Onderstepoort J. vet. Res., 50, 257–265 (1983)

SCANNING ELECTRON MICROSCOPY OF *STRONGYLUS* SPP. IN ZEBRA

H. J. ELS[1], F. S. MALAN [2] and ROSINA C. SCIALDO-KRECEK[3]

ABSTRACT

ELS, H.J., MALAN, F. S. & SCIALDO-KRECEK, ROSINA C., 1983. Scanning electron microscopy of *Strongylus* spp. in zebra. *Onderstepoort Journal of Veterinary Research*, 50, 257–265 (1983).

The external ultrastructure of the anterior and posterior extremities of the nematodes, *Strongylus asini, Strongylus vulgaris, Strongylus equinus* and *Strongylus edentatus*, was studied with scanning electron microscopy (SEM). Fresh specimens of *S. asini* were collected from the caecum, ventral colon and *vena portae* of *Equus burchelli* and *Equus zebra hartmannae; S. vulgaris* from the caecum, colon and *arteria ileocolica* of *E. burchelli; S. equinus* from the ventral colon of *E. z. hartmannae* and *S. edentatus* from the caecum and ventral colon of both zebras, during surveys of parasites in zebras in the Etosha Game Reserve, South West Africa/Namibia, and the Kruger National Park, Republic of South Africa.

The worms were cleaned, fixed and mounted by standard methods and photographed in a JEOL JSM–35C scanning electron microscope (SEM) operating at 12kV. The SEM showed the following differences: the tips of the external leaf-crowns varied and were fine and delicate in *S. asini*, coarse and broad in *S. vulgaris* and, in *S. equinus* and *S. edentatus*, closely adherent, separating into single elements for half their length. The excretory pores showed only slight variation, and the morphology of the copulatory bursae did not differ from those seen with light microscopy. The genital cones differed markedly: *S. asini* had a ventral triangular projection and laterally 2 finger-like projections: in *S. vulgaris* there were numerous bosses on the lateral and ventral aspects of the cone; in *S. equinus* 2 finger-like processes projected laterocaudally; and in *S. edentatus* 2 pairs of papilla-like processes projected laterally on the ventral aspects, and a pair of rounded projections and a pair of hair-like structures adorned the dorsal aspects. The most significant micrograph was the shedding of the cuticle of the 4th moult of a female *S. asini*.

INTRODUCTION

The 4 species of *Strongylus* parasitizing zebra are: *Strongylus equinus* Mueller, 1780, *Strongylus asini* Boulenger, 1920, *Strongylus edentatus* (Looss, 1900) and *Strongylus vulgaris* (Looss, 1900). For some years we have been studying the parasites of zebra *Equus burchelli* in the Kruger National Park, Republic of South Africa, and both *Equus zebra hartmannae* and *E. burchelli* in the Etosha Game Reserve in South West Africa/Namibia (Malan, De Vos, Reinecke & Pletcher, 1982; Scialdo, Reinecke & De Vos, 1982; Scialdo-Krecek, 1983). Specimens of *Strongylus* spp. collected from these zebras were used in the present study.

Kikuchi (1976 a,b) has described some structures of *S. vulgaris* and *S. equinus* with the aid of the scanning electron microscope (SEM). In this paper we compare the anterior and posterior extremities of the 4 species with the aid of the SEM. The external ultrastructure of these nematodes from zebra has not previously been described.

MATERIALS AND METHODS

Collection

Necropsy techniques were similar to those described by Malan, Reinecke & Scialdo (1981 a, b) and Malan *et al.* (1982). The different species were collected from organs of zebra as follows:

(i) *S. asini:* caecum, ventral colon and *vena portae* of *E. burchelli* and *E. z. hartmannae*.

(ii) *S. vulgaris:* caecum, colon and *arteria ileocolica* of *E. burchelli*.

(iii) *S. equinus:* ventral colon of *E. z. hartmannae*.

(iv) *S. edentatus:* caecum and ventral colon of *E. burchelli* and *E. z. hartmannae*.

Cleaning and fixing

Freshly collected *Strongylus* spp. were placed in physiological saline (0,85 % NaCl solution) and each individual worm was thoroughly washed either in saline or sodium cacodylate buffer for 5–30 min. Washing was done in an Ehrlenmeyer flask either at 27 °C (room temperature) or at 37 °C in a water-bath.

Specimens were then fixed in 2 % glutaraldehyde containing 0,1 M sodium cacodylate and 0,1 M sucrose adjusted to pH 7,2. Subsequently the specimens were washed twice in 0,1 M sodium cacodylate buffer with 0,1 M sucrose at room temperature for 15 min. The specimens were post-fixed in 1 % OsO_4 containing 0,15 M sodium cacodylate buffer for 1–2 h at room temperature. A washing in 0,15 M sodium cacodylate buffer followed, and in some cases sonification was applied at this stage (modified from Brunck, Collins & Arro, 1981; Wollweber, Stracke & Gothe, 1981).

Some of the specimens were infiltrated for 1 h at room temperature with 1 % tannic acid containing 0,05 M sodium cacodylate buffer at pH 7,2. This was followed by 3 washings in 0,85 % saline for 5 min and then a 2nd incubation in 0,5 % uranyl acetate in distilled water containing 45 mg/100 mℓ sucrose for 1 h at room temperature. A final rinse was carried out in saline for 5 min. These infiltration steps were later added to improve the existing method. Examples of this procedure are demonstrated in Fig. 4, 8, 16, 20, 23 & 24.

All the specimens were dehydrated in 50 %, 70 %, 100 % (twice) acetone for 15 min in each concentration and either transferred to Freon F113 or retained in 100 % acetone. They were then critical point-dried with CO_2 in a critical point-drying apparatus. The specimens were mounted on stubs and coated with a thin Au layer in a sputter coater.

These specimens were examined and photographed in a JEOL JSM-35C scanning electron microscope operating at 12kV.

RESULTS

External leaf-crown (anterior end)

An *en face* view of the external leaf-crowns of the 4 species showed marked indentations of the mouth collar (Fig. 1–4). The elements of the external leaf-crowns can be seen in Fig. 5–8. The elements of the external leaf-crowns differed as follows:

S. asini: they were fine and slender, close to each other for most of their length, but formed pairs for the last 30 μm (Fig. 5).

[1] Department of Physiology, Medical University of Southern Africa, 0204 Medunsa, Republic of South Africa
[2] Hoechst Research Farm, P.O. Box 124, 1320 Malelane, Republic of South Africa
[3] Department of Parasitology, Faculty of Veterinary Science, P.O. Box 12580, 0110 Onderstepoort, Republic of South Africa

Received 5 July 1983—Editor

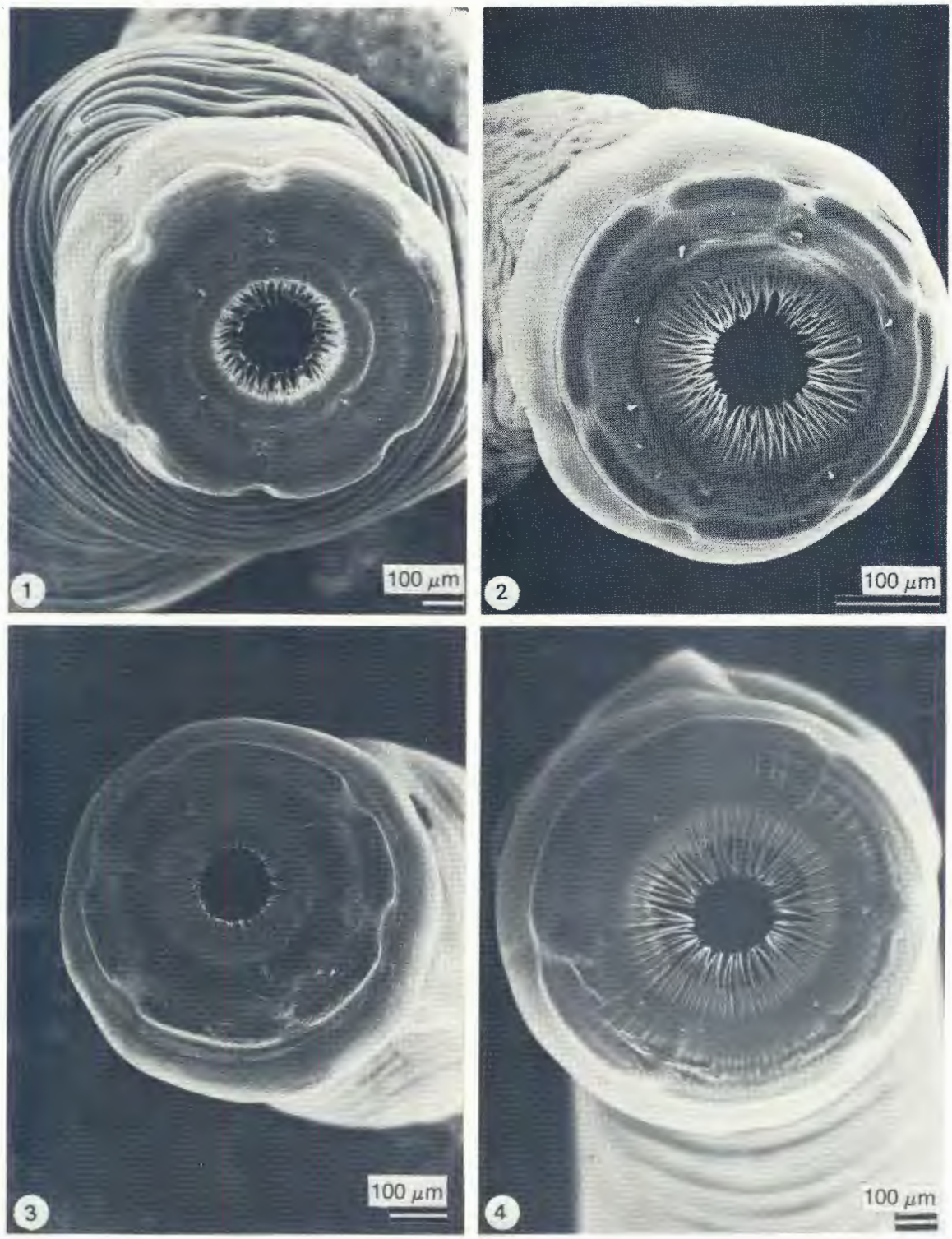

FIG. 1–4 Scanning electron micrographs of *en face* view of *Strongylus* spp., (1) *Strongylus asini*, (2) *Strongylus vulgaris*, (3) *Strongylus equinus*, (4) *Strongylus edentatus*

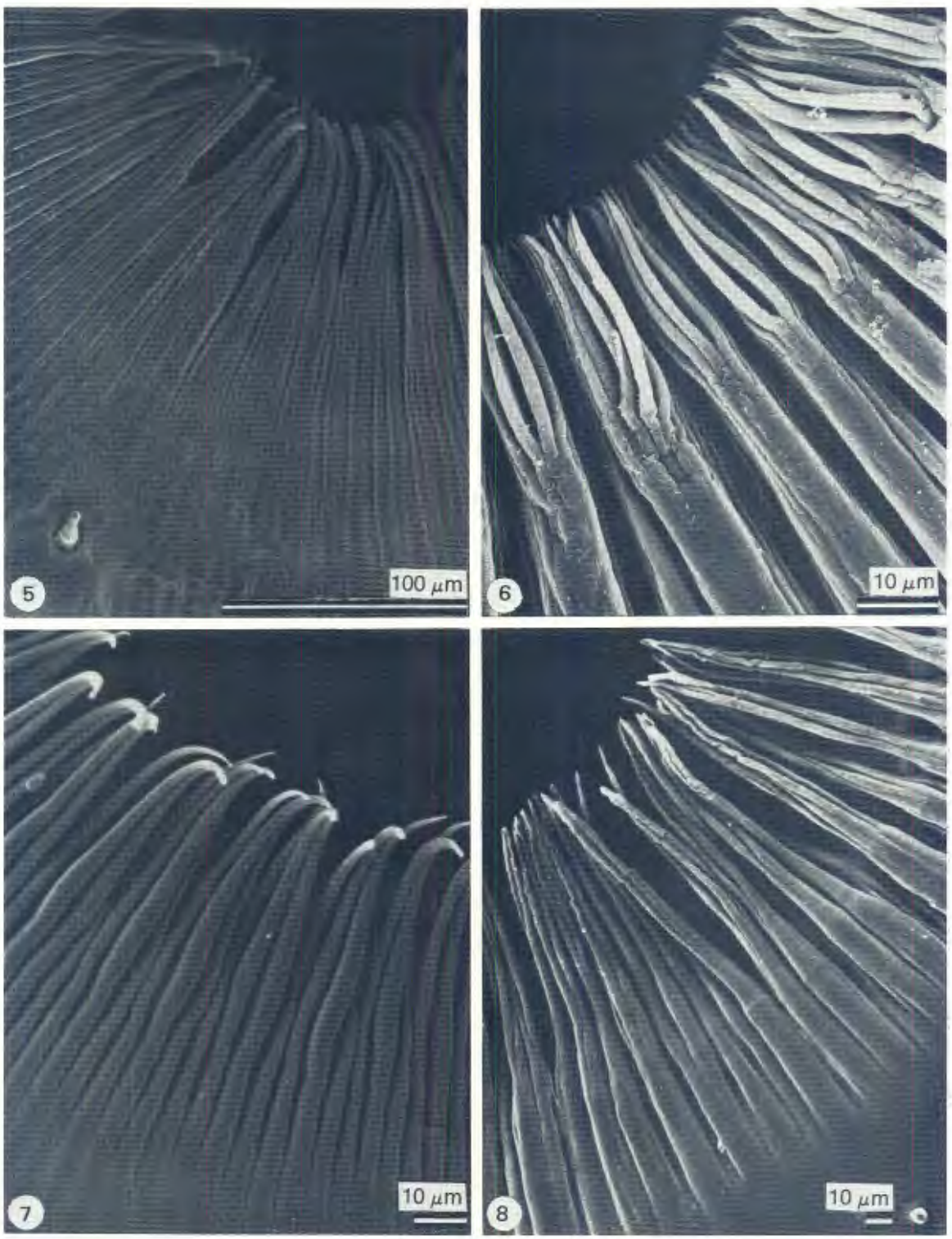

FIG. 5–8 SEM of elements of the external leaf-crowns of *Strongylus* spp., (5) *S. asini*, (6) *S. vulgaris*, (7) *S. equinus*, (8) *S. edentatus*

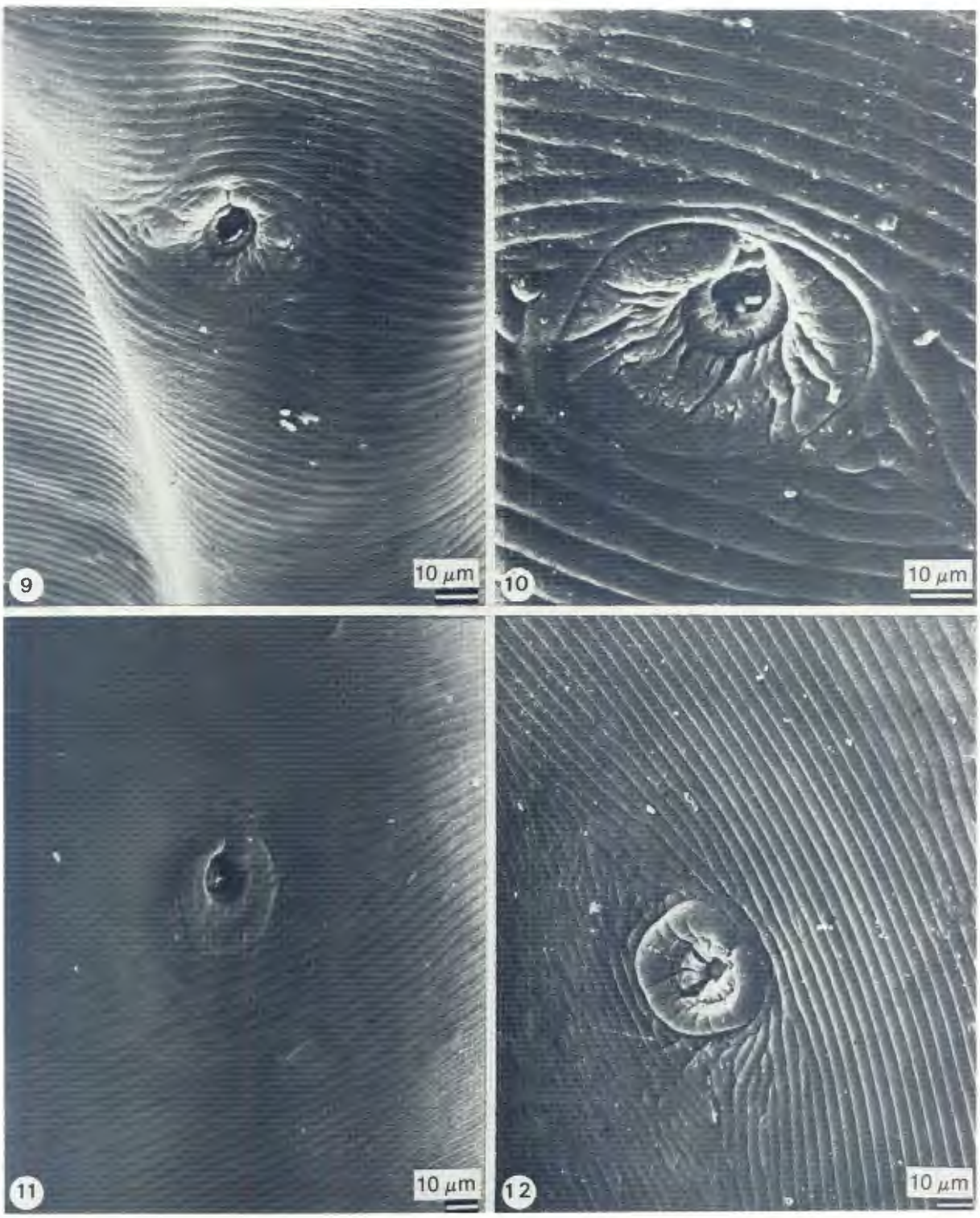

FIG. 9–12 SEM of excretory pore of *Strongylus* spp., (9) *S. asini*, (10) *S. vulgaris*, (11) *S. equinus*, (12) *S. edentatus*

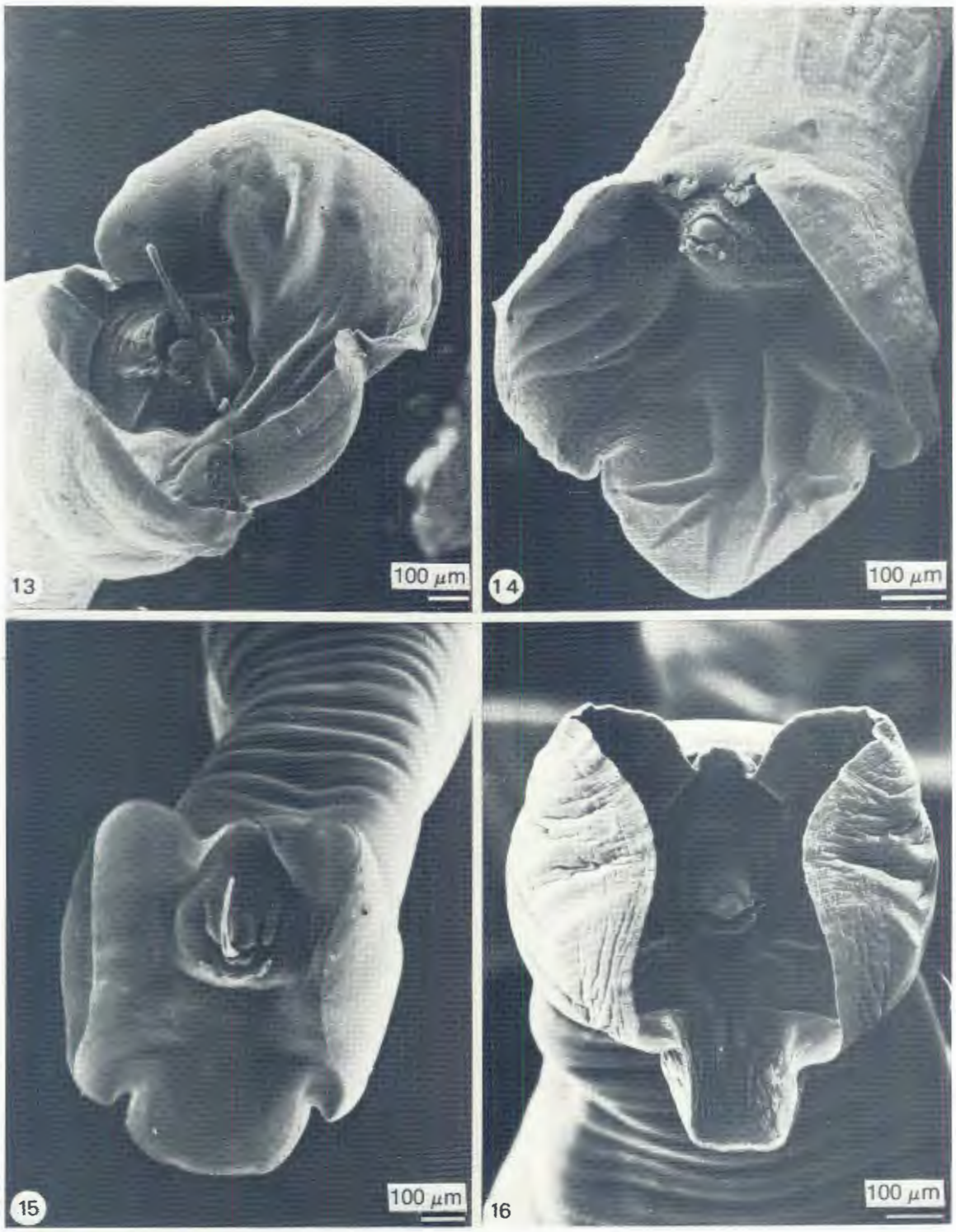

FIG. 13–16 SEM of dorsal ray of male bursa of *Strongylus* spp., (13) *S. asini*, (14) *S. vulgaris*, (15) *S. equinus*, (16) *S. edentatus*

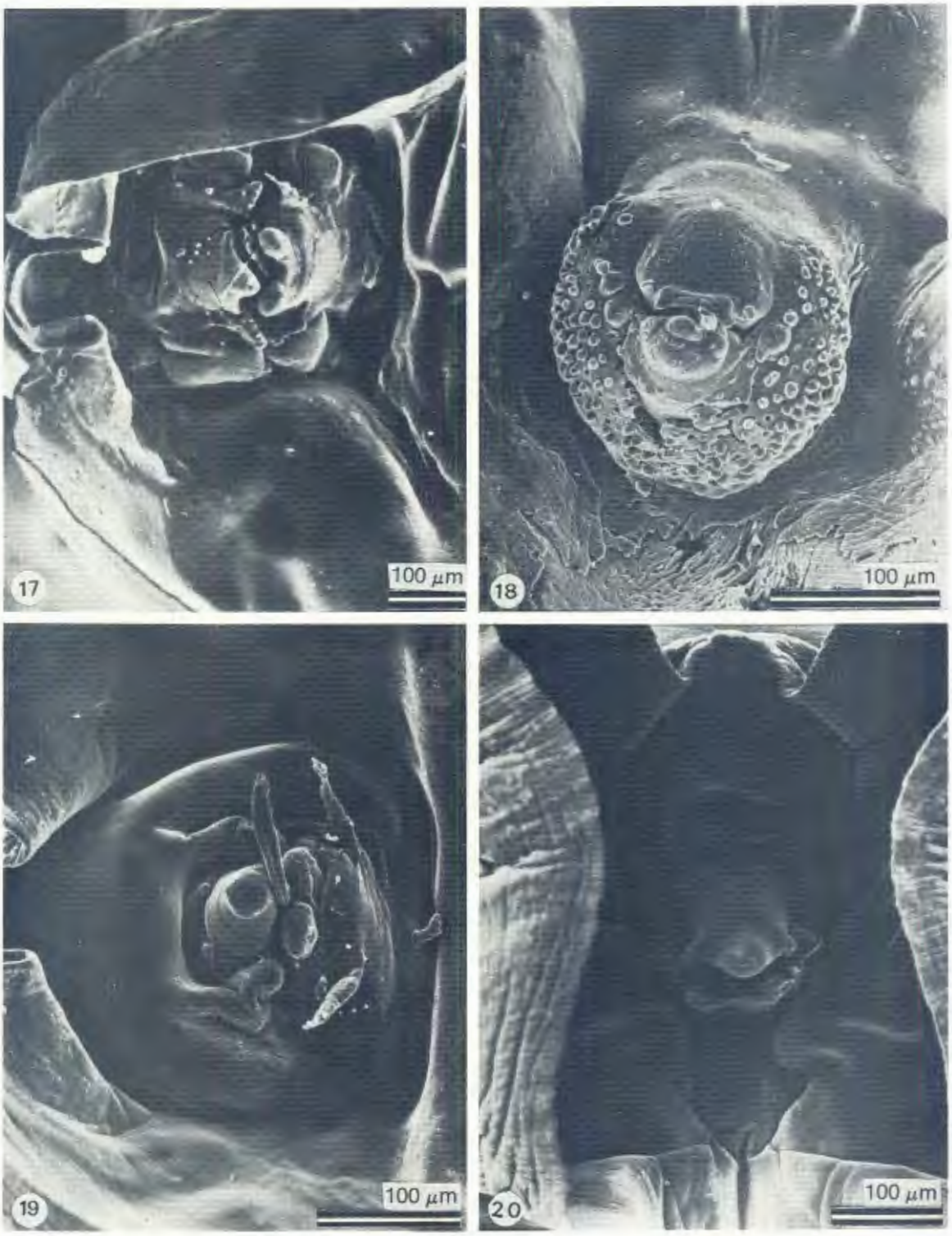

FIG. 17–20 SEM of genital cone of male bursa of *Strongylus* spp., (17) *S. asini*, (18) *S. vulgaris*, (19) *S. equinus*, (20) *S. edentatus*. (Note: the ventral sides of the bursa faces to the left of the photograph in Fig. 17 & 19, and to the top of the photograph in Fig. 18 & 20)

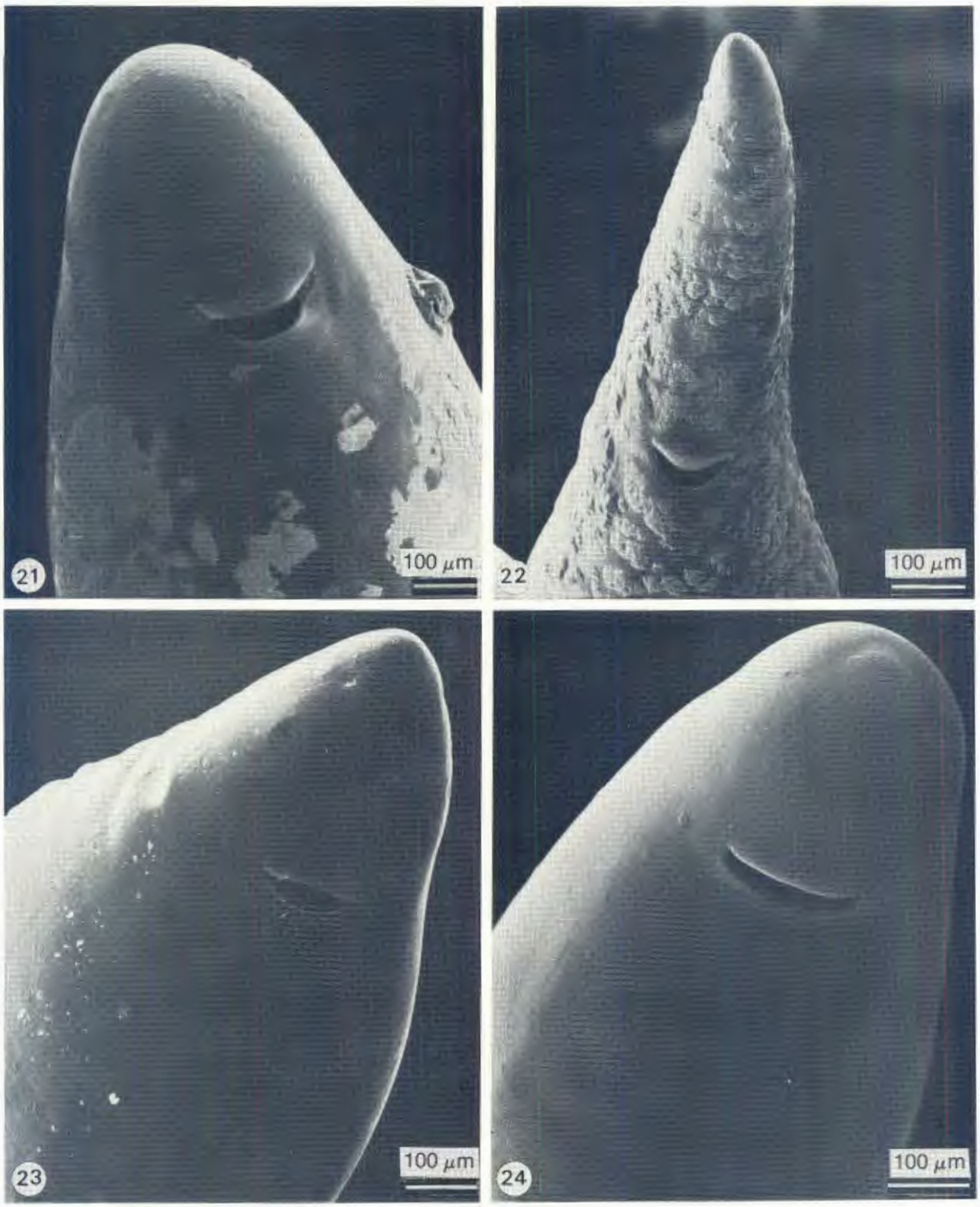

FIG. 21–24 SEM of female posterior extremity with anal opening of *Strongylus* spp., (21) *S. asini*, (22) *S. vulgaris*, (23) *S. equinus*, (24) *S. edentatus*

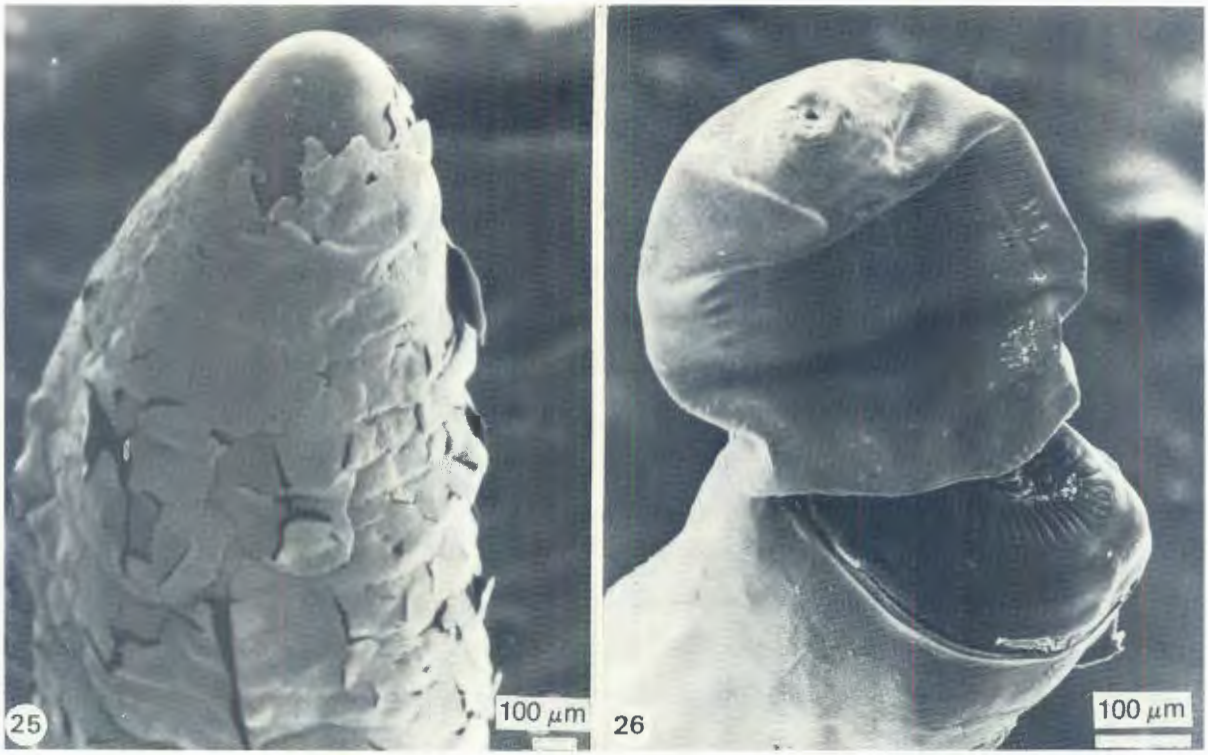

FIG. 25–26 SEM of *Strongylus* spp. undergoing the 4th moult, (25) *S. asini*, (26) *S. vulgaris*

S. vulgaris: the elements of the leaf-crowns were coarse and broad at the base (10–12 μm wide), remaining as a single element, then narrowing slightly (8 μm) before splitting into 8–10 coarse filaments 35–40 μm from the tip (Fig. 6).

S. equinus: the elements were closely adherent at the base, but separated into pairs or single elements for about the last half of their length. The petals were fine but thicker than those of *S. asini* (Fig 7).

S. edentatus: at the base 2 or 3 elements were closely adherent, separating into filaments at their tips (Fig. 8).

Excretory pore

The excretory pores were 10 μm or less in diameter (Fig. 9–12). Those of *S. asini* were depressed, but those of the other species had grooves around them. The excretory pore of *S. edentatus* was most prominent.

Copulatory bursa (posterior extremity)

S. asini: These had the smallest bursa and, in Fig. 13, one spicule was extruded.

S. vulgaris: The dorsal lobe was triangular, with prominent dorsal rays (Fig. 14).

S. equinus: The bursa was the largest of the 4 species and the dorsal lobe square (Fig. 15).

S. edentatus: The dorsal lobe was short and not rounded (Fig. 16).

Genital cone

S. asini: Ventrally there was a triangular and dorsally 2 finger-like projections on the genital cone (Fig. 17).

S. vulgaris: Numerous bosses were present on the ventral and lateral aspects of the cone (Fig. 18).

S. equinus: Two finger-like processes projected laterocaudally beyond the cone, and one spicule extruded (Fig. 19).

S. edentatus: Ventrally were 2 papilla-like processes projecting laterally to each side of the genital cone. Slightly cranially and further laterally were 2 more papilla-like processes. Dorsally were 2 moderately rounded structures and cranially to these 2 fine hair-like structures projecting laterally. The opening through which the spicules passed was clear (Fig. 20).

Female tail

With the exception of *S. vulgaris* (Fig. 22), which had a long slender tail (600 μm long from the anus to the tip of the tail), there was little variation between species.

S. asini: The female undergoing a 4th moult showed the tegument of the 4th stage flaking from the tail (Fig. 25).

S. vulgaris: the cuticle of the anterior end of the 4th stage larvae became detached in a single piece to reveal the external leaf-crown of the 5th stage (Fig. 26).

DISCUSSION

The most important morphological characteristics of these species are the differences in the size of the worms, the structures in buccal capsule (teeth and dorsal gutter) and the copulatory bursa of the male. In addition to these microscopic features, SEM reveals external morphological features which confirm that these are 4 different species.

The ultrastructure shows clear differences in the size and arrangement of the elements of the external leaf-crowns, the excretory pores and the structures of the genital cones. While the cervical papillae of *S. asini, S. equinus* and *S. vulgaris* were evident, we were not able to demonstrate these structures on *S. edentatus*.

The SEM revealed a genital cone which seemed to include part of the cloacal wall extruding caudally and surrounding the tip of the spicule(s). Reinecke (personal

communication, 1983) postulates that this is an ejaculatory duct or primitive penis which enters the vulva and vagina with the spicules during copulation.

This is the first report of *S. asini, S. equinus* and *S. edentatus* in the mountain zebra, *E. z. hartmannae*.

The moulting of the 4th stage is clearly illustrated with SEM. The tegument is literally flaking from the female *S. asini* during the 4th moult, and the anterior end of the 4th stage is lifting off to release the 5th stage of *S. vulgaris*. These are significant illustrations of the moulting process (Fig. 25–26).

Those specimens that were placed in tannic acid and uranyl acetate were better preserved and there was notably less collapse and deformation of the tegument.

The external structures of the surface of parasitic strongyles differ markedly between species, and SEM makes it possible to compare these structures.

ACKNOWLEDGEMENTS

We wish to thank the University of Pretoria, the Council for Scientific and Industrial Research and the Department of Agriculture and Fisheries of South Africa for financial assistance. The technical assistance of Miss Henriëtha Soer and Mrs C. Roper is gratefully acknowledged. The authors are also grateful to Mrs E. L. Visser for her co-operation, Prof. R. K. Reinecke for assistance in preparing the manuscript, Dr V. de Vos, Kruger National Park, and Dr Ian Hofmeyer, Etosha National Park, and their staffs for their co-operation in processing material, and to Dr Theuns van Wyk for his assistance.

REFERENCES

BRUNCK, U., COLLINS, V. P. & ARRO, E., 1981. The fixation, dehydration, drying and coating of cultured cells for SEM. *Journal of Microscopy*, 123, 121–131.

KIKUCHI, S., 1976 a. (Scanning electron microscopy of nematodes of mammals and birds. *Strongylus vulgaris* and *Stephanurus dentatus*.) *Journal of Veterinary Medicine, Tokyo* No. 657, 214–219.

KIKUCHI, S., 1976 b. (Scanning electron microscopy of nematodes of mammals and birds. *Strongylus equinus* and *Ancylostoma caninum*.) *Journal of Veterinary Medicine, Tokyo* No. 658, 275, 277, 281–282.

MALAN, F. S., REINECKE, R. K. & SCIALDO, ROSINA C., 1981 a. Recovery of helminths post-mortem from equines. I. Parasites in arteries, subperitoneum, liver and lungs. *Onderstepoort Journal of Veterinary Research*, 48, 141–143.

MALAN, F. S., REINECKE, R. K. & SCIALDO, ROSINA C., 1981 b. Recovery of helminths post-mortem from equines. II. Helminths and larvae of *Gasterophilus* in the gastro-intestinal tract and oestrids from the sinuses. *Onderstepoort Journal of Veterinary Research*, 48, 145–147.

MALAN, F. S., DE VOS, V., REINECKE, R. K. & PLETCHER, J. M., 1982. Studies on *Strongylus asini*. I. Experimental infestation of equines. *Onderstepoort Journal of Veterinary Research*, 49, 151–154.

SCIALDO, ROSINA, C., REINECKE, R. K. & DE VOS, V., 1982. Seasonal incidence of helminths in the Burchell's zebra. *Onderstepoort Journal of Veterinary Research*, 49, 127–130.

SCIALDO-KRECEK, ROSINA C., 1983. Studies on the parasites of zebras. I. Nematodes of Burchell's zebra in the Kruger National Park. *Onderstepoort Journal of Veterinary Research* 50, 111–114.

WOLLWEBER, L., STRACKE, R. & GOTHE, U., 1981. The use of a simple method to avoid cell shrinkage during SEM preparation. *Journal of Microscopy*, 121, 185–189.