

ULTRASTRUCTURE OF THE ADULTS OF *BUNOSTOMUM PHLEBOTOMUM* (NEMATODA: ANCYLOSTOMATIDAE)

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The morphology of the male and female of Bunostomum phlebotomum are described based on scanning electron microscope (SEM) observations. The attachment of the worms to the small intestinal mucosa and during the copula were observed. Structures of the bucal capsule and genital organs were also studied.

Key words: Nematoda – Ancylostomatidae – *Bunostomum phlebotomum* – ultrastructure

Bunostomum phlebotomum (Railliet, 1900) is a economically important parasite of livestock. The hookworm is commonly found in warm and moist areas throughout the world (Williams et al., 1983). The bloodsucking activity of the worm can cause severe pathology in young animals. In human individuals the third instar larvae of *B. phlebotomum* can cause cutaneous larva migrans (Mayhew, 1947).

In Brazil, *B. phlebotomum* occurs in bovines and bubalines in many States, and most frequently parasitizes young cattle (Guimarães et al., 1975; Costa et al., 1985).

The morphology of this species and other Ancylostomatidae were well studied under light microscope (Rep, 1963), but based on SEM *B. phlebotomum* was described in a research note (Malan et al., 1986). This paper records morphological features of male and female of *B. phlebotomum* under SEM.

MATERIALS AND METHODS

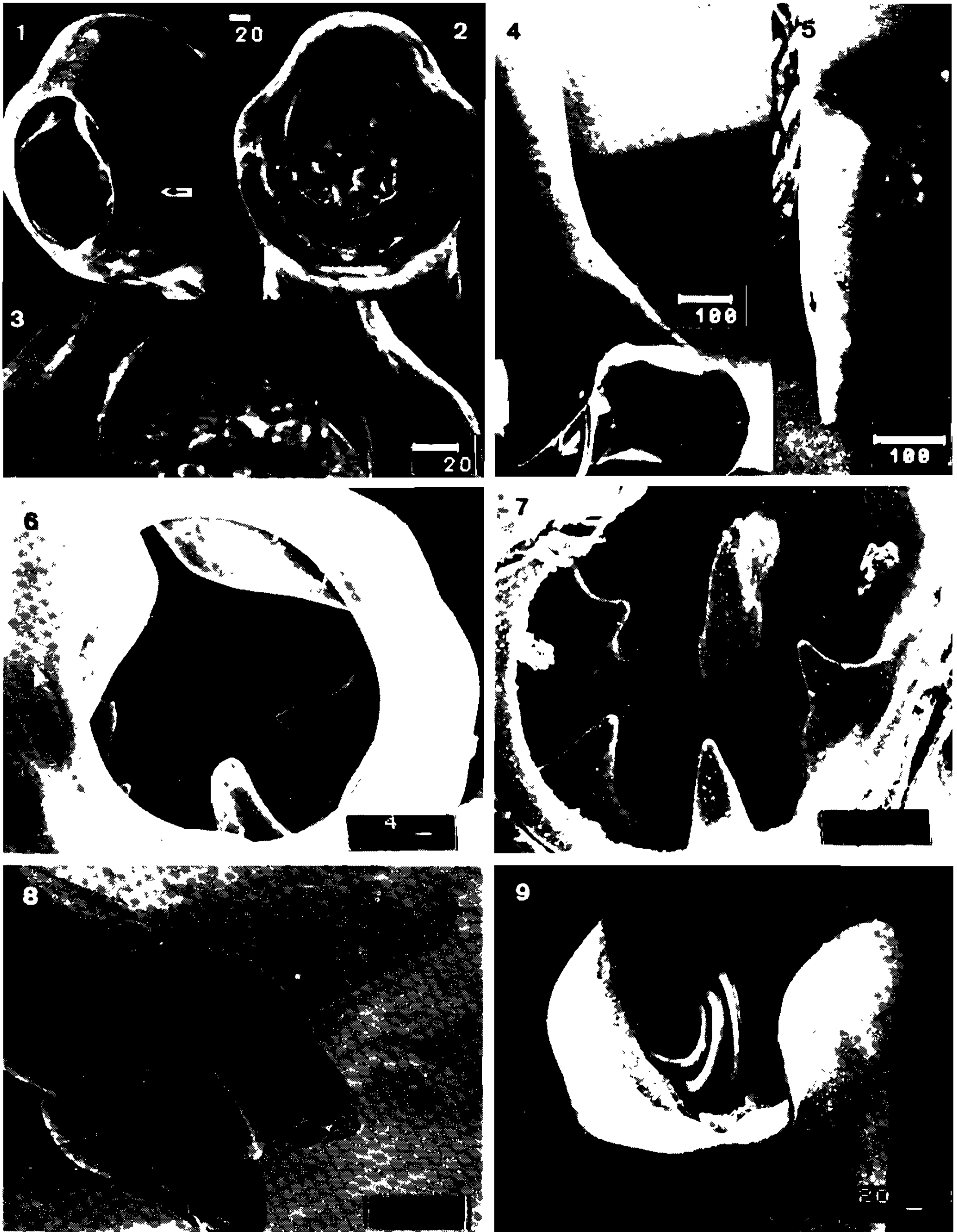
Adults hookworms and pieces of small intestine with attached worms were obtained from naturally infected young cattle from Igarapé, State of Minas Gerais, Brazil. The specimens were previously washed in 0.1 M phosphate buffer pH 7.4 (PB) and fixed with 2.5% glutaraldehyde in PB at 4 °C for 4 h. After rewashing in PB, the specimens were post-fixed with 1% osmium tetroxide in PB at 4 °C for 2 h. Afterwards, the specimens were dehydrated in a graded ethanol series (60, 70, 80,

90, 100 and 100%), dried by CO₂ critical point, coated with gold and examined in a SEM. The sections across of the worm's mouth were made with a barbed blade after postfixation. The terminology used is based on Gibbons (1986).

RESULTS

Bunostomum phlebotomum is recurved anteriorly and has the mouth opening situated anterior-dorsally. On the internal margin of the oral opening, there are a pair of long cutting plates ventrally and another small pair, dorsally (Figs 1-3, 5). The large buccal capsule bears a single, deep dorsal cone (subdorsal strong tooth), two subventral-lateral lancets (or mamiliform strong teeth) and two subdorsal-lateral lancets (strong teeth) (Figs 6-7). The dorsal cone has a small opening at the flattered top and a longitudinal furrow in posterior base that reaches the oesophageal opening. The opening of the amphidial gland is shown in Figs 1 and 6.

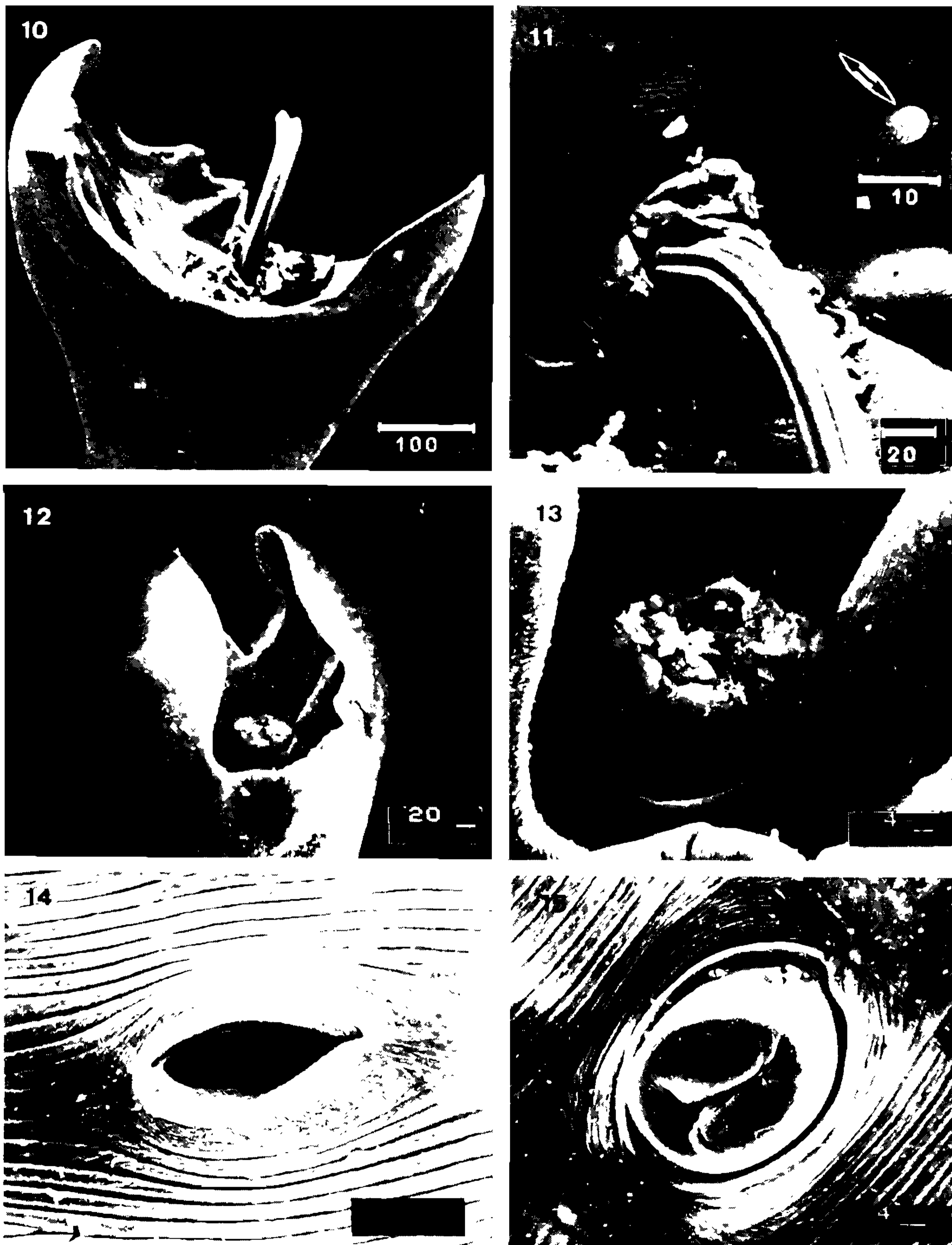
The male copulatrix bursa is well developed (Figs 4, 8-9). It has two lateral lobes and one dorsal lobe that is partly divided (Fig. 10). The slender and long spicules are externally fused, and have longitudinal striations on the surface and two diverge tips (Figs 8-9). Usually the bursa shows the cloaca with outside spicules (Figs 10-11) and sometimes with invaginated spicules (Fig. 12). The genital cone, which surrounds the cloaca, is structurally very simple. In the proconus, ventral region of the cloaca, there are two tubercled appendages, and two in dorsal region (one of which is hidden by debris) (Fig. 13). The presence of bursal papillae, antero-lateral and externo-dorsal,



SEM photographs of adults of *Bunostomum phlebotomum*. Fig. 1: cephalic region (amphidial opening arrowed), latero-anterior view. Fig. 2: cephalic region, anterior view. Fig. 3: close up of ventral plates. Fig. 4: male tail. Fig. 5: female tail (phasmidial opening arrowed). Fig. 6: oral opening showing the plates, dorsal cone and lancets. Fig. 7: section of the mouth showing the depth of buccal capsule with dorsal cone and lancets. Fig. 8: copulatrix bursa (spicular pits arrowed), ventral view. Fig. 9: copulatrix bursa showing the lateral lobes and spicules, ventral view.

is easily observed (Figs 10, 19). In the internal face of the bursa there are two papillae near the cloaca, situated ventrally (Fig. 11) and,

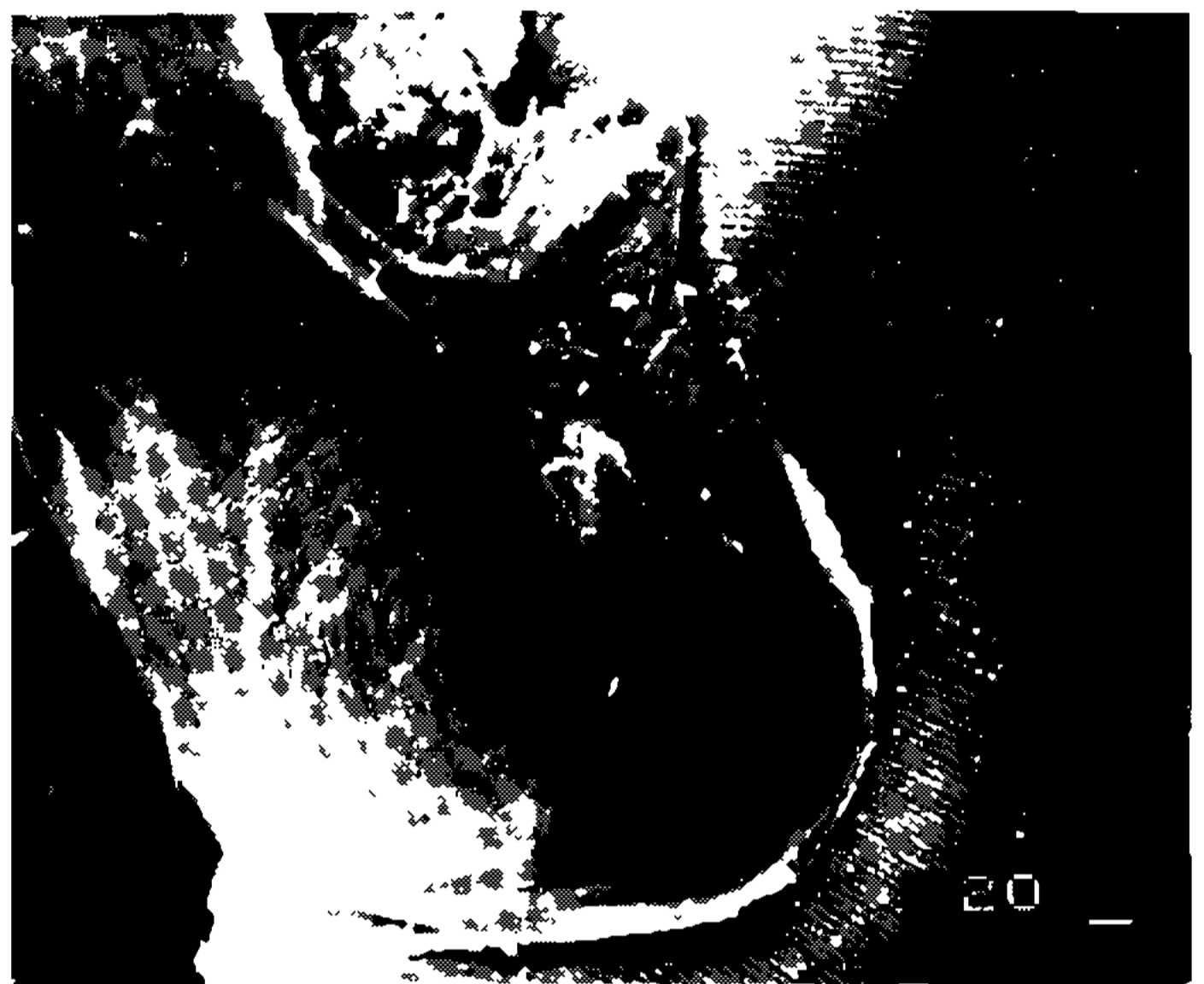
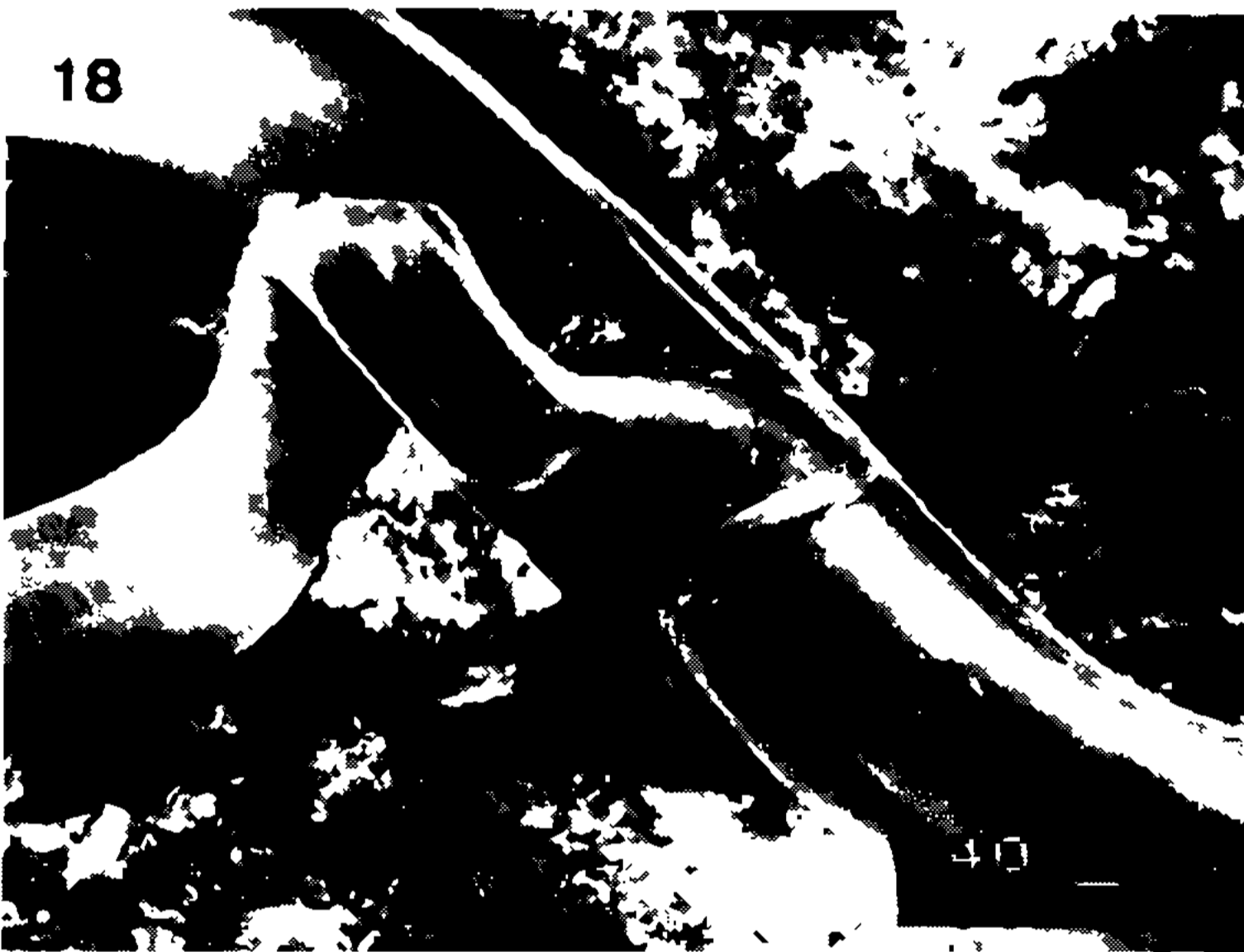
higher up, there are two pairs of similar papillae (one behind the other) on either side of ventral margin of the bursa.



SEM photographs of adults of *Bunostomum phlebotomum*. Fig. 10: copulatrix bursa showing the lateral and dorsal lobes, and emergent spicules (anterior-lateral papilla arrowed). Fig. 11: cloaca and emergent spicules (high magnification showing a papilla on the internal face of bursa). Fig. 12: copulatrix bursa with invaginated spicules, bearing the genital cone, latero-ventral view. Fig. 13: genital cone. Fig. 14: female genital opening. Fig. 15: female with everted vagina.

The transverse genital opening of the female, in the middle of the anterior half, interrupts the cuticular striations (Fig. 14). Some-

times the everted vagina is observed (Fig. 15). The anal opening is followed by a long and tapering tail without a terminal spine (Fig. 5).



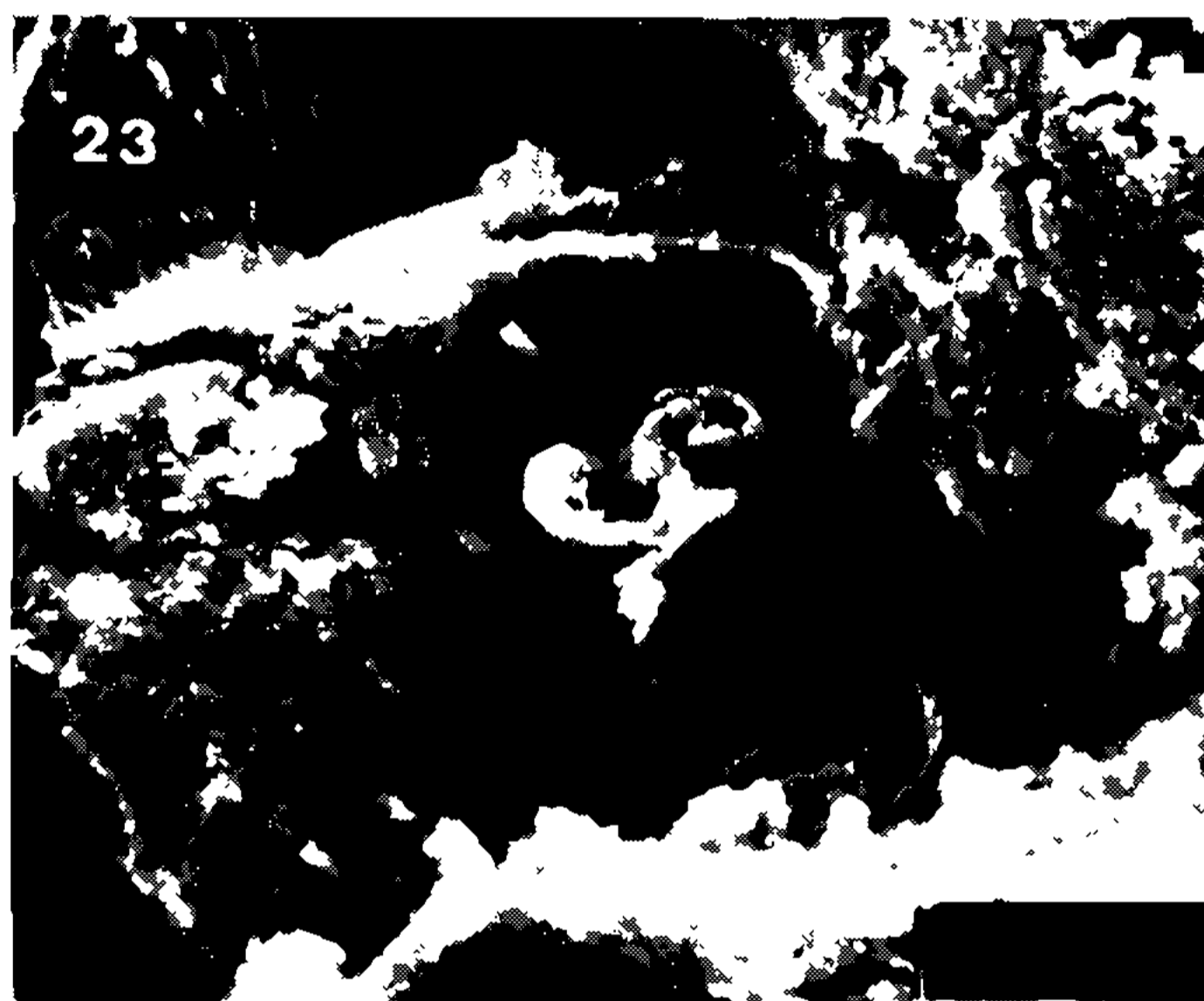
SEM photographs of adults of *Bunostomum phlebotomum*. Fig. 16: worm in copula (arrowed) fixed to intestinal mucosa. Fig. 17: worm fixed to intestinal mucosa. Fig. 18: copula of the worms. Fig. 19: copulatrix bursa around the female genital opening (externo-dorsal papilla arrowed).

The opening of the phasmidial gland is visible in Fig. 5.

The attachment of worms to the small intestine, causing lesions, is shown in Figs 16 and 17. When attached to the intestine, the worms copulate (Fig. 18). When copulating, the male copulatrix bursa expands its lateral and dorsal rays, which are fixed around the female genital opening (Fig. 19). After provoking the separation of the copula a sticky substance is visible around the female genital opening which is fixed firmly the male bursa (Fig. 20). The structure of the sectioned spicules shows them as a narrow tube coated with a striated membrane (Fig. 21). Introduced in the female genital opening, a section of the two spicules is shown in Figs 22 and 23.

DISCUSSION

Bunostomum phlebotomum, as all species of Bunostominae, has cutting plates in the mouth opening, similar to those described in *Necator americanus* and *B. trigonocephalum* by SEM (Yoshida et al., 1974a; Wilfrede & Lee, 1981). Although the distribution of the deep teeth in buccal cavity of *B. phlebotomum* is like that observed in *B. trigonocephalum* (Wilfrede & Lee, 1981) and *N. americanus* (Gibbons, 1986), the dorsal cone, characteristic of Bunostominae, is not long and sharp as described in those two species. Based on Rep (1963) the dorsal gutter, a generic feature of some Ancylostomatidae, e. g. *Ancylostoma*, *Uncinaria*, *Hypodontus*, is replaced by dorsal cone in other species of this family, e. g. *B.*



SEM photographs of adults of *Bunostomum phlebotomum*. Fig. 20: provoking separation of copula. Fig. 21: section of spicules. Fig. 22: cement substance around female genital opening. Fig. 23: spicules inserted in female genital opening.

phlebotomum, *B. trigonocephalum*, *Grammocephalus clathratus*, *Monodontella okapiae*, *N. americanus*. The small opening in the flattened top of *B. phlebotomum* is an entrance opening of dorsal cone if compared with those species above mentioned (Rep, 1963).

Bunostomum phlebotomum has the dorsal cone like that of *G. pachyscelis*, and a longitudinal furrow on the posterior base of the dorsal cone was shown in *N. americanus* (Gibbons, 1986). The amphidial opening of *B. phlebotomum* is situated as in other Ancylostomatidae: *N. americanus* (Yoshida et al., 1974a); *Ancylostoma duodenale* (Yoshida et al., 1974b); *A. braziliense* and *A. caninum* (Zaman, 1983); *B. trigonocephalum*, *Globocephalum urosubulatus* and *G. pachyscelis* (Gibbons, 1986).

Compared with *N. americanus* (Gibbons, 1986) the copulatrix bursa of *B. phlebotomum*

neither has the dorsal lobe almost completely divided nor the spicular pits closed and barbed. Although many bursal papillae have been described, the presence of papillae on the internal face of the bursa has not previously been described in Ancylostomatidae. The simple genital cone of *B. phlebotomum* is quite distinct from those of Trichostrongylidae species, which bear different structures and papillae (Stringellow, 1970; Gibbons & Khalil, 1983). The comparison between the genital cone of the nine species of Ancylostomatidae, described by Setasuban (1975), and *B. phlebotomum* show distinct differences. This species neither has two pointed pairs of papillae situated dorso-laterally nor two pairs in the latero-ventral region of cloaca, as found in *Ancylostoma ceylanicum* and *A. braziliense*. The pair of semilunar membranc-like and the two pairs of long and one pair of small papillae described in *A. kusimaense* were not observed in *B. phlebotomum*. Strong difference occur between

B. phlebotomum and *A. malayanum* because this species has six small round papillae and also three pairs of long finger-like processes on the genital cone. The pattern of the genital cone of *A. duodenale*, *A. tubaeforme*, *A. caninum*, *Agriostomum vryburgi* and *Cyclo-dontostomum purvisi* has similarly two pairs of papillae (one longer than other) and differs from that of *B. phlebotomum*.

As in the female of *B. phlebotomum*, the absence of a terminal tail spine occurs also in *G. pachycelis* and *N. americanus* (Gibbons, 1986). but the terminal spine is a typical character in females of *Ancylostoma*, e. g. *A. duodenale* (Yoshida et al., 1974b), and is also present in *G. urosubulatus* (Gibbons, 1986). Malan et al. (1986) considered the female tail of *B. phlebotomum* as having a pointed end, and an illustration by SEM the relation between length and width of the tail is unlike that of *B. phlebotomum*.

Sometimes hookworms cause severe lesions in the small intestine of their hosts. By SEM these traumatic lesions of *B. phlebotomum* can be seen to be like those described for *N. americanus* in hamsters (Leite, 1991). During copula the position of male bursa is equal to that observed in *A. caninum*; and that the narrow tubular appearance and divergent tips of the spicules, as well as the position of the phasmidial opening occur as in others parasitic Nematoda (Gibbons, 1986).

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