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ORIGINAL ARTICLE

Ultrastructural studies on *Setaria digitata* by scaning electron microscopy

Lingala Raju Kumar · Manchukonda Udaya Kumar

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Abstract Ultrastructural details of adult male and female worms of *Setaria* species collected from the peritoneal cavities of bovines slaughtered in and around Hyderabad, Telangana, India were studied using scanning electron microscopy. The worms exhibited peribuccal crown with round mouth opening encircled by a pair of lateral appendages and slightly notched dorso and ventro projections at the anterior end where as bosses and fine cuticular striations were seen all over the body. Posterior end of female worm ended in a round knob with a pair of lateral appendages whereas three pairs of precloacal, a pair of adcloacal, three pairs of postcloacal papillae, a central papillae just in front of the cloaca, a single tongue shaped spicule emerging from the cloaca, and a phasmid in front of a pair of lateral appendages were seen at the posterior end of male worm. The ultrastructural details confirmed the worms as *Seteria digitata.*

Keywords *Setaria digitata* · SEM · Bovine

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L. Raju Kumar · M. Udaya Kumar
Department of Veterinary Parasitology, College of Veterinary Science, Sri Venkateswara Veterinary University, Rajendranagar, Hyderabad 500 030, Andhra Pradesh, India
e-mail: vety0526@gmail.com

L. Raju Kumar
Veterinary Dispensary, Maddur Mandal, Warangal District, Warangal, Telangana, India

M. Udaya Kumar (✉)
Department of Veterinary Parasitology, College of Veterinary Science, Sri PV Narsimha Rao Telangana State University for Veterinary, Animal and Fishery Sciences, Korutla, Karimnagar 505 326, Telangana, India
e-mail: udaiku@gmail.com; udaiku@yahoo.co.in

Introduction

About 43 species of *Setaria* (Yatawara et al. 2007) were identified so far in more than 100 countries of world including India. Among setarial worms *S. digitata, S. labiato-papillosa, S. cervi, S. marshali, S. yehi* have been reported worldwide. There are two setaria species infecting cattle and buffaloes, viz. *S. digitata* and *S. labiato-papillosa*, the most common being *S. digitata* (Shoho 1958) which commonly dwells in peritoneal cavities without any apparent pathogenicity but in heavy infections it causes mild fibrinous peritonitis (Jayasinghe and Wijesundera 2003). The microfilariae (L_1) found in the blood stream of host causes microfilariosis in bovine natural hosts (Sigraskar et al. 1999) and infectious form causes cerebrospinal nematodosis in unnatural hosts i.e., sheep, goat and horses (Rhee et al. 1994). Morphological studies of the worms not only help in species identification but also form the basis for taxonomy, phylogenetic establishment and phylogenetic position of the parasites (Chabaud and Bain 1994). Traditionally worms are identified with the aid of light microscopy on cleared worms but specific identification of the species poses problem (Yadav and Tandon 1991) in mixed infections because these worms lack specific distinguishable characters which can be recognized by light microscopy (Rhee et al. 1994). Therefore the present study has been undertaken to study the fine ultrastructural details of male and female worms of *S. digitata* by scanning electron microscopy (SEM).

Materials and methods

Setaria worms of both sexes were collected from the peritoneal cavity of bovines slaughtered at Chengicherla,

Hyderabad and Alana slaughter house, Zaheerabad, Telangana State and transported to the laboratory in Phosphate buffered saline (pH 7.2). Worms were cleaned several times in PBS (pH 7.2) to remove host material and blood contamination (Dhas et al. 1993).

The anterior and posterior ends of the worms were cut, cleared in lactophenol for identification of the worm by light microscopy. Later the worms were prepared for SEM as per the procedure of Rhee et al. (1994) with minor modifications. Cleared head and tail ends of the worms were fixed in 2.5 % gluteraldehyde in 0.1 M phosphate buffer (pH 7.2) for 24 h at 4 °C and postfixed in 2 % osmium tetroxide for 4 h. Later samples were dehydrated in graded ethyl alcohol and dried to critical point drying in CPD unit. The processed samples were mounted over the stubs with double carbon conductivity tape, and a thin layering of gold coat over the samples was done by using an automated sputter coater (model-JOEL JFC-1600) for 3 min and scanned under scanning electron microscope (SEM—model: JOEL-JSM 5600) at required magnifications as per the standard procedures at RUSKA Lab's College of Veterinary Science, Sri Venkateswara Veterinary University, Rajendranagar, Hyderabad, Telangana State, India.

Results and discussion

Milky white coloured, thread like nematodes with rounded anterior end and coiled posterior end (Soulsby 1982) collected alive from the peritoneal cavities of slaughtered bovines were tentatively identified as setarid worms. The female worms were comparatively longer ranging from 90 to 120 mm (104 ± 8.0) and wider ranging from 0.5 to 0.86 mm (0.59 ± 0.05) than the length (30–40 mm (35 ± 2) and width (0.5–0.6 mm (0.41 ± 0.02) of male worms. The dimensions of *S. digitata* recorded in the present study were falling in the range reported by Innes and Shoho (1953), Nakano et al. (2006) and Kim et al. (2010).

Tentative identification of the lactophenol cleared anterior end of the worms revealed the presence of a chitinous peribuccal crown (Subhachalat et al. 1999) with a pair of circular lateral lips adjacent to buccal aperture and also with dorsal and ventral projections at the anterior end. Our observations were matching with the descriptions of Whitlock (1960) for *S. digitata.* Similarly, lactophenol cleared tapering posterior end of female worm ending in a smooth knob with a pair of lateral appendages anterior to the knob were in confirmity with the observations of Rhee et al. (1994) and Subhachalat et al. (1999) where as male worm having three pairs of pre-cloacal, a pair of adcloacal, three pairs of postcloacal papillae, a single spicule and a pair of lateral appendages nearer to the tip were on line with the observations of Subhachalat et al. (1999) and Nakano et al. (2006).

There has been difficulty in the identification of species when a multiple infection by different species of *Setaria* i.e., *S. digitata*, S. *labiato-papillosa* and *S. cervi* occur in the natural infections of cattle and buffaloes in India. Earlier Subhachalat et al. (1999) made attempts to differentiate between *S. digitata* and *S. marshali* by SDS-PAGE analysis. However, the SEM has its own advantage of high resolution of the ultrstructure of *Setaria* species thereby facilitating definitive species identification as briefly discussed in literatuere by Shoho and Uni (1977) and Almeida et al. (1991). Hence, scanning electron microscopic studies were conducted to avoid any such possible misidentifiction of the species under light microscopy.

The head of both male and female worms of *S. digitata* exhibited uniform roundness of the buccal opening with adjacent circular lateral lips without any indentations (Kim et al. 2010), and somewhat depressed dorso and ventro projections of the peribuccal crown at the tips (Fig. 1). The findings were in accordance with the observations of Rhee et al. (1994) and Yadav and Tandon (1991). The resolution of above structures could be improved many folds in SEM compared to those under light microscopy. Horizontal bands and longitudinal striations present on entire length of the worm (Fig. 2) could be viewed only in SEM but not under light microscope. Cuticular bosses were seen all over the cuticle but more in the posterior end than in anterior end. The genital opening of female worm was situated on the ventral surface at a distance of 285 μm from the anterior end (Fig. 3).

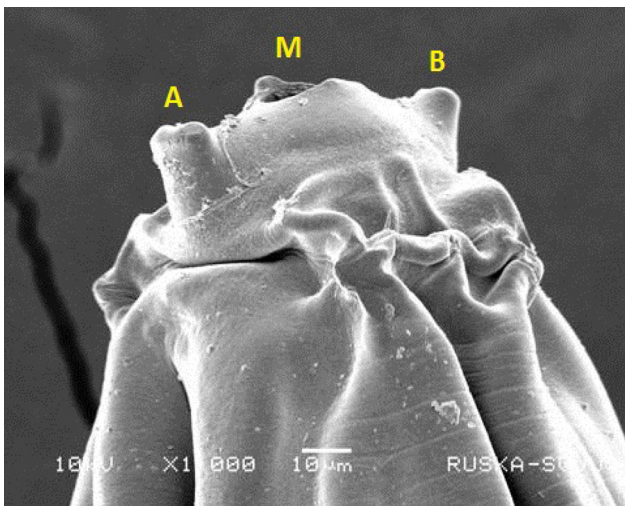

Fig. 1 SEM photograph showing the anterior end of *S.digitata. M* round buccal opening, *A* and *B* dorsal and ventral projecions

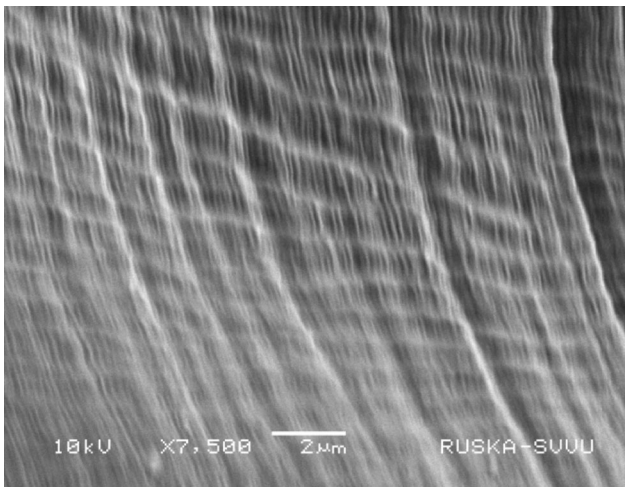

Fig. 2 SEM photograph showing the *horizontal bands* and *longitudinal striations* on the cuticle of *S.digitata*

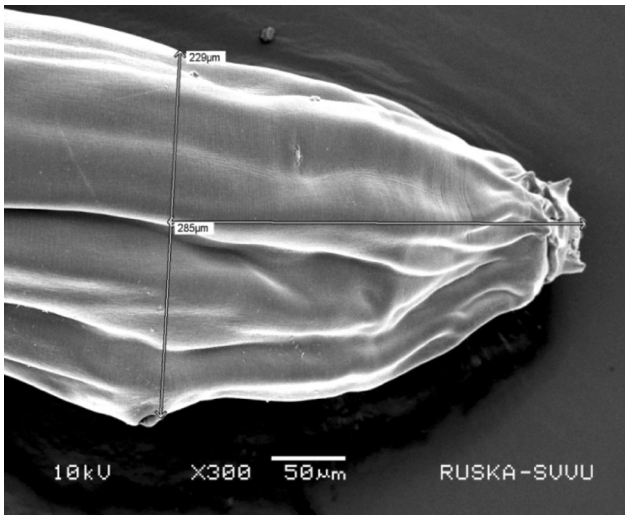

Fig. 3 SEM photograph showing the genital opening of female worm

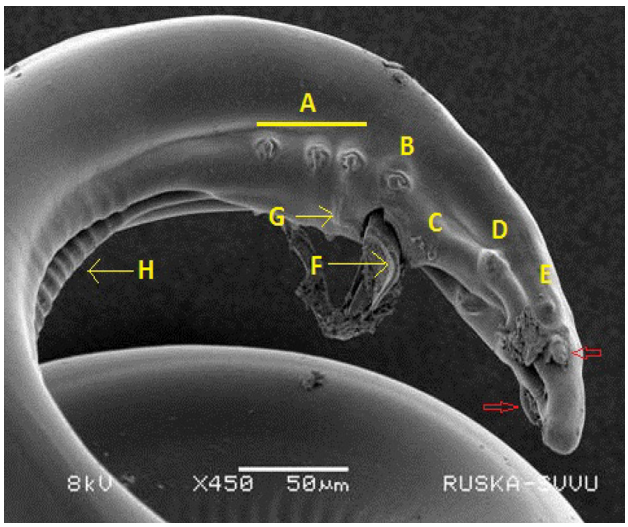

Fig. 4 SEM photograph showing the tail end of male *S.digitata* *A* three pairs of pre-cloacal papillae *B* one pair of adcloacal papillae *C*, *D*, *E* post-cloacal papillae *F* spicule *G* single papillae *H* cuticular ornamentations *red colour arrows* indiacate lateral appendages

Fig. 5 Scanning electron microscopic photograph showing the *loose spirally coiled* posterior end of female *Setaria digitata*

Male *S. digitata* had a coiled spiral end consisting of characteristically arranged sexual papillae. Of the caudal papillae, three pairs each of pre and post cloacal and a pair of adcloacal papillae were present, in addition a central papillae situated in front of the cloaca. A single tongue shaped spicule was seen projecting from the cloacal orifice (Fig. 4). The characteristic horizontal ornamentations of the cuticle known as ventral bands were seen in front of the cloaca. Our findings were similar to those described by Rhee et al. (1994) and Yadav and Tandon (1991). The observations of spirally curved posterior end of female *S. digitata* (Fig. 5) bearing a pair of small lateral appendages near the tail tip and terminating into a rounded knob (Fig. 6) were in confirmity with those reported by Rhee et al. (1994), Yadav and Tandon (1991), Fujita et al. (1985) and Kim et al. (2010). Though the out line of knob appeared smooth under light microscopy, the irregualr, horizontal foldings on the cuticle at the junction of knob (Fig. 7) and tail and irregularly arranged tubercles on the cuticle of posteior end (Fig. 8) could be appreciated only in SEM. Phasmids were seen at the upper junction of lateral appendages. Majority of our observations on *S. digitata* were in confirmity with those reported by Yadav and Tandon (1991) and Rhee et al. (1994).

In the present investigation, *S. digiata* was precisely identified by SEM using the criteria such as shape of the mouth opening, structure of the lateral lips, dorsal or ventral projections, pattern of finer longitudinal striations, ventral

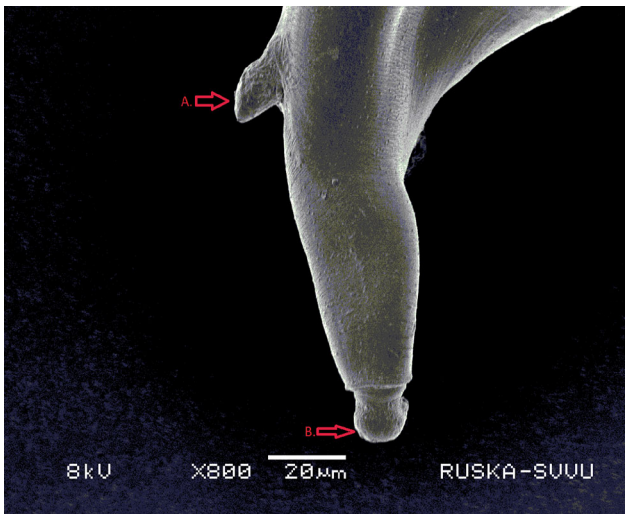

Fig. 6 Scanning electron microscopic photograph showing the *A* knob and *B* lateral appendages on the tail end of female *Setaria digitata*

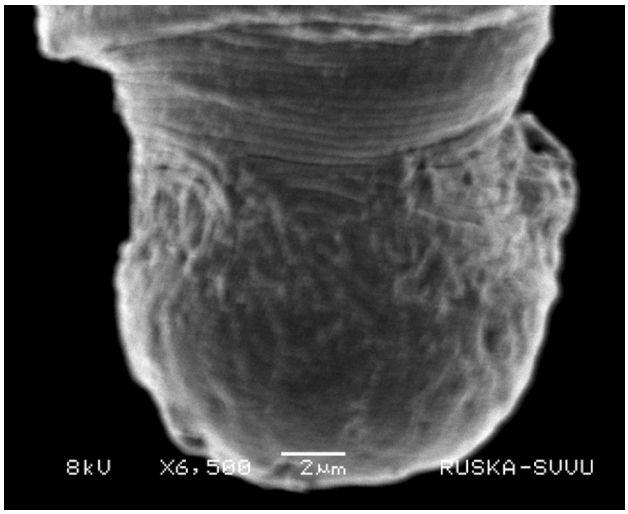

Fig. 7 SEM photograph showing the *horizontal cuticular foldings* at the junction of tail end and knob at the posterior end of female *S.digitata*

bands, topography of the tail, number and arrangement of the caudal papillae in male tail and terminal end of the female and amphids, etc., confirming the previous reports of Shoho and Uni (1977) and Almeida et al. (1991). A better view of morphological features of *S. digitata* could be achieved in SEM than in light microscopy.

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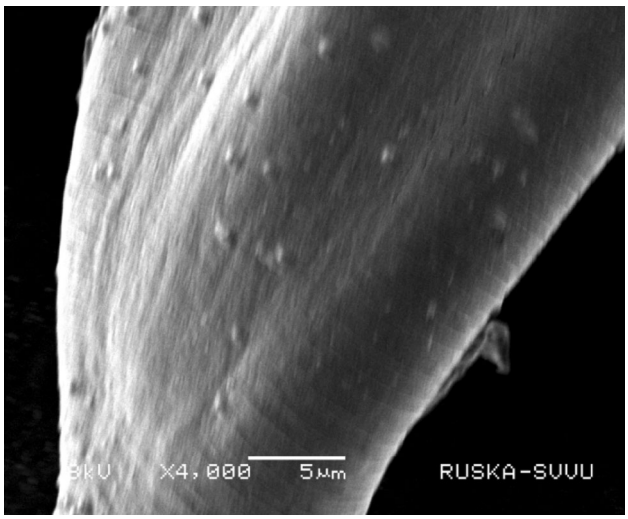

Fig. 8 Scanning electron microscopic photograph showing the *irregularly arranged tubercles* on the cuticle of posterior end of *S.digitata*

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