

Ultrastructural study on morphology of *Schistosoma spindale* by Scanning electron microscopy (SEM)

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Abstract: The present study was undertaken to investigate the detailed morphological features along with morphometry of different structures of *Schistosoma spindale* (Adult flukes) which were recovered by a perfusion technique and visualized by Scanning Electron Microscopy (SEM). The length of spines on the oral sucker and ventral suckers were 2.6 μm and 2.5 μm , respectively. The measured width of aspinose area beneath the ventral sucker, rim of the ventral sucker and tegumental papillae were 5.4, 22.5 μm and 3 μm , respectively. Males have a well-defined gynaecophoric canal, originating just below the ventral sucker and extending up to the posterior end of the body, continued as a marked conical projection. The ventral surface of the oral sucker was completely covered with numerous spines. The ventral sucker was pedunculated, round, thick-rimmed and the inner side contained numerous pointed spines directed towards the center of the ventral sucker. The tegument surface of *S. spindale* showed ridged layers with large unciliated and pit like papillae which were recorded more in posterior end. Thus, Scanning Electron microscopy (SEM) provided indepth ultrastructural morphological details of *Schistosoma spindale* which was in accordance with that of previous studies, would be applicable for its differentiation with other species (*S. mansoni*, *S. bovis*, *S. haematobium*, *S. japonicum*).

Keywords: Gynaecophoric canal, *Schistosoma spindale*, Scanning Electron Microscopy, Oral sucker, Ventral Sucker

INTRODUCTION

Schistosomiasis, a disease of veterinary and medical importance caused by *Schistosoma* spp is characterized by frequent diarrhoea with blood and mucous, weight loss and weakness in animals and causes significant morbidity, mortality and economic loss in cattle population (Niaz *et al.*, 2015). It is a neglected tropical disease that ranks second to malaria in terms of human suffering in the tropics and subtropics (Inobaya *et al.*, 2014) and is now well recognized as the fifth major helminthosis of domestic animals in the Indian sub-continent (Sumanth *et al.*, 2004). In 2011, an estimated 243 million people in 78 countries were living in areas of high risk for the disease (Barry *et al.*, 2013) and affected more than 200 million people worldwide (Colley *et al.*, 2014). Schistosomes are elongate, unisexual and dimorphic trematodes which inhabit the

blood vessels of their hosts (Soulsby, 2006) and hence commonly known as blood flukes. The commonly occurring species in India are *Schistosoma nasale* and *S. spindale* in cattle, *S. indicum* in sheep and equines and *S. incognitum* in pigs (Latchumikanthan *et al.*, 2014). In bovines, the *S. spindale* and *S. indicum* generally inhabit the portal and mesenteric veins and meant to cause visceral schistosomiasis. The infection may be present in subclinical form but the severely affected animal show symptoms of diarrhoea and may develop anaemia. Intestinal schistosomiasis due to *Schistosoma spindale* is an economically important blood fluke infection widespread in India and other developing countries which is manifested as a chronic diarrhoeic disease if a large number of worm pairs inhabit the mesentery (Agarwal and Southgate, 2000). A significant prevalence of *S. spindale* infection among cattle, goats and buffaloes has been reported

based on slaughter house studies in South India (Sumanth *et al.*, 2004; Ravindran *et al.*, 2007). In India, Jeyathilakan *et al.* (2008) reported 30.7% of prevalence of *S. spindale* in cattle.

The detection and identification of these helminths are done by morphological characterization. Moreover, a complete understanding of micro-morphological features plays an important role in the development of vaccines (Degheidy and Shalaby, 2010) and alterations in the ultrastructure of schistosome worms are useful for evaluation of anti-schistosomal drugs (El-Shabasy *et al.*, 2015) for which there is a necessity of understanding normal ultrastructural morphology. Scanning Electron microscopy (SEM) allows better characterization of taxonomically significant structures and is important for generating a more precise species identification and the confirmation, description and/or re-description of species of nematodes (Rebello *et al.*, 2012). Previously, tentative identification of *Schistosoma spindale* was carried out by Hossain *et al.*, (2015) on the basis of morphology and morphometric analysis by light microscopy but detailed ultrastructural study regarding the morphology of *S. spindale* was limited. So, the present research study laid special emphasis on detailed morphological characterization of *Schistosoma spindale* by scanning electron microscopy (SEM) in comparison with other schistosomes viz., *S. mansoni*, *S. bovis*, *S. haematobium*, *S. japonicum*.

MATERIALS AND METHODS

Ethical approval: The present study was conducted after approval by the Institutional Animal Ethics Committee bearing Ref: 698/ CPCSEA dated October 01, 2002 F.No. 25/60/2010- AWD/Veterinary College/ Hyderabad).

Collection of schistosomes: The present study was carried out at Ruska Labs, Rajendranagar (Longitude: 78.4168° E, Latitude: 17.3235° N), Hyderabad during January to May, 2014. Bovine mesenteries immediately after slaughter were examined for the presence of blood flukes by holding them against the sunlight. The positive mesenteries were brought to the laboratory, washed in normal saline and the parasites were recovered by a perfusion technique in which little amount of phosphate buffered saline (PBS) (pH 7.4) was injected into the vein by making a small hole and the contents were squeezed from the other side. The recovered

worms were washed thrice in PBS and subjected to light microscopy (TXS08-04B). The recovered male and female flukes of *Schistosoma* were differentiated into *S. spindale* and *S. nasale* based on their morphological features like presence or absence of tuberculations on the cuticle, number of testes in the male, shape of the eggs present inside the uterus etc.

Processing of samples for SEM: A total number of six samples which were confirmed to be *S. spindale* by light microscopy were subjected for SEM for further studies. The worms were fixed in 2.5% Gluteraldehyde in 0.1M phosphate buffer (pH 7.2) for 24 hrs at 4°C and post fixed in 2% aqueous Osmium tetroxide for 4 hr, dehydrated in series of graded alcohols (50, 70, 90 & 100%) and dried with critical point of drying (CPD) unit. The processed samples were mounted over the stubs with double-sided carbon conductivity tape, and a thin layer of gold coating over the samples was done by using an automated sputter coater (Model- JOEL JFC- 1600) for 3 minutes and scanned under Scanning Electron Microscope (SEM-Model: JOEL JFC- 5600) at required magnifications (70X, 100X, 300X, 400X, 600X, 2500X, 3,000X, 5,000X & 10,000X) (as per the standard procedures at RUSKA Lab, College of Veterinary Science, SVVU, Rajendranagar, Hyderabad, India.

RESULTS

Light microscopy: Under light microscopic studies male worms with smooth cuticle, having 3-5 testes (Fig. 1) and female with 15-20 spindle-shaped eggs with terminal spine inside the uterus (Fig. 2) were confirmed as *S. spindale*. Similarly, *S. indicum* male worms were identified by their spiny cuticle (Fig. 3) and female worms having oval-shaped eggs with spine at posterior end (Fig. 4 & 5).

Scanning electron microscopy

Oral sucker: The oral sucker of the male worm was hollow, triangular and subterminal in position. It has a mouth with thick muscular rim without spines (Fig. 9). The ventral surface of the oral sucker was completely covered with numerous spines measuring 2.6 µm in length (Fig. 7) (Table No. 1). Irrespective of the position of spines on the oral sucker all spines were directed downwards into the aperture of the oral cavity (Fig. 8).

Ventral sucker: The ventral sucker was larger than

Table. 1. Mean (± S.E) of Different structures of *S. spindale* measured by Scanning Electron Microscope (SEM)

S.No	Different structures	Mean ± S.E (n=6)*
1.	Spines on oral sucker (L, µm)	2.6 ± 0.02
2.	Spines on ventral sucker (L, µm)	2.5 ± 0.02
3.	Aspinose area beneath the ventral sucker (W, µm)	5.4 ± 0.01
4.	Rim of ventral sucker (W, µm)	22.5 ± 0.03
5.	Tegumental Papillae (W, µm)	3 ± 0.04

L=Length; W=Width; *Values are mean ± S.E and values in parenthesis are the no. of samples analysed by Scanning Electron Microscope (SEM)

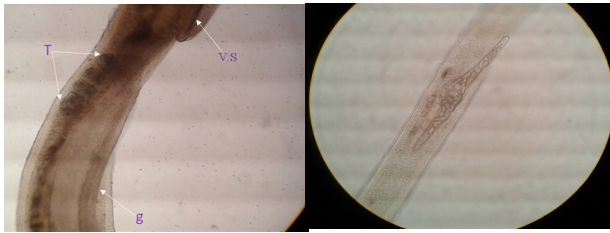


Fig. 1. Photomicrograph of anterior end of *S. spindale* male showing 3-5 testes (T), ventral sucker (V.S) and gynaecophoric canal (g). 400X.

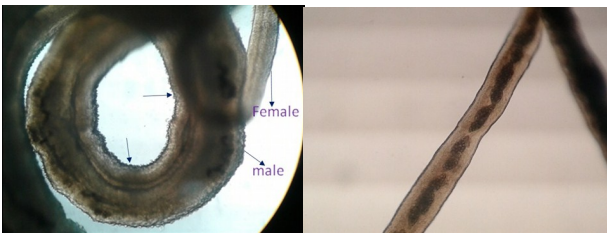
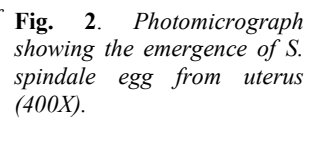


Fig. 2. Photomicrograph of *S. spindale* egg from uterus (400X).

Fig. 3. Photomicrograph showing the tuberculated/spiny cuticle of *S. indicum*. 100X.

Fig. 4. Photomicrograph showing the eggs inside the uterus of *S. indicum*. 100X.



Fig. 5. Photomicrograph showing oval shaped egg with terminal spine inside uterus of *Schistosoma indicum*. 400X.

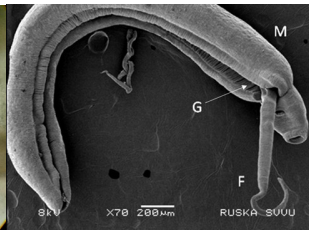


Fig. 6. SEM photograph showing the lateral view of thick male and slender female in a coupula stage. Gynaecophoric canal (G), Female (F), Male (M) of *S. spindale*. 70X.

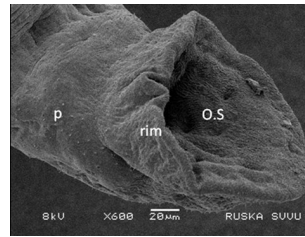


Fig. 7. SEM photograph of *S. spindale* oral sucker (O.S) showing sharp spines, muscular rim and scattered large papillae (p) on the lateral side. 600X.

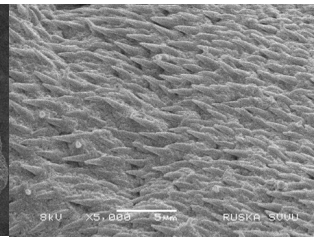


Fig. 8. SEM photograph of *S. spindale* oral sucker (O.S) showing sharp spines on oral sucker of *S. spindale*. 5000X.

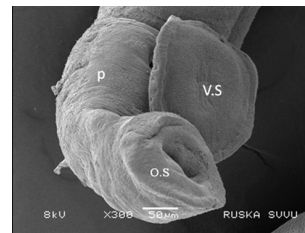


Fig. 9. SEM photograph of *S. spindale* with scattered large papillae (p) between oral sucker (O.S) and ventral suckers (V.S) on lateral side. 300X.

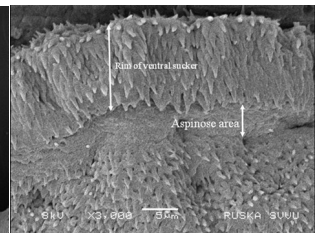


Fig. 10. SEM photograph showing the spines directed towards center of ventral sucker, double headed arrows showing the rim of ventral sucker and aspinose area below the rim of *S. spindale*. 3000X.

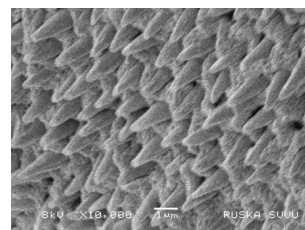


Fig. 11. SEM photograph showing blunt spines on the ventral sucker of *S. spindale*. 10,000X

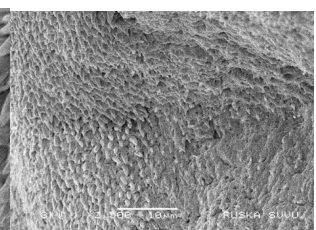


Fig. 12. SEM photograph showing the spines of ventral sucker and the apparently absence of spines in the central part (arrows) of the sucker in *S. spindale*. 2,500X.

the oral sucker, pedunculated, round, thick-rimmed and situated below the oral sucker (Fig. 9). Numerous unciliated papillae were noticed between the lateral aspects of oral and ventral suckers (Fig. 7 and 9) and sharply pointed spines (2.5 µm in length) (Table No. 1) directed towards the center of ventral sucker were present on the rim of ventral sucker having a width of 22.5 µm (Table No. 1). An aspinose area (5.4 µm) was observed (Fig. 10) beneath the lower border of the rim of a ventral sucker. Followed by aspinose area another area of spines comparatively blunt (Fig. 11) than the spines of oral sucker and rim of ventral sucker were observed towards the centre of ventral sucker. There is a gradual reduction in the number of spines towards the centre of ventral sucker, showing apparently aspinose area (Fig. 12).

Gynaecophoric canal: The width of the body of male worm increases and folded ventrally just behind the ventral sucker to form gynaecophoric canal (Fig. 6). The tegument lining of gynaecophoric canal consisted

of thick, evenly arranged transverse ridges and numerous papillae (Fig. 13).

Tegument: Under higher magnification, the tegument of *S. spindale* showed ridged layers with unciliated and pit-like papillae of 3 µm in diameter (Fig. 14) (Table 1) and the number of papillae were more in posterior part than the anterior part of the body whereas the spines were not observed in between papillae. The cuticular ridges in female were smooth, perforated, compact, dense, coarsely placed when compared to male worm. The ratio of epidermis to papillae is more in female (Fig. 15) than the male worm.

Posterior end: The posterior part of male worm ended in wide conical projection (Fig. 17)

DISCUSSION

The male and female *S. spindale* worm subjected for SEM studies were in coupula stage. The morphological features observed in this study are in accordance with the research findings of Narain and Mahanta, (1999).

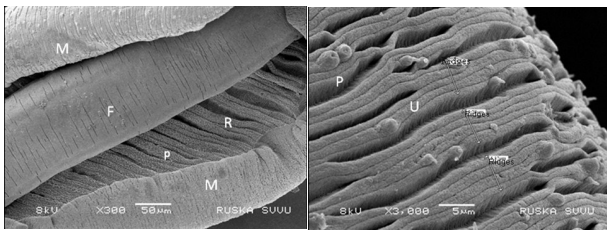


Fig. 13. SEM photograph of ventro-lateral view of gynaecophoric canal of male (M), showing the female (F) lodged inside and ridges of the canal (R) with numerous papillae (P) in *S. spindale*. 300X.

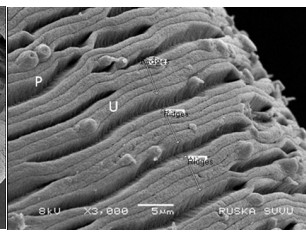


Fig. 14. SEM photograph of epidermis of male *S. spindale* showing the ridges, unciliated papillae (U) and pit like papillae (P). 3000X.

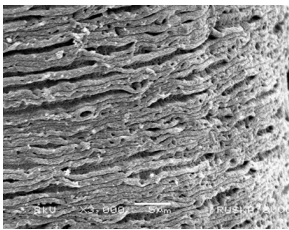


Fig. 15. SEM photograph of epidermis of female *S. spindale* showing the smooth and perforated ridges. 3000X.

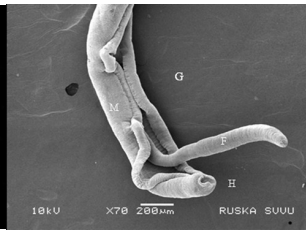


Fig. 16. SEM photograph of *S. spindale* - Thick male (M) and slender female (F) in copula stage, G, gynaecophoric canal, H. Posterior end. 70X.

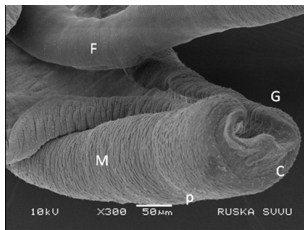


Fig. 17. SEM photograph showing posterior end of *S. spindale* male. Note more number of papillae (p). Female (F) lodged in gynaecophoric canal (G), which extends to poster end and continued to form a conical projection (C). 300X.

Hossain *et al.*, (2015) reported *S. spindale* female having spindle-shaped eggs with the terminal spine in the uterus which was in accordance with that of the present study.

In *S. spindale*, the anterior region was occupied by both oral and ventral sucker. Ventral sucker was larger than oral sucker which was similar to the structure of *S. mansoni* as reported by Machado-Silva *et al.* (1997). Machado-Silva *et al.* (1997) also reported that the anterior border of the oral sucker of *S. mansoni* presented an area covered by several small sharp spines whereas the present morphological study of *S. spindale* revealed that the rim of oral sucker does not contain any spines while the ventral surface contains numerous spines which are directed downward into the oral aperture. In *S. haematobium*, the oral sucker has spines directed towards the oesophagus (Kuntz *et al.*, 1976) and in case of *S. japonicum* it shows a rim with spines of variable size and sharpness (Sakamoto *et al.*, 1977). Regarding the ventral sucker of *S. spindale*, the rim

contains sharp and pointed spines. An aspinose area is present below the lower border of the rim which is followed by an area of blunt spines. In *S. mansoni*, the extremity of the ventral sucker presented two spiny regions and some sensorial papillae distributed between the two rows of spines (Machado-Silva *et al.*, 1997) whereas in *S. japonicum* it possesses spines smaller than the oral sucker (Sakamoto *et al.*, 1977).

The tegument of *S. spindale* showed ridged layers and pit-like papillae which are more in the posterior part. In *S. mansoni*, the dorsal tegument presented tubercles with numerous spines (Machado-Silva *et al.* 1997, Kamel and Bayaomy, 2017). In *S. bovis*, the dorsal and dorso lateral surface is devoid of spines (Southgate *et al.*, 1986). The tegument of *S. haematobium* is moderately rough (Kuntz *et al.*, 1976), where as in case of *S. japonicum* there were no spines and tegumental ridges were compact and regular with sensory papillae in the middle of the integument (Yang *et al.*, 2012).

The lining of gynaecophoric canal in *S. spindale* consists of thick, evenly arranged transverse ridges and numerous papillae and in *S. haematobium* the surface of canal possesses irregular ridging with scattered pits (Kuntz *et al.*, 1976) and the surface of the canal is ridged and covered with small, irregularly arranged spines (Skelly and Wilson, 2006) and in *S. japonicum*, irregularly directed spines is distributed inside the gynaecophoric canal (Sakamoto *et al.*, 1977). The posterior part of the male worm ends in wide conical projection, and similar findings were observed by Agrawal (2000).

Conclusion

The morphological characteristics of *S. spindale* like an oral sucker, ventral sucker, tegumental papillae and gynaecophoric canal studied by Scanning Electron microscopy (SEM) not only provided indepth ultra-structural morphological details but also enabled it to be differentiated from other Schistosoma spp (*S. mansoni*, *S. bovis*, *S. haematobium*, *S. japonicum*).

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