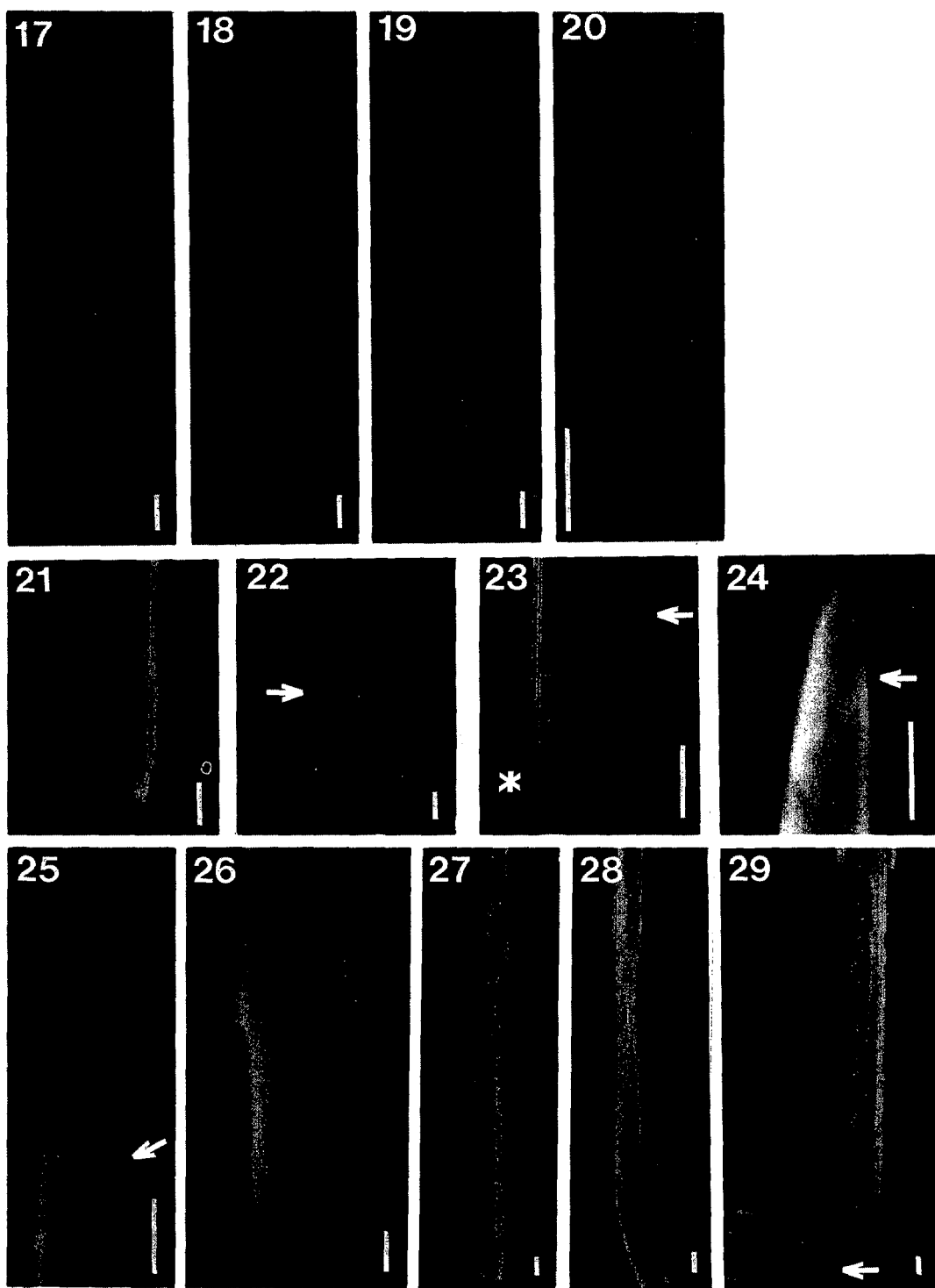
*Xiphinema* spp.  
(Figs. 1–16)

Stylet shapes were not different enough to discern one *Xiphinema* species from another except by size. Odontostyles are

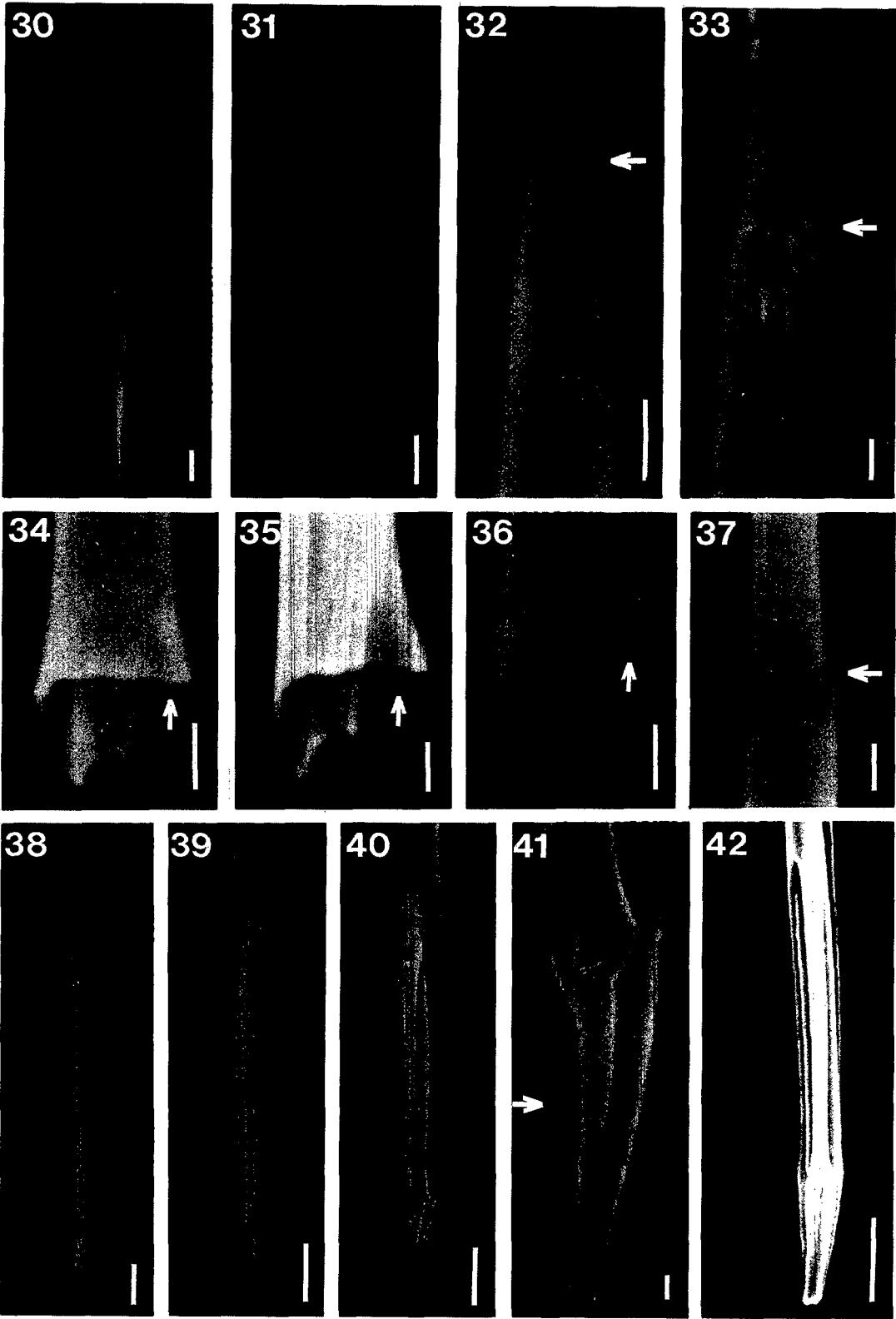
elongate (Fig. 2) and odontophores have well-developed basal flanges (Fig. 1). The stylet aperture is dorsally located and about 1  $\mu$ m from the odontostyle tip (Fig. 9). The odontostyle has an external collar which surrounds an internal extension of the odontostyle and the anterior end of the odontophore (Figs. 5, 6). By light microscope observations, the odontostyle–odontophore junction (O–OJ) appears forked, but when viewed dorsally by SEM it is V-shaped (Figs. 3, 4). A dorsal seam is evident at the V-shaped O–OJ (Figs. 3, 4). In some specimens, this seam extends anterior to the odontostyle aperture (Fig. 9) and posteriorly to the odontophore basal flanges (Fig. 10). The O–OJ appears as slightly rounded or as a transverse slanted line when viewed laterally (Figs. 7, 8). Three well-developed basal flanges and three sinuses were present in all the species studied (Figs. 10–16). The ventral sinus was longer than the two subdorsal sinuses which were similar in length (Figs. 10, 11).

*Longidorus* spp.  
(Figs. 17–29)

Two different stylet shapes were observed among the four *Longidorus* spp. (Figs. 17, 18). The odontostyle apertures are lo-



Figs. 17–29. SEM photographs of excised stylets of *Longidorus* species. 17) Stylet, *L. diadecturus*. 18) Stylet, *Longidorus* from Beaver Dam. 19) Odontostyle, *L. diadecturus*. 20) Odontostyle base, *L. diadecturus*. 21) Odontostyle base, *Longidorus* from Beaver Dam. 22) Odontostyle–odontophore junction (O–OJ) (arrow), lateral view, *Longidorus* from Beaver Dam. 23) O–OJ (arrow) and girdling furrow (\*) (arrow), lateral view, *L. diadecturus*. 24) Odontostyle aperture (arrow), *L. diadecturus*. 25) Odontostyle aperture (arrow), *Longidorus* from Beaver Dam. 26) Odontophore, *L. diadecturus*. 27) Odontophore, *Longidorus* from White River. 28) Odontophore, *Longidorus* from War Eagle. 29) Odontophore with connected esophageal lumen (arrow), *Longidorus* from Beaver Dam. Scale bars = 1  $\mu$ m (20–29) and 10  $\mu$ m (17–19).



cated about 1  $\mu\text{m}$  from the tip in *L. diadecturus* (Fig. 24) and 4  $\mu\text{m}$  in the *Longidorus* from Beaver Dam (Fig. 25). Odontostyles are elongate with a bluntly rounded basal end (Figs. 19–21). Collar development was not detected in *Longidorus* species (Fig. 21), except for *L. diadecturus* which has a weakly developed collar (Fig. 20). The O–OJ appears as a slanted transverse line when viewed laterally (Figs. 22, 23). The anterior odontophore surrounds the internal odontostyle extension and extends to the slanted transverse line (Figs. 22, 23). The base of the internal odontostyle extension is denoted by a girdling furrow in *L. diadecturus* (Fig. 23) not seen in other *Longidorus* species. The odontophore base of *L. diadecturus* has weakly developed flanges (Fig. 26), while the other three species have blunt rod-shaped bases without flanges (Figs. 27–29). The three odontophore sinuses of *L. diadecturus* are approximately equal in length (Fig. 26). The three *Longidorus* species with rod-shaped odontophores have one longer ventral sinus and two shorter subdorsal sinuses (Figs. 27–29). The esophageal lumen lining continues anteriorly inside the basal flanges of *L. diadecturus* (Fig. 26), whereas it begins at the odontophore bases in the other species (Fig. 29). Undissolved tissue of the esophageal lumen lining connected to the odontophore base of the *Longidorus* from Beaver Dam by a single strand of tissue suggests that it is arranged in a tight spiral (Fig. 29).

*Californidorus* spp.  
(Figs. 30–42)

All *Californidorus* studied have similar stylet morphology, an elongate odontostyle, and an odontophore with a short

horn-shaped extension (Figs. 38–42). The odontostyle aperture is ca. 1  $\mu\text{m}$  from the anterior tip (Fig. 32). Odontostyle bases have collars similar to those seen in *Xiphinema* (Figs. 31, 34–36). In dorsal view, the O–OJ appears V-shaped (Fig. 33), and laterally it appears as a slanted transverse line (Fig. 37). The dorsal seam of the odontostyle extends anteriorly to the odontostyle aperture (Fig. 32) and posteriorly to about the odontophore midpoint (Fig. 38). *Californidorus* odontophores have horn-shaped extensions that are ridged (Figs. 38–41). The odontophores have one long ventral sinus and two shorter subdorsal sinuses. Each sinus has a midridge extending most of its length (Fig. 42). The horn-shaped extension appears to have six ridges and grooves formed when each of the three sinus walls splits into two extension ridges separated by a midgroove, whereas each sinus midridge appears to split and fuse into the anterior end of the extension ridges just anterior to the separating groove between the three paired sets of ridges (Figs. 38–42).

#### DISCUSSION

Several morphological differences and similarities were observed among the stylets of the three genera studied. Significant differences in stylet morphology were not detected within species of *Xiphinema* and *Californidorus*. Two different odontophores were present in the *Longidorus* species. The odontophore of *L. diadecturus* had weakly developed basal flanges (Fig. 26), and the other three species had rod-shaped odontophores (Figs. 27–29). Similar variations in odontophore morphology were reported by Swart and Heyns (12)

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Figs. 30–42. SEM photographs of excised stylets of *Californidorus* species. 30) Stylet, *C. cylindricaudatus*. 31) Odontostyle, *C. pinguicaudatus*. 32) Odontostyle aperture (arrow), *C. cylindricaudatus*. 33) Odontostyle–odontophore junction (O–OJ) (arrow), ventral view, *C. cylindricaudatus*. 34–36) Odontostyle base collars (arrows) and internal extensions of *C. cylindricaudatus*, *C. clavicaudatus*, and *C. pinguicaudatus*, respectively. 37) O–OJ (arrow), ventral view, *C. cylindricaudatus*. 38) Odontophore, dorsal view, *C. cylindricaudatus*. 39) Odontophore, ventral view, *C. clavicaudatus*. 40) Odontophore, ventral view, *C. cylindricaudatus*. 41) Odontophore base and terminal horn-shaped extension (arrow), *C. cylindricaudatus*. 42) Odontophore terminus, ventral view, *C. cylindricaudatus*. Scale bars = 1  $\mu\text{m}$  (32–37, 41) and 10  $\mu\text{m}$  (30, 31, 38–40, 42).

among species of *Longidorus*, *Longidoroides*, and *Paralongidorus*. Heyns *et al.* (9) observed weakly developed basal flanges in the stylet of *L. fursti* Heyns, Coomans, Hutsebaut & Swart. The esophageal lumen lining in the *Longidorus* from Beaver Dam (Fig. 29) appeared similar to the undissolved lumen structures described by Swart and Heyns (12).

Odontostyle apertures were ca. 1  $\mu\text{m}$  from the anterior tip in the species studied, except the *Longidorus* from Beaver Dam; its aperture was ca. 4  $\mu\text{m}$  from the tip (Fig. 25). This indicates that the position of the aperture is variable between *Longidorus* species. Variation in the location of stylet aperture has been reported for *Meloidogyne* species (Eisenback, pers. comm.).

*Xiphinema* and *Californidorus* species, while belonging to different families, have similar odontostyle and O-OJ morphology. Morphology of both is different from the *Longidorus* species studied. The odontostyle bases of *Xiphinema* and *Californidorus* have well-developed external collars (Figs. 5, 6, 34–36) which were much reduced or lacking in *Longidorus* (Figs. 20, 21). The V-shaped O-OJ observed in *Xiphinema* and *Californidorus* showed some differences in the type of connection. In *Xiphinema* the odontostyle collar overlaps on the outside of the odontophore (Figs. 3, 4, 7, 8), and in *Californidorus* the anterior portion of the odontophore appears to overlap the odontostyle collar (Figs. 33, 37). The O-OJ in *Longidorus* species show that the odontostyle and odontophore are abutted with the anterior extension portion of the odontophore thinly surrounding the internal odontostyle extension (Figs. 20–23).

Dorsal seams were observed on the odontostyles of all three genera and on the odontophores of *Xiphinema* and *Californidorus*. These seams are well-depicted in cross section by transmission electron microscopy for several *Xiphinema* species (1, 11, 13, 14), and morphological comparison can be made with the SEM pictures of this study.

The ventral odontophore sinus was the longest in all species studied, except *L. dia-*

*decturus* where all three sinuses were approximately equal in length. In *Xiphinema*, according to Robertson and Taylor (11), "the ventral sinus is the largest and extends anteriorly to a point of weakness in the cuticle of the odontophore; during the moulting process the replacement stylet moves forward from the oesophagus into the anterior stomadaeum through the sinus."

Observations of the stylets of *Californidorus* revealed morphological structures of the odontophore base and the presence of a horn-shaped extension not seen with light microscopy. The horn-shaped extension was seen only in *Californidorus* (Figs. 38–42); this may be a structure present in Nor-diidae and lacking in Longidoridae.

Coomans (4) postulated that the more posterior the position of the guide ring and the more robust the odontostyle, the more developed the odontophore base. This was supported in our study. *Californidorus* and three of the *Longidorus* species with guide rings far forward had weakly developed odontophore bases (Figs. 27–29, 39–42). The guide ring of *L. diadecturus* was more posterior and the odontophore had a weakly developed base with small flanges (Fig. 26). In *Xiphinema*, the guide ring was the furthest posterior and the odontophore basal flanges were well developed (Figs. 10–16). Further comparative studies on the stylet ultrastructure of Dorylaimida species with elongate odontostyles would be helpful in understanding group relationships.

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