

Scanning Electron Microscopic Study of *Proisorhynchoides arcuatus* (Linton, 1900) (Bucephalidae: Digenea)

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Proisorhynchoides arcuatus (Linton, 1900) from the intestine of *Pomatomus saltator* (L.) from the Atlantic coast of the State of Rio de Janeiro is studied by scanning electron microscopy, with detailed description of tegumental spines. Comments on the synonymy of this species with *Bucephalopsis callicotyle* Kohn, 1962 are made. The tegument of adult *P. arcuatus* presents scale like and serrated spines and unciliated sensory papillae, distributed over the body surface and is compared with other digenetic trematodes.

Key words: *Proisorhynchoides arcuatus* - *Bucephalopsis callicotyle* - fish parasites - *Pomatomus saltator* - Brazil - scanning electron microscopy

Proisorhynchoides arcuatus was described as *Bucephalopsis callicotyle* in Brazil by Kohn (1962) from *Pomatomus saltator* and posteriorly referred by Gomes et al. (1972), Rego et al. (1983) and Wallet and Kohn (1987) from the same host and locality.

Srivastava and Chauhan (1973) published the historical review of the genus *Proisorhynchoides* Dollfus, 1929 with its synonyms *Bucephalopsis* (Diesing, 1855) Nicoll, 1914, *Neobucephalopsis* Dayal, 1948 and *Bucephaloides* Hopkins, 1954.

Bray (1984) considered *B. callicotyle* Kohn, 1962 as a synonym of *P. arcuatus* (Linton, 1900) considering that both species present the same morphological characteristics, although the size of anterior sucker in *P. arcuatus* of Linton (1940) is smaller than in *B. callicotyle*.

Recent investigations using scanning electron microscopy (SEM) have revealed ultrastructural features of several adult trematodes, demonstrating that the tegumental surfaces varied among the species. From the family Bucephalidae up to now, only few members had been studied by SEM (Pandey & Tewari 1984, Halton & Johnston 1982).

In the present paper, the surface ultrastructure of *P. arcuatus* was studied by SEM for the first time.

MATERIALS AND METHODS

Pomatomus saltator (L.) were obtained from fishermen of "Praia da Ribeira, Ilha do Governador" and "Praia de Copacabana", Atlantic coast of the State of Rio de Janeiro, Brazil. From the 36 specimens of fish examined, 17 were parasitized by 1-24 specimens of *P. arcuatus*.

For light microscope observations, 28 specimens were fixed and preserved in AFA (Alcohol, Formalin, Acetic acid), stained in alcoholic-acid carmine of Langeron, dehydrated in ethyl alcohol, cleared in beachwood creosote and mounted in Canada balsam. Drawings were made with the aid of a Leitz microscope drawing attachment. The photo was taken in a Zeiss Axiophoto microscope. The specimens were deposited in the Helminthological Collection of "Instituto Oswaldo Cruz" (CHIOC) No. 33220 to 33230.

The specimen No. 30742 of *P. arcuatus* studied by Gomes et al. (1972) and deposited in CHIOC as *Bucephalopsis callicotyle* was also examined and measured.

For SEM two worms were fixed in 2.5% glutaraldehyde phosphate buffer and post fixed for 1 hr in 1% osmium tetroxide in the same buffer, dehydrated in graded ethanol, critical point dried and coated with gold. The observations were made using a Zeiss SEM DSM 940.

RESULTS

Proisorhynchoides arcuatus
(Linton, 1900) Bray, 1984
(Figs 1-14)

Host: *Pomatomus saltator* (L.), common named "enxova" (Pomatomidae)

Site: intestine

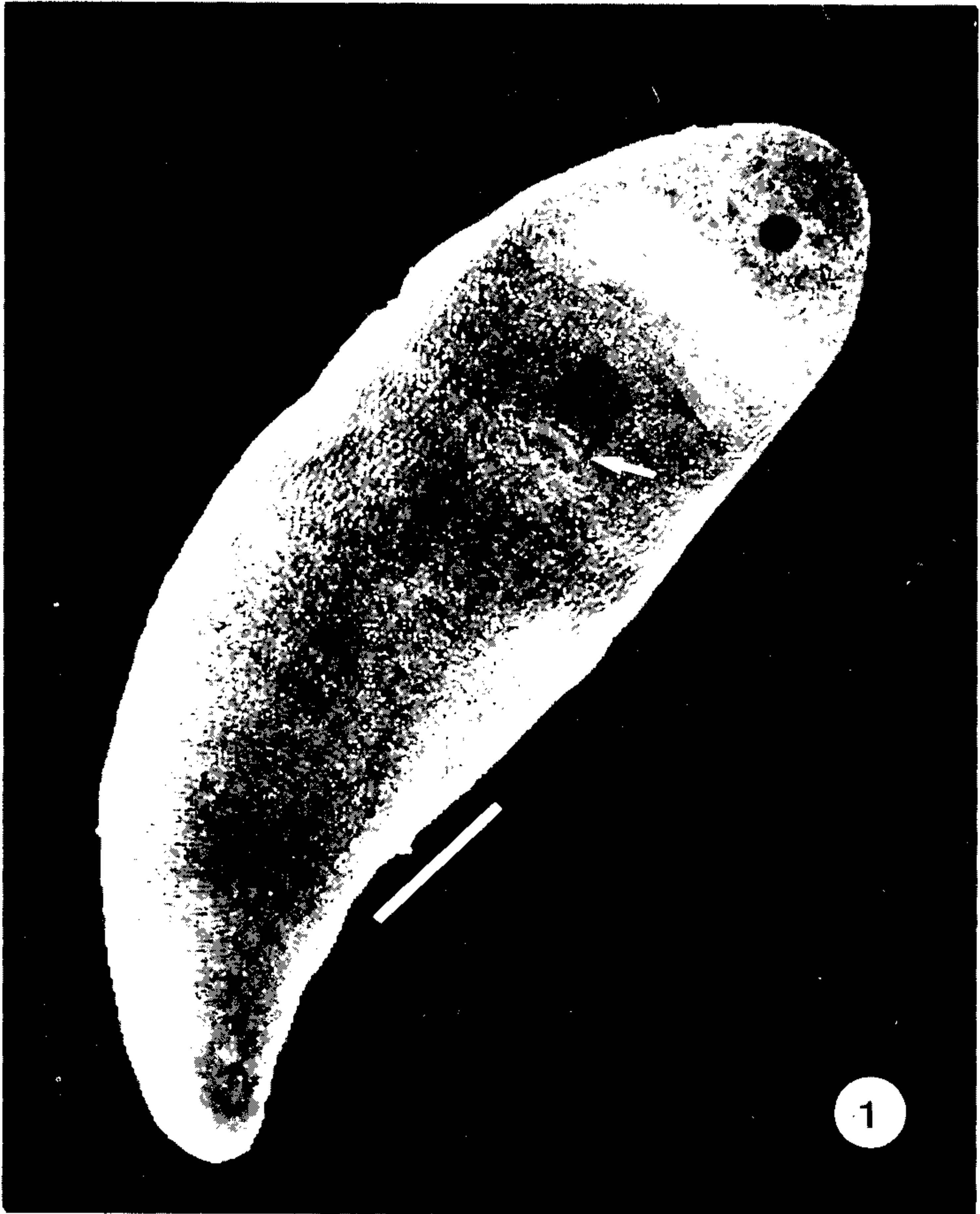
The morphology and measurements of the specimens studied by light microscopy are in agreement with the material described by previ-

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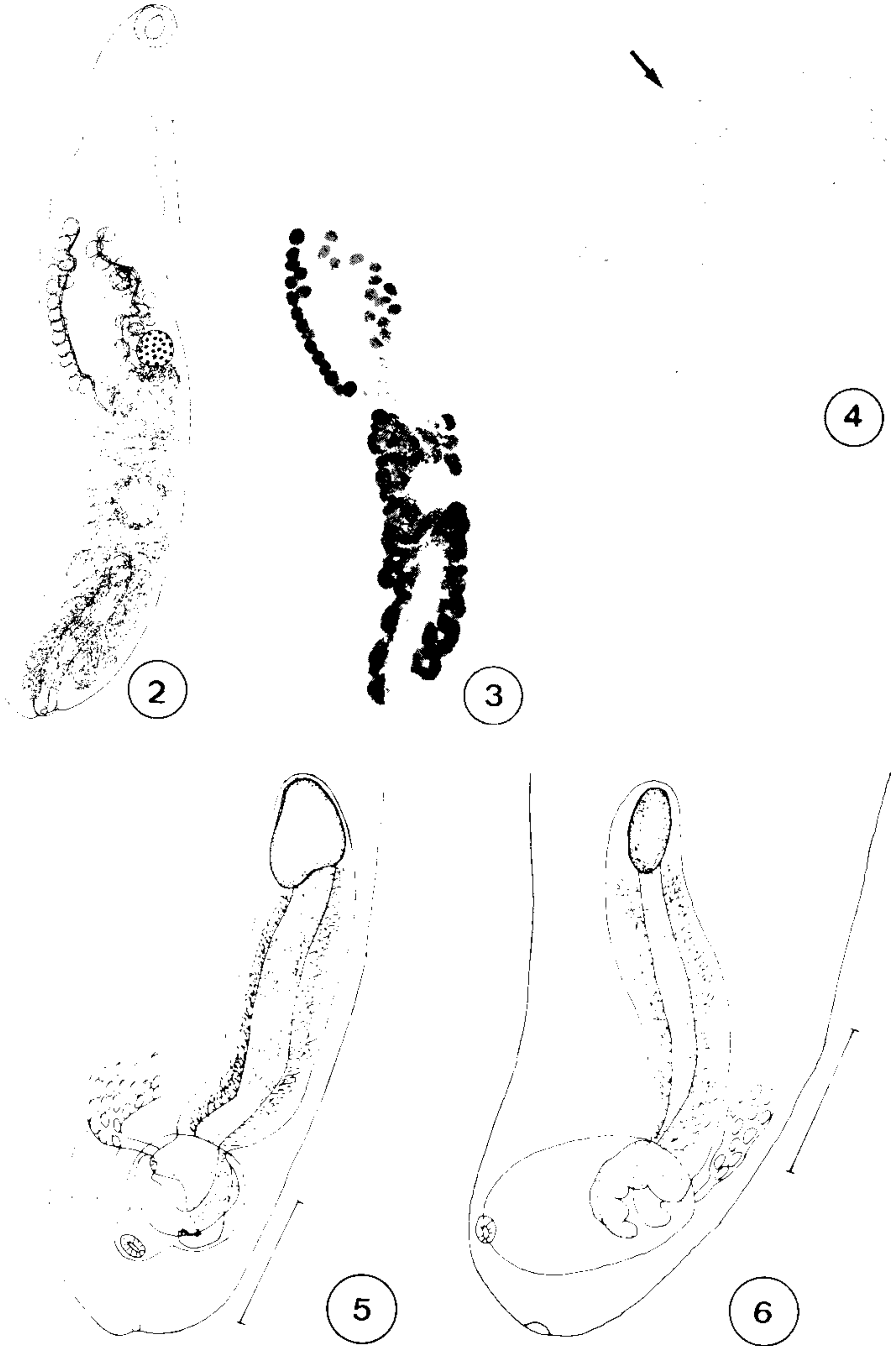
Prosorhynchoides arcuatus (Linton, 1900) Fig. 1. SEM micrography, whole worm. Mouth aperture arrowed. Scale-bar: 100 μ m

ous authors from the same host and locality (Kohn 1962, Wallet & Kohn 1987).

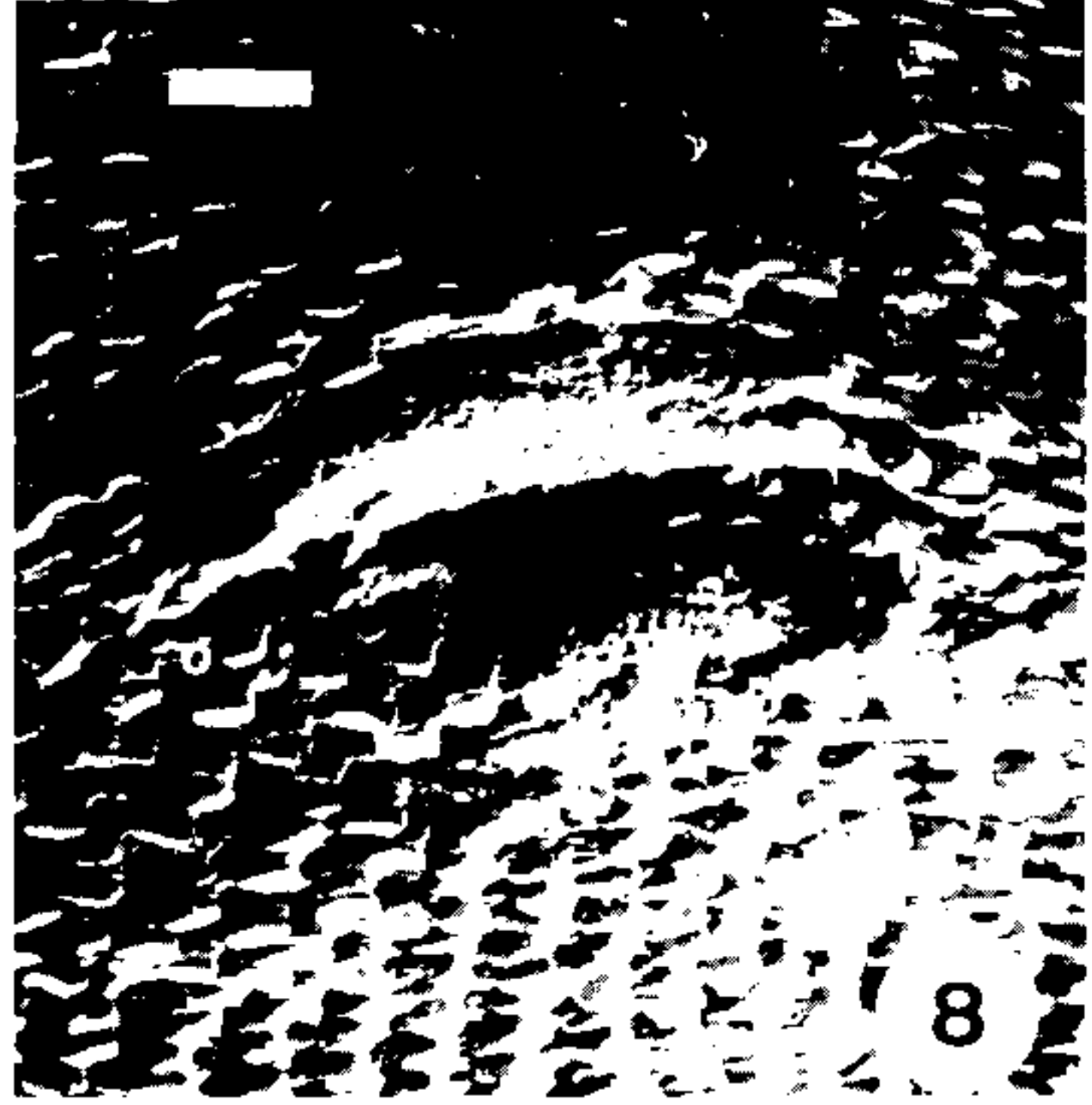
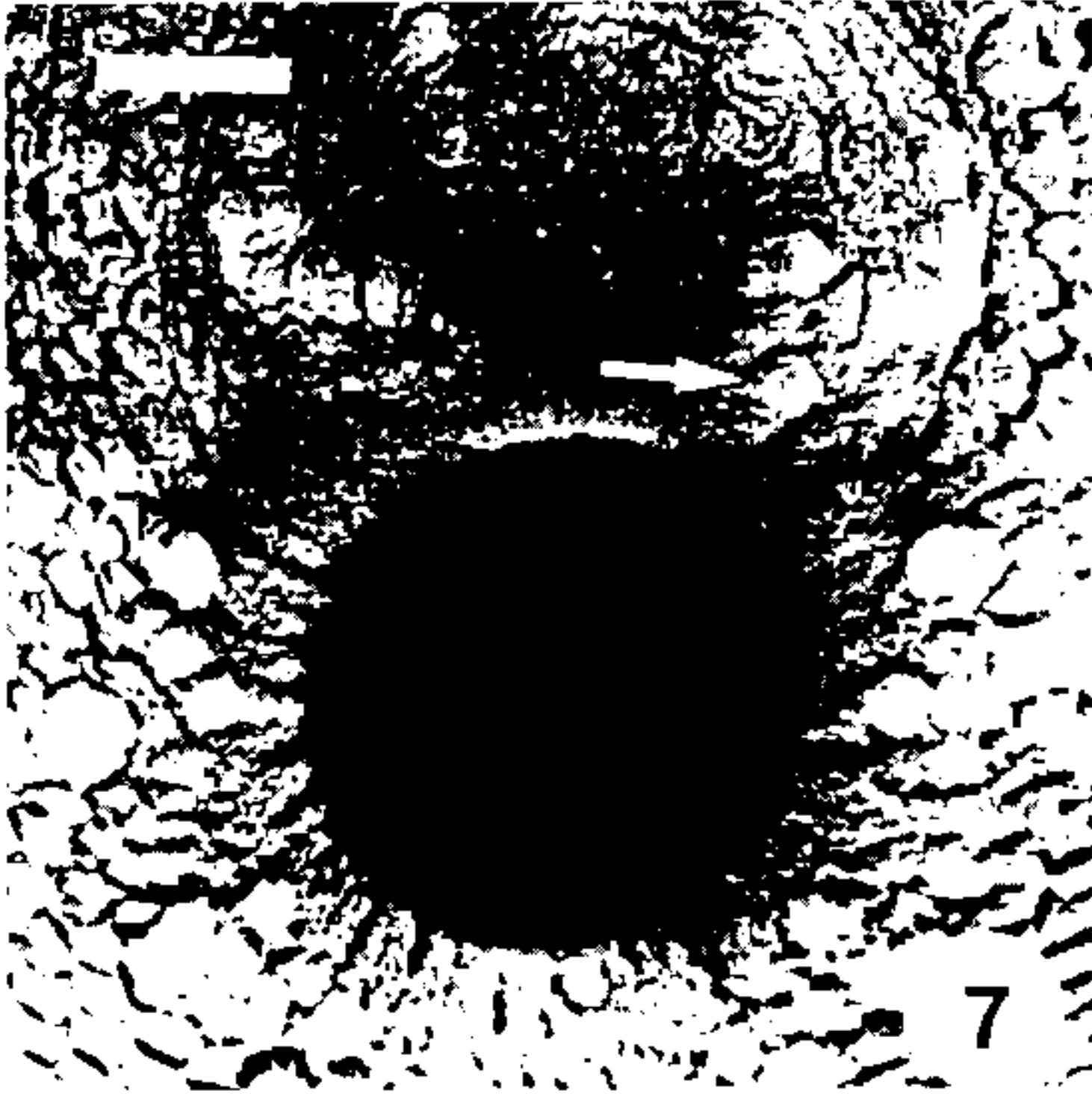
By SEM observations, the body is elongated, with rounded extremities or tapered posteriorly (Fig. 1), while by light microscopy, compressed specimens present a more elongated body (Figs 2, 3). The anterior sucker is subterminal, with rounded opening (Fig. 7). Mouth aperture elliptical (Fig. 8), opening ventrally in the anterior half of body length (Fig. 1,

arrow). Genital atrium located in the posterior region of body, can be observed as a protuberance in the surface (Fig. 9). By light microscopy we observed genital lobes sub-equal, projecting into genital atrium. Excretory pore terminal, situated below genital atrium (Fig. 9).

The body tegument presents transversal ridges at regular intervals formed by cobblestone-like areas (Fig. 10), encircling the worm. The



Prosorhynchoides arcuatus (Linton, 1900) - Fig. 2: whole worm, ventral view. Scale-bar: 500 μ m. Fig. 3: photomicrography of whole worm, ventral view, X 35. Fig. 4: photomicrography of anterior region of body, showing anterior sucker and spines with pointed appearance. X 53. Figs 5-6: posterior region of body, showing cirrus sac with different aspects of genital atrium and genital lobes. Scale-bars: 200 μ m



SEM micrographs of *Prosorhynchoides arcuatus* - Fig. 7: anterior sucker surrounded by sensory papillae (arrow). Scale-bar: 10 μ m. Fig. 8: mouth aperture. Scale-bar: 10 μ m. Fig. 9: genital atrium (ga) and excretory pore (ep). Scale-bar: 10 μ m. Fig. 10: body surface and uniciliated sensory papillae (arrow). Scale-bar: 5 μ m.

whole body surface of the parasite is covered with numerous flattened tegumental spines, arranged in regular rows among the ridges. In the anterior and middle regions of the body, the spines overlap completely and obscure the structure of underlying tegument (Fig. 13).

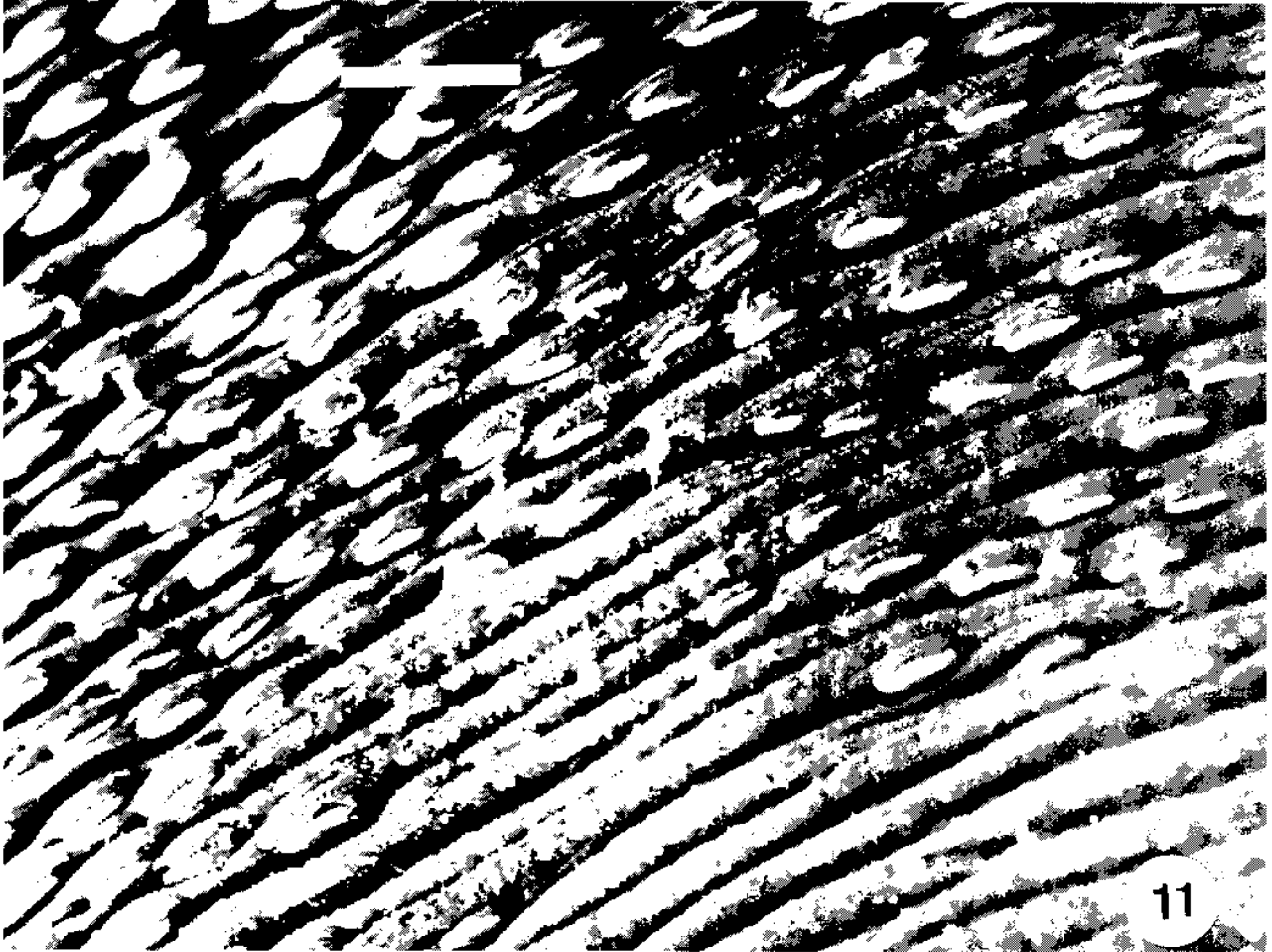
The spines, when observed in light microscope preparations, seem to be single pointed (Fig. 4) and at higher magnification, by SEM, they appear multipointed, with up to 30 points which remain the same in all regions of body surface (Fig. 14).

On the posterior region of the body the spines observed were located inside the folds of the tegument (Fig. 12), probably due to the state of con-

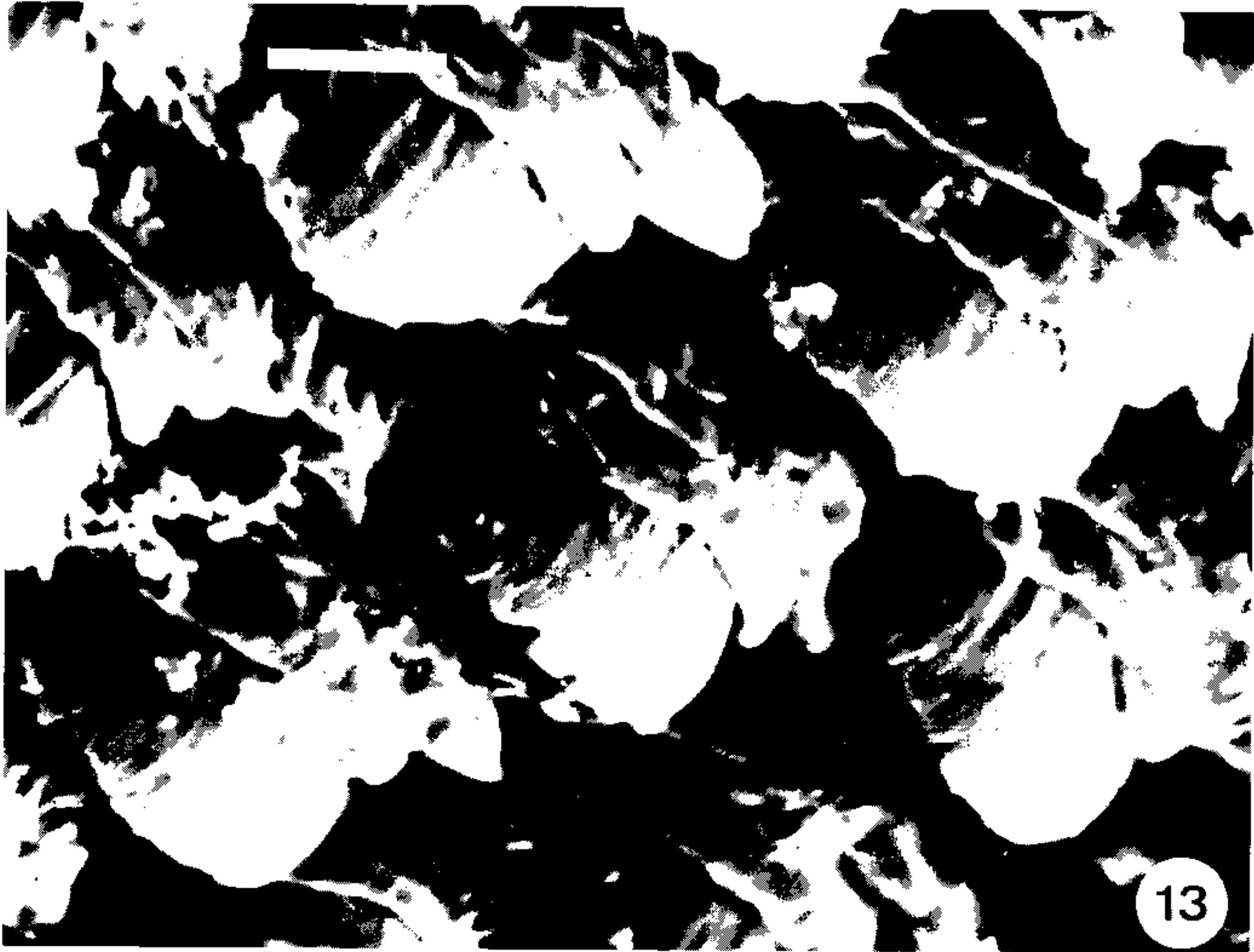
traction of body. By light microscopy this surface appears to be devoid of spines.

Sensory papillae are observed all over the body surface, consisting of an elevated area of tegument surrounding a pit from which arise a single retractil cilium (Figs 10, 12).

The tegument of the rims of the anterior sucker is similar to the surface tegument in the cobblesome units, but differs in that the ridges are longitudinal and lack the spines. The tegument of the inner surface of this sucker has also a cobblesome appearance, without spines. Uniciliated sensory papillae are present around this sucker, more numerous than at the rest of body surface.



SEM micrographs of *Proisorhynchoides arcuatus* - Fig. 11: tegument of the posterior region of body with spines outer and inner of tegumental folds. Scale-bar: 10 μ m. Fig. 12: high resolution of surface of posterior part of body with spines inside the tegumental folds and uniciliated sensory papilla (arrow). Scale-bar: 2 μ m.



SEM micrographs of *Prosorhynchoides arcuatus* - Fig. 13: tegument of middle ventral surface of body with spines. Scale-bar: 2 μ m. Fig. 14: high resolution of tegumental serrated spines. Scale- bar: 1 μ m.

The tegument around openings of the genital atrium and excretory pore also form longitudinal striations, devoid of spines.

DISCUSSION

Yamaguti (1970) described *P. arcuatus* from *Sarda orientalis* from Hawaii in the genus *Bucephalopsis*. In his paper he refers "genital lobe projecting into genital atrium, with its tip turned back on itself; near the base of this lobe is attached a curved horn-like projection on the right side and another shorter scoop-shaped process on the left side. Genital pore opening ventrally near posterior tip of body". In our specimens we observe two subequal genital lobes projecting into the genital atrium (Figs 5, 6) but we could not identify the "horn-like projection" and the "scoopshaped process" referred and figured by Yamaguti.

Examining the specimen studied by Gomes et al. (1972), we observe that the anterior sucker measures 180 by 220 and the eggs 15-17 by 12-15 instead of 260-280 and 26-31 x 20-26, respectively, as referred by these authors. The ratio of the anterior sucker and body length of the Brazilian specimens are larger than the formerly described from different hosts and localities (Linton 1900, 1940, Ward 1954, Yamaguti 1970). Although there are differences in the ratio of anterior sucker/ body length and in the structures of the genital lobes, we prefer to maintain the synonymy of *B. callicotyle* with *P. arcuatus* proposed by Bray (1984), considering the other similar morphological characteristics, until the type material of *P. arcuatus* can better be studied.

The cobblestone-like surface of *P. arcuatus* is similar to that described for *Megalodiscus temperatus* (Stafford, 1905) by Nollen and Nadakavukaren (1974) and for *Gorgoderina attenuata* (Stafford, 1902) by Nadakavukaren and Nollen (1975).

The presence of scale-like spines was revealed by SEM also in other digeneans from different families, associated to the gastrointestinal tract (Lee et al. 1984, Pandey & Tewari 1984, Fujino et al. 1989, Srisawangwonk et al. 1989, Hong et al. 1991, Koie 1992). Although the spines in *P. arcuatus* possess the same distribution all over the body surface, with the same number of points, this feature is different in other digeneans. *Echinostoma revolutum* (Froelich, 1802) has scale-like spines, but they are not serrated (Smales & Blankespoor 1984); *Heterophyopsis continua* Onji and Nishio, 1916 presents a great difference between the spines of body: at the anterior end they present 15-17 points, decreasing posteriorly (Hong et al. 1991). In *Haplorchis pumilio* (Looss, 1896) the size of the scale-like spines decreases at the anterior and posterior regions of body while in *Metagonimus*

yokogawai (Katsurada, 1912) they change to slender spines posterior to ventral sucker (Srisawangwonk et al. 1989). The spines of *M. yokogawai* and *M. takahashii* are serrated and single or double pointed posteriorly (Fujino et al. 1989) and no spines are present around the excretory pore, as in *P. arcuatus*. In *Pygidiopsis ardea* Koie, 1990 the multipointed spines decrease in size posteriorly (Koie 1992). *Leucochloridium* sp. (on 55-day-old specimens) presents spines which were found to have up to 20 points on the posterior ventral part (Bakke 1976).

The tegumental spines of *P. arcuatus* with up to 30 points, appear to be similar to *P. karvei* Bhalerao, 1937 although the number of points varies from 3 to 25 in the latter species (Pandey & Tewari 1984). Several observations demonstrated that tegumental spines are more single pointed on the surface of juveniles than of adults (Koie 1977, 1992, Lee et al. 1984). The single spines on juvenile flukes probably function in locomotion and anchorage (Hong et al. 1991) and the more pointed and enlarged spines are related to abrasion of host tissue for feeding and to a less degree for anchorage (Koie 1977).

According to Bennett (1975b) the spines are cytological structures which are not present in either of the common liver fluke's hosts, thus making them unique targets for antihelminthic drugs.

Bakke (1976) has stated that the absence of spines on the suckers may indicate the necessity for a smooth seal against the host mucosa as found in other digeneans. Differing from *P. arcuatus*, the dorsal surface of *Leucochloridium* sp. is aspinose with no functional role in maintenance of position in the host. The spines must also have a very irritating effect on the host's intestinal mucosa, according to Davies (1979).

Sensory papillae with a long and short cilium were found on the surface of other digenetic trematodes, as in adults of *H. pumilio* referred by Srisawangwonk et al. (1989), in *E. revolutum* and *Isthmiophora melis* by Smales and Blankespoor (1984), in juveniles and adults of *M. yokogawai* by Lee et al. (1984), in *H. continua* by Hong et al. (1991) and in rediae and cercariae of *Ribeiroia marini* by Page et al. (1980).

The unciliated papillae of the body surface of *P. arcuatus* appear to be similar to the type A papillae of *Clonorchis sinensis* (Cobbold, 1875) studied by Fujino et al. (1979). According to these authors the papillae have tango and/or rheoreceptive functions and may serve to orientation, feeding and assessment of the host environment. Bennett (1975a) stated that "the cilium may be capable to recording the direction and degree of any pressure acting on spines".

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