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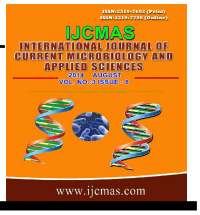

Original Research Article

Scanning Electron Microscopic observations of Rumen Amphistome (Trematoda) in buffalo (*Bubalus bubalis*) in Udaipur, Rajasthan

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A B S T R A C T

The amphistomes *Cotylophoron cotylophorum and Orthocoelium scoliocoelium* (Trematodes) was studied by using a scanning electron microscope (SEM). Adult worms were collected from rumen of freshly slaughtered buffalo (*Bubalus bubalis*). Observations demonstrated that the *Cotylophoron cotylophorum* has conical pyriform body and *Orthocoelium scoliocoelium* has small stumpy, oval, slightly curved body at the ventral side. In *Cotylophoron cotylophorum* oral opening is terminal and surrounded by wrinkled and ridged tegument which forms concentric elevated rings encircling the oral opening. Acetabulum is sub terminal and the tegument covering outer border of the sucker is smooth whereas the inner lining of the acetabular tegument is rough in a bee- comb like structure. In *Orthocoelium scoliocoelium* oral sucker in circular form present anterior sub terminal region having 7-8 concentric rings of tegumental folds and ventral sucker are also in circular form and present in posterior extremity known as acetabulum. *Cotylophoron cotylophorum* has mid ventral genital sucker found first third of the body and surrounded by a tire- shaped elevation of the tegument. Whereas in *Orthocoelium scoliocoelium* genital sucker is absent but genital papillae is present and situated at ventral region on anterior middle position. Here the tegumental folds are arranged in radial pattern and some papillae are also visible here. Based on these results, we can use morphological characters for the species differentiation for the future. SEM study of *Cotylophoron cotylophorum* and *Orthocoelium scoliocoelium* will be helpful in identification and taxonomic characterization. Due to this parasites have been received special attention to study the ultrastructural, adaptive features and functions of worms.

K e y w o r d s

Amphistomes, *Cotylophoron cotylophorum*, *Orthocoelium scoliocoelium*, *Bubalus bubalis* and scanning electron microscope.

Introduction

Amphistomes are digenetic trematodes parasite found in domestic as well as wild ruminants and causes severe pathogenicity in cattle and indirectly responsible for massive economic losses to the cattle farmers. These worms are small, stumpy

body shape having two or more suckers. Yamaguti (1971) has listed 62 species of paramphistomes in the ruminants of various countries of the world whereas Dinnik (1964) recorded 32 species in Africa. In India 20 species were described and classified by Dutt (1980). Paramphistomiadae family includes worms which are endo-parasites of digestive tract and other visceral organs of mammalian hosts having different genera and species. All the paramphistomes are differ in shape, size, number, size of suckers, cuticle, oral organ, oesophagus, intestinal caeca, excretory vesicle, genital bulb, male and female reproductive organs. The basic outline of the structure of paramphistomes comprises thick fleshy body with posterior acetabulum, terminal mouth without oral pouches, nonspinous cuticle, excretory pore is dorsal and either anterior or posterior to Laurer's canal.

The use of scanning electron microscope (SEM) magnifications has enable scientists to make more use of surface characters. Thus many characters are available for solving taxonomic problems. The surface topography of few digenean trematodes that are infected cattle and vertebrates were studied in past few decade: *Phyllodistomum conostomum* (Bakke and Lien 1978 & Bakke *et al*. 1982), *Fasciola hepatica* (Bennet, 1975 a and b), *Orthocoelium, Cotylophoron, Paramphistomum, Calicophoron* and *Gigentocotyle sps.* (Eduardo 1980b and c, 1983 & 1984), *Echinostoma revolutum* (Smales and Blankespoor 1984), *Concinnum epomopis* (Otubanjo, 1985), *Phyllodistomum umblae* (Bakke and Bailey 1987), *Paramphistomum epiclitum*, *Gastrothylax cruminifer*, *Orthocoelium sps* (Tandon and Maitra 1981, 1983 and 1987), *Gastrodiscoides hominis* (Tandon and Maitra 1983 and Brennen *et al.* 1991), *Zogocotyl lunata* (Irwin *et al*. 1991), *Balanorchis anastrophus* (De Fatima *et al.* 1992), *Transversotrema licinum* (Abdul-Salam and Sreelatha 1992), *N. aegyptensis* (Shalaby and Hassanine 1996), *R. angusticolle, T. arripidis, B. belonae, P. sigani* (Shalaby and Hassanine 1997), *Cotylophoron cotylophorum* (Veerakumari and Munuswamy 1999 and Nahla *et al*., 2012), *Glyianchen volubilis* (Lee *et al*. 1985 and Abdou 2000), *Stephanostomum egypticum* (Abdou and Ashour 2000), *Probolocoryphe uca* (Abdul-Salam and Sreelatha, 2000), A*llodidymozoon operculare, Didymocystoides singularis, Platocystoides polyester* (Sethukarasi and Veerakumari 2007), *Paramphistomum cervi* (Sharma and Hanna 1988; Payarachun et al. 2010; Shaheen & Eman 2012 and Saowakon *et al*. 2013).

Amphistomes *Cotylophoron cotylophorum* and *Orthocoelium scoliocoelium* are commonly occurring species severely infected ruminants of southern Rajasthan belong to sub-family Paramphistomatinae and Orthocoelinae respectively. The general characters of Paramphistomatinae are small, curved, conical or pyriform body with well-developed oral organ without pouches strongly developed posterior sub terminal acetabulum, distinct genital bulb, Laurer's canal opening posterior to excretory vesicle. The key characters of group of Orthocoelinae are generally small, slightly curved body, weakly developed acetabulum**,** and oral sucker without pouches, excretory vesicle not crossing the Laurer's canal, genital bulb small and parasite in alimentary tract of mammals (Yamaguti 1971; Dutt 1980; Tandon and Maitra 1987 and Tandon & Roy 2002).

In an epidemiological survey the prevalence of *Cotylophoron cotylophorum* in buffaloes was 61.60% and *Orthocoelium scoliocoelium* was 63.44% (Swarnakar *et al.*

2014). As the knowledge the detail SEM structure of *Cotylophoron cotylophorum* and *Orthocoelium scoliocoelium* is very little. Due to this parasites have been received special attention to study the ultrastructural, adaptive features and functions of worms. The present study is to determine the structure of *Cotylophoron cotylophorum* and *Orthocoelium scoliocoelium* by SEM, which will be helpful in identification and taxonomic characterization.

Materials and Methods

Live amphistomes *Cotylophoron cotylophorum* and *Orthocoelium scoliocoelium* (Trematodes) were removed from the rumen of freshly slaughtered buffaloes at the local zoo abattoir and from various slaughtered shops of Udaipur. These parasites were washed several times in distilled water to make them free from debris and mucous. Then they were transferred into physiological saline (0.9% NaCl) for their maintenance. Then parasites were fixed 12 hrs at 4^{o}C in 3.5% glutaraldehyde in 0.2M sodium cacodylate buffer at pH 7.2 fixative (Bancroft and Stevens, 1977). Further the worms were post fixed at 4^{o}C for 1 hour in 1% osmium tetraoxide prepared in 0.1 M phosphate buffer at pH 7.2 and dehydrated in acetone ascending series and then transferred to 100% acetone for one hrs. and dried. Later on the worms were fixed and glued on metal stubs, coated with gold in vacuum and examined by using ZEISS Scanning Electron Microscope (SEM).

Results and Discussion

The SEM photomicrograph revealed that the body of adult *Cotylophoron cotylophorum* is beaned shaped with slightly corrugated surface. The oral opening is terminal and surrounded by wrinkled and ridged tegument which forms concentric elevated rings encircling the oral opening. Acetabulum is sub terminal and the tegument covering outer border of the sucker is smooth whereas the inner lining of the acetabular tegument is rough in a bee-comb like structure (Fig. 1). The mid ventral genital sucker is found first third of the body and surrounded by a tire- shaped elevation of the tegument (Fig. 2).

The SEM observations demonstrated that the normal adult *Orthocoelium scoliocoelium* (*O. scoliocoelium*) has elongated oval and slightly ventrally curved body. The body of adult is dorsally convex and almost straight at ventral. It has two suckers; oral sucker in circular form present anterior sub terminal region and ventral sucker are also in circular form and present in posterior extremity known as acetabulum. The genital pore is situated in ventral side at the anterior third of the body. The ventral and lateral side of *O. scoliocoelium* reveal acetabulum, genital pore and tegumental folds (Fig.3). Everted genital papillae with radial tegumental folds and knob like papillae were observed on ventral surface of the worm (Fig. 4). The tegumental surface in both the amphistome is highly corrugated with transverse folds alternating with grooves and furrows and is spineless, which is exceptional character of trematodes.

Use of scanning electron microscopy is a very supportive instrument in the study of cellular structure of animal biology in general, thus enhances the knowledge of biologists. In trematodes parasites, all morphological structures are very important in the context of their systematic classifications. These characters includes; body contour, position and shape, size of oral and ventral suckers, presence of tegumental folds, ridges and furrows on the tegument, types of papillae present on

tegument and around suckers, tegumental structures like spines and holes. The importance of SEM lies in its ability to provide three dimensional images with high magnification that allow understanding the spatial relationships among surface structures. It could be used to separate species that appear morphologically identical when examined under light microscope, validate and demonstrate differences between species. The tegumental alteration and structures of digenetic trematodes are facilitate adaptive characters to the individual genus (Adibi *et al*. 1988). The tegument plays important role in protection, absorption, excretion, transport and osmoregulation. It is the surface which is in direct contact with host's tissue along with the body fluids (Otubanjo 1985; Sharma & Hanna 1988; Brennen *et al*. 1991; Panyarachun *et al*. 2010; Shaheen & Eman 2012 and Saowakon *et al*. 2013).

All the trematodes have species specific arrangement of tegumental folding, ridges and furrows. This character facilitates increased surface area for absorption and stretching capability. In present study, transversally arranged tegumental folds having prominent ridges and furrows were observed which shows similarity with other paramphistomes studied by scientists. Amongst other species of amphistome Tandon and Maitra (1987) reported broader grooves in the extreme ends of body and narrow grooves at the middle portion in *Orthocoelium dewasi*. Bakke (1978 and 1979) reported different types of hole on tegumental surface of *Leucochlodium sps.* Brennen (1991) documented rough, concentrically arranged tegumental folds with clearly visible ridges and furrows bearing numerous tightly packed tubercles and extended into the oral cavity in *Gastrodiscoides hominis* and in *Platocystoides polyester*, furrows and ridges radiating from central portion of dorsal surface (Sethukarasi and Veerakumari 2007) and finger like projection of tegumental folds were observed in *Glyianchen volubilis* (Abdou 2000). The present research may indicate that the position of oral sucker, acetabulum and genital bulb tegumental folds are topographical characters which are considered as generalised taxonomic character of maximum trematodes. The present parasites *C. cotylophorum* and *O. scoliocoelium* are characterised by concentric arrangement of tegumental folds encircling oral sucker and less number is found near acetabulum and genital pore leads to the conclusion that these folds may be used in the site location within the host rumen and small intestine. This position brings them into contact with the plug of host tissue formed by the fluke at the attachment site (Hoole and Mickell 1981 and Ashour, 1994 & 1995).

The SEM study of *C. cotylophorum* and *O. scoliocoelium* revealed in present study shows similarity in few basic structures with many digeneans; *O. devasi, O. tamilansis* (Tandon and Maitra 1987), *N. aegyptensis* (Shalaby and Hassanine 1996), *R. angusticolle, T. arripidis, B. belonae, P. sigani* (Shalaby and Hassanine 1997), *P. interruptus* and *G. volubilis* (Abdou *et al*. 1999b and 2000), A*llodidymozoon operculare, Didymocystoides singularis, Platocystoides polyester* (Sethukarasi and Veerakumari 2007), *Paramphistomum cervi* (Payarachun *et al*. 2010) and the above finding shows that the species of *C. cotylophorum* and *O. scoliocoelium* inhabiting rumen of buffalo exhibit important variations in their surface ultrastructure. Diversity in the surface ultrastructure is significant with regards to the adaptation of the *C. cotylophorum, O. scoliocoelium*. Such studies on the other ruminant parasites are necessary because many of them are responsible to cause paramphistomiasis.

Fig.1 SEM photomicrograph of ventral surface of *Cotylophoron cotylophorum* showing position of oral sucker (OS), genital sucker (GS), posterior sub- terminal acetabulum (AC) and tegumental folds (TF) x100µm

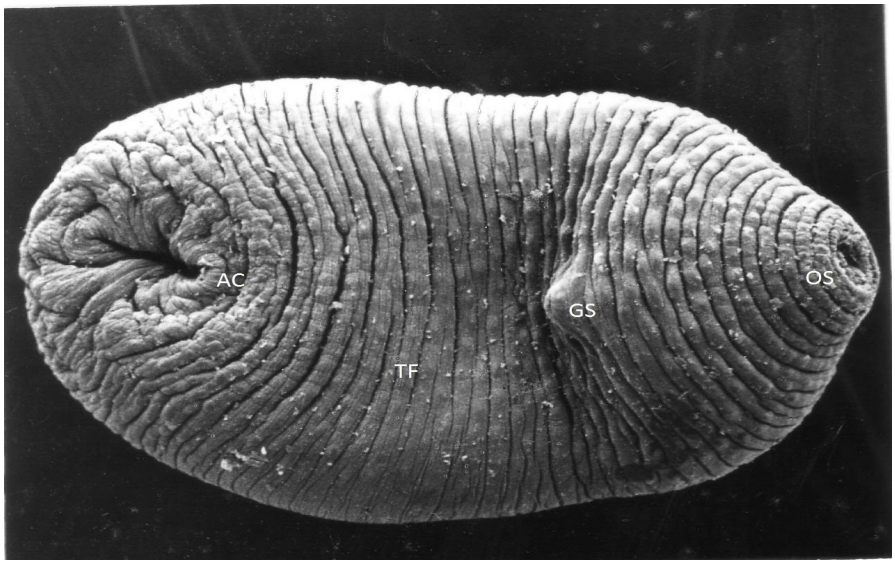


Fig.2 SEM photomicrograph showing genital sucker (GS) and tire-shaped arrangement of tegumental folds (TF) x80 µm

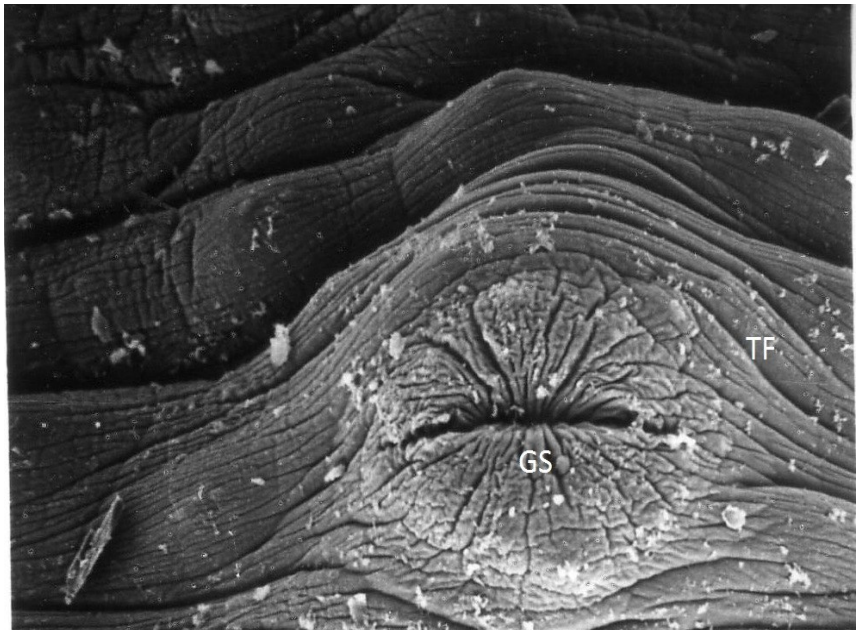


Fig.3 SEM photomicrograph of ventral surface of *Orthocoelium scoliocoelium* showing oral sucker (OS), genital papillae (GP), acetabulum (AC) and tegumental folds (TF) x100µm.

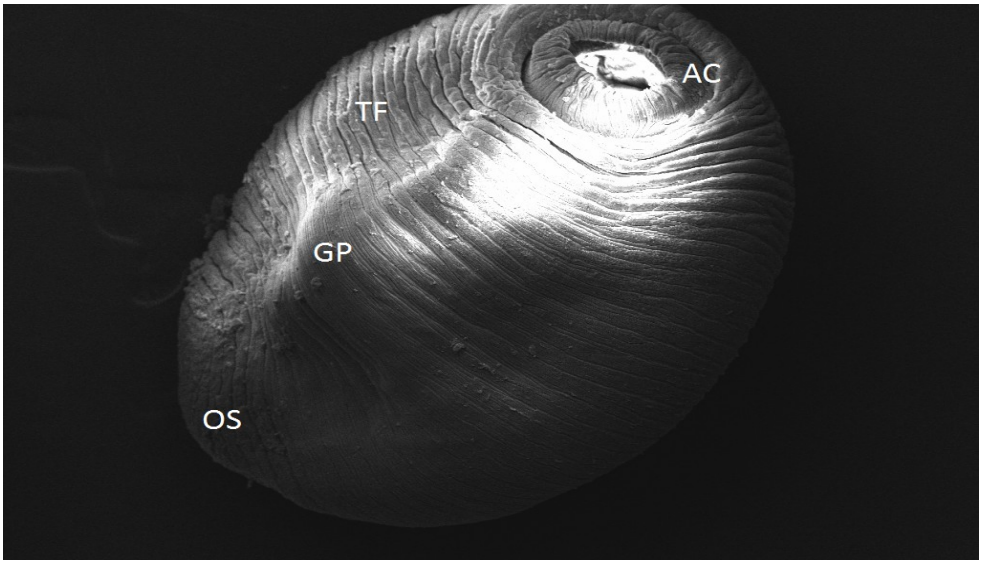
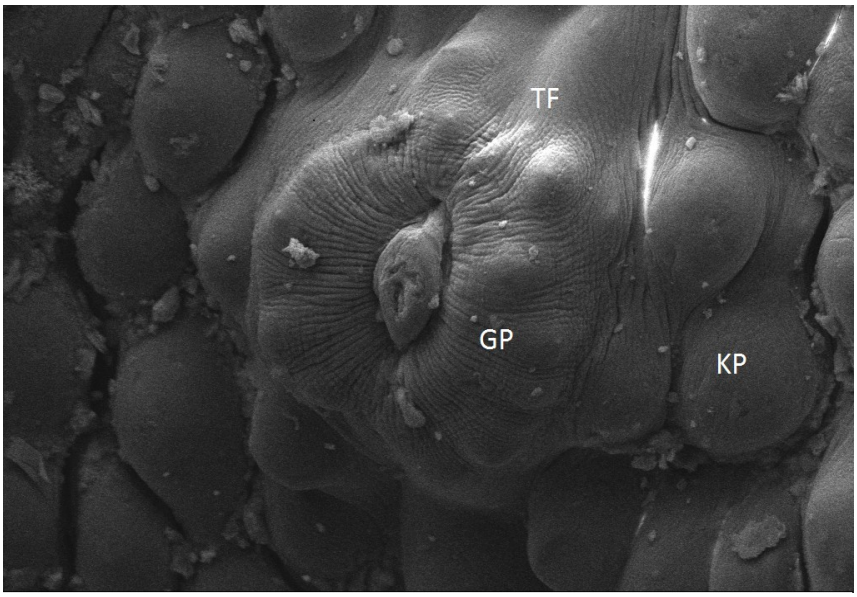


Fig.4 SEM photomicrograph of genital papillae (GP) of *Orthocoelium scoliocoelium* having radially arranged tegumental folds (TF), and knob like papillae (KP) x80 µm



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