

ELECTRON-MICROSCOPIC INVESTIGATIONS ON THE MORPHOLOGY OF THE RECRUDESCENT *PARAMPHISTOMUM (CALICOPHORON) DAUBNEYI* TREMATODE

INVESTIGAȚII ELECTRONO-MICROSCOPICE ASUPRA MORFOLOGIEI TREMATODULUI RECRUDESCENT *PARAMPHISTOMUM (CALICOPHORON) DAUBNEYI*

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ABSTRACT | REZUMAT

Paramphistomosis is caused both by adult forms of trematodes located in the rumen and reticulum and by young forms which are more pathogenic and found in the abomasum and duodenum.

During the last decade, the disease has met a recrudescence both in the case of sheep and cattle. The economic loss, caused by decreased productivity, mortality and expenses regarding prophylaxis and treatment, is increased.

Scanning electron microscopy (SEM) has revealed that the surface of the tegument has an undulated aspect on all body sides, with transversal folds, alternating with multiple grooves and spines are absent. When using high amplification, the surface of the fold appears to be made of multiple micro-folds or crests, separated by micro-grooves or grooves. The undulations and invaginations of the ventral surface are also more extensive on the dorsal area of the body. Both the anterior sucker and the posterior one show thick lips with transversal folds and no spines. The genital pore is situated in the anterior third of the body. There are two types of sensory papillae, present on the surface of the *Paramphistomum* parasites: type 1 is bullous, measuring 10 – 15 µm diameter at the base, with a mamelonated shape and type 2, with similar shapes and sizes, presenting a short cilium on top. Usually, these sensory papillae appear in large groups, each having between 5 and 20 units, according to body-length. In contrast with the other parts of the surface, the posterior end of the dorsal surface does not have papillae.

Scanning electron microscopy highlights the spatial image of the body and its components (organs). Tegument layers and other very small components, such as sensory organs, can also be analysed.

Keywords: *Paramphistomum daubneyi*, tegument, scanning electron microscopy (SEM)

Paramfistomoza este determinată de formele adulte ale unor trematode cu localizare în rumen și rețea, iar formele tinere, și mai patogene, se localizează în abomas și duoden.

În ultima decadă s-a constatat o recrudescență a bolii, atât la ovine cât și la bovine. Pierderile economice sunt mari prin scăderea producțiilor, mortalitate și cheltuielile cu profilaxia și combaterea.

Prin scanarea electrono-microscopică (SEM) s-a observat că suprafața tegumentului apare în toate părțile corpului foarte ondulantă, cu pliuri transversale care alternează cu multiple caneluri și nu prezintă spini. La amplificare mare, suprafața pliului este compusă din microfalduri sau creste separate prin microșanțuri sau șanțuri. Onduleurile și invaginațiile suprafeței ventrale sunt, de asemenea, mai extinse decât pe suprafața dorsală a corpului. Atât ventuza anterioară cât și cea posterioară au buze groase cu pliuri transversale, fără spini. Porul genital este situat în treimea anterioară a corpului. Există două tipuri de papile senzoriale la suprafața paramfistomilor: tipul 1 este de formă buloasă măsurând 10 – 15 µm în diametru la bază, având formă mamelonată, iar tipul 2 are o formă și o dimensiune similară prezentând un cil scurt deasupra. De obicei, aceste papile senzoriale apar în grupuri mari, fiecare având între 5 și 20 de unități, în funcție de lungimea corpului. Spre deosebire de celelalte părți ale suprafeței, capătul posterior al suprafeței dorsale nu are papile.

Microscopia electronică pune în evidență o imagine spațială a corpului și a componentelor acestuia (organe). De asemenea, pot fi analizate straturile tegumentare și alte componente de dimensiuni mici, cum ar fi organe senzoriale.

Cuvinte cheie: *Paramphistomum daubneyi*, tegument, scanare electrono-microscopică (SEM)

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Ruminants are usually highly receptive to a large number of parasite species, from various classes. Thus, in Romania, at least when discussing the western part, there has been evidence of Protozoa infestations (4, 5, 9, 10, 11, 14, 25) and gastrointestinal hel-

minths infestations (12, 17, 22, 23) in both domestic and wild ruminants (3, 7, 14, 15). Among these infestations, during the last decade, a recrudescence of paramphistomosis was noticed, a fact that can also be seen as a re-emergence. The disease is characterised by acute gastroenteritis, with an increased morbidity and mortality rate, especially in the case of young animals (13, 16, 18, 21, 26).

Recent studies suggest that *P. daubneyi* has become the dominant species of rumen fluke throughout Europe. (6, 8, 27).

It is a well-known fact that the tegument of helminths has a component in direct contact with the body tissues and fluids of the host. The tegument has many functions that contribute to homeostasis such as absorption of nutrients, osmo-regulation, protection against digestive enzymes of the host, and against the host immune reactions as well as sensory perception. The understanding of the tegument structural organization appears essential for the conception of any drug or vaccine meant to destroy the parasite via its tegument.

The present study aimed to provide a thorough study of the morphological characteristics of the tegument of adult *Paramphistomum* (*Calicophoron*) *daubneyi* using scanning electron microscopy (SEM).

MATERIALS AND METHODS

Collection of adult parasites



Fig. 1. Identification and collection of *Paramphistomum*

Paramphistomum daubneyi adults were collected from the rumen of infested sheep (Fig. 1) that were slaughtered for consumption in households from Timis County. Following identification, the parasites were washed several times using NaCl 0.85% solution and processed immediately. The parasites were fixated using alcohol-formalin-acetic acid (AFA) fixative, stained using Semichon carmine, differentiated in alcohol-acid, dehydrated using serial concentrations of ethanol, cleaned using xylene followed by species

identification based on their morphological characteristics.

Scanning electron microscopy (SEM)

The adult parasites were fixated in 2.5% glutaraldehyde, buffered in sodium cacodylate 0.1M, pH 7.2, at 4 °C for at least 2 hours. The parasites were washed three times with the same buffer solution and fixated subsequently in 1% osmium tetroxide and sodium cacodylate buffer 0.1M, pH 7.2, at 4°C for 1 hour (Fig. 3). The parasites were washed three times with distilled water and dried. Next, the parasites were placed on aluminium support plates (Fig. 4) and covered in gold particles using an ionic pulverisation device - Quorum SC7620 Sputter Coater- for 2 minutes. The samples were examined using a Hitachi TM3000 Tabletop Microscope (Fig. 2) functioning at 5 and 15 kV (19).



Fig. 2. Hitachi TM3000 Tabletop Microscope



Fig. 3. Parasites ready to be examined with the electronic microscope

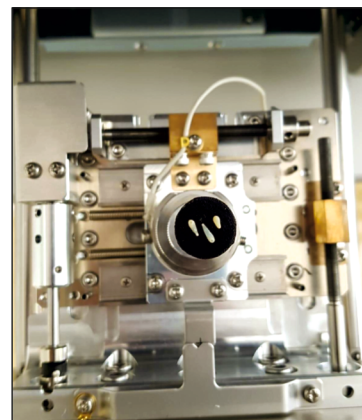


Fig. 4. Examination of the parasites using the electronic microscope

RESULTS AND DISCUSSIONS

Ventral surface

The anterior region of the ventral surface shows undulations and major transversal folds alternating with grooves (Fig. 5 A and B). The major folds are made of a network of micro-folds and interlaced crests, separated through micro-grooves or grooves of variable sizes (Fig. 5 C). Spines are not present in this region.

The major folds show clusters of papillae which are thought to be sensory receptors (Fig. 5 C). Each papilla appears as a large dome or bulb, measuring 10-15 μm diameter at the base. There are two types of pa-

pillae: type 1 has a mamelon-like tip and type 2 shows a small cylinder on the mamelon-like tip.

The anterior (oral) sucker has a thick muscular edge covered in wide, transversal folds which show numerous rows of papillae clustered in large groups (Fig. 5 B and C). Type 1 and type 2 papillae appear in a single unit or groups of two-three units on the anterior and lateral parts. There are large groups of papillae inside the pharynx and in the genital canal but not in the ventral sucker (Fig. 6).

The surface of the medial and lateral parts of the body also appears undulated, with major folds separated through major grooves. The surface of every main

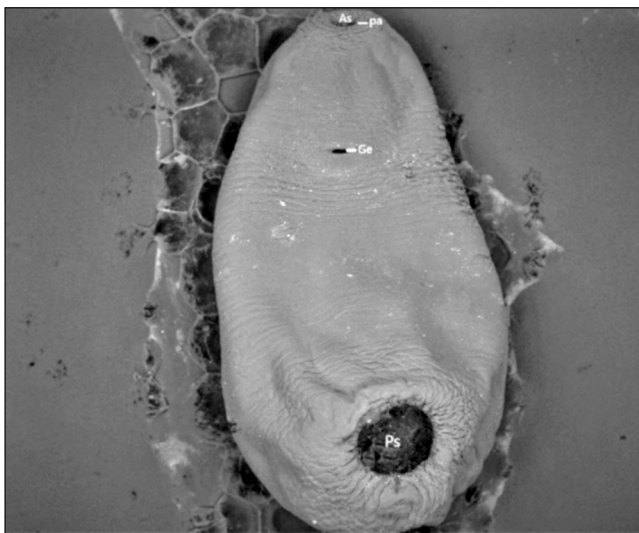


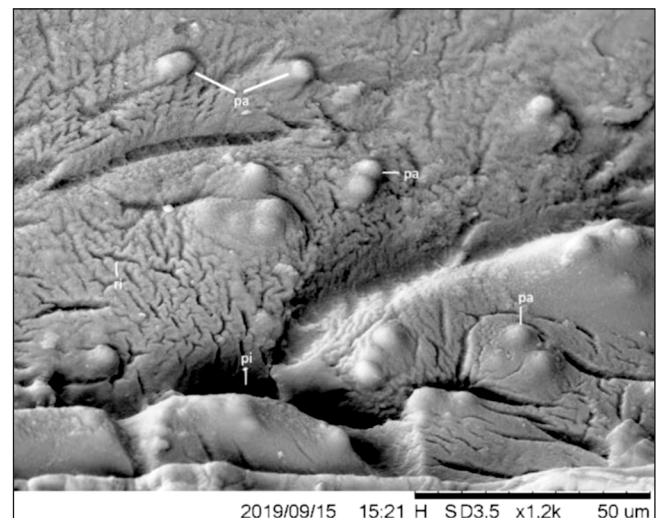
Fig. 5A. The whole body of an adult *Paramphistomum daubneyi* showing the ventral surface with anterior (As) and posterior suckers (Ps) surrounded by folds bearing rows of ventral papillae (pa), and opening of genital canal (Ge) at the anterior third.



Fig. 5B. The ventral surface showing the anterior sucker (As) surrounded by rows of papillae (pa) and major folds (fo) as well as major grooves (gr)

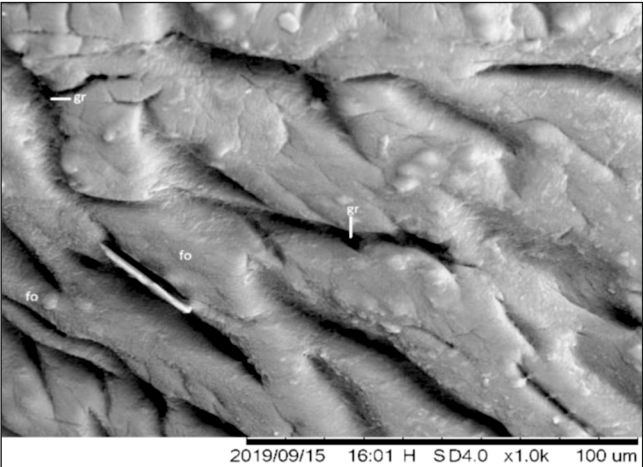
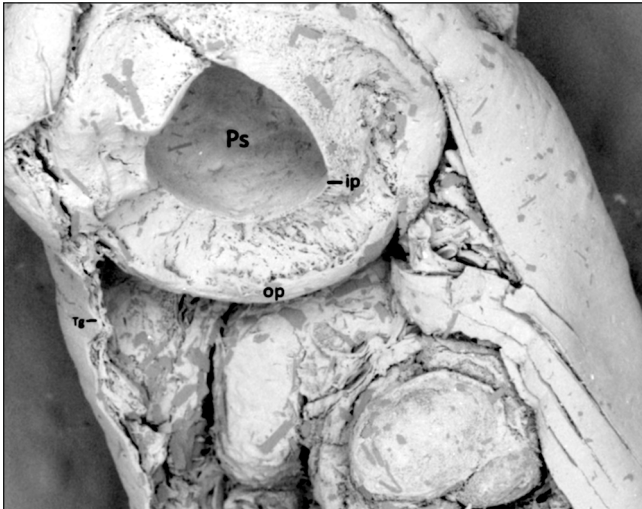
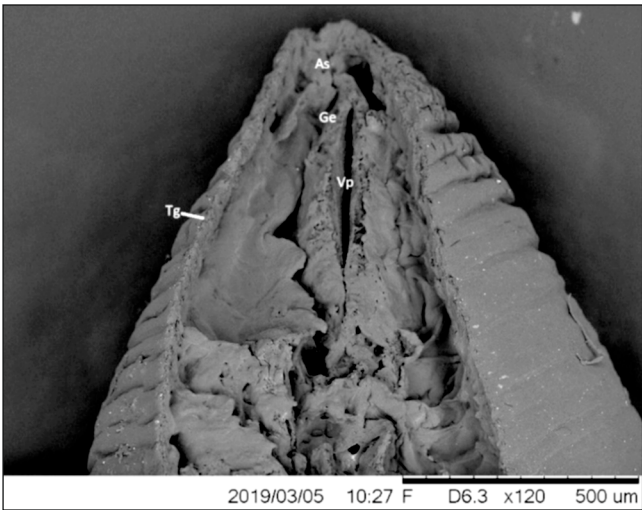


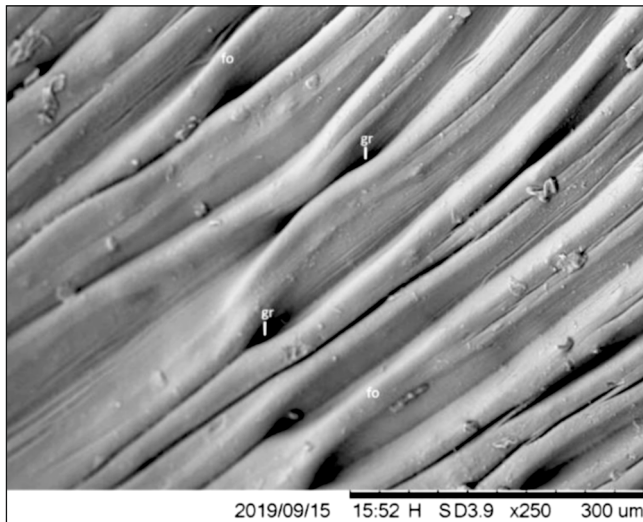
Fig. 5C. The surface of major fold shows clusters of papillae (pa), a series of alternating microfolds or ridges (ri) as well as microgrooves or pits (pi).



fold is made of micro-folds or interface crests, separated by micro-grooves or grooves. The close-up view

the dorsal area lacks papillae (Fig. 10).





matodes generally, especially in *F. hepatica* and *F. gigantica* (2), *Opisthorchis viverrini* (2) and *Schistosoma* spp. (20, 24). The ridges and grooves are well developed on all the regions of the parasite surface, except for the posterior part of the dorsal surface which tends to be flat. Also, the surface of all the tegument regions is increased through the presence of numerous short microvilli suggesting a high level of absorption capacity in all the tegument regions except for the dorsal surface of the posterior region. Similar to an *O. viverrini* adult (2), all the sides of *Paramphistomum daubneyi* are spineless. This suggests that the parasites taken into study do not require spines to move around through the rumen and reticulum because they can anchor themselves on the wall of the parasitized organ by firmly attaching themselves with the help of their suckers. On the other hand, *Fasciola* spp. and *Schistosoma* spp. adults have numerous surface spines, indicating the fact that they can move more through the biliary canals of the liver or blood vessels (2, 24). Most of the time, *Paramphistomum daubneyi* adults probably remain attached to the rumen or reticulum wall, using their relatively large and muscular posterior suckers which are surrounded by type 1 and type 2 papillae that can serve as tactile receptors similar to those seen in *F. gigantica* (2). In what regards these sensory receptors, there is a possibility that the type 1 papilla is the pressure receptor due to its bulla-like aspect and lack of cilia, while the type 2 papilla can play a tactile/chemoreceptive role due to the presence of a single cilium (1).

CONCLUSIONS

Scanning electron microscopy is a valuable tech-

nique used for differentiating morphology characteristics of *Paramphistomum*. Scanning electron microscopy highlights the spatial image of the body and its components (organs). Tegument layers and other very small components, such as sensory organs, can also be analysed. Anterior region of the adult trematode provides the main morphology characteristics in different species using scanning electron microscopy (SEM).

REFERENCES

1. Anuracpreeda P., Wanichanon C., Sobhon P., (2008), *Paramphistomum cervi*: Antigenic profile of adults as recognized by infected cattle sera. *Exp Parasitol*, 118:203-207
2. Dangprasert T., Khawsuk W., Meepol A., Wani-
chanon C., Viyanant V., Upatham E.S., Wongratanacheevin S., Sobhon P., (2001), *Fasciola gigantica*: surface topography of the adult tegument. *J Helminthol*, 75:43-50
3. Dărbăuş G., Afrenie M., Hotea I., Imre M., Morariu S., (2014), Endoparasites in mammals from seven zoological gardens in Romania. *J Zoo Wildl Med*, 45(2):239-246
4. Dărbăuş Gh., Cosoroabă I., Oprescu I., Morariu S., (2001), Epidemiologie de la cryptosporidioză chez les animaux dans l'Ouest de la Roumanie. *Revue Med. Vet.*, 152(5):399-404
5. Dărbăuş Gh., Hotea I., Oprescu I., Morariu S., Brudiu I., Olariu R.T., (2011), Toxoplasmosis seroprevalence in cats and sheep from Western Romania. *Revue Med Vet*, 162(6):316-320
6. Gordon D.K., Roberts L.C.P., Lean N., Zadoks R.N., Sargison N.D., Skuce P.J., (2013), Identification of the rumen fluke, *Calicophoron daubneyi*, in GB livestock: possible implications for liver fluke diagnosis. *Vet Parasitol*, 195:65-71
7. Hora F.S., Genchi C., Ferrari N., Morariu S., Mederle N., Dărbăuş Gh., (2017), Frequency of gastrointestinal and pulmonary helminth infections in wild deer from western Romania. *Vet Parasitol: Regional Studies and Reports*, 8:75-77
8. Huson K.M., Oliver N.A., Robinson M.W., (2017), Paramphistomosis of ruminants: an emerging parasitic disease in Europe. *Trends Parasitol*, 33:836-844
9. Imre K., Luca C., Costache M., Sala C., Morar A., Morariu S., Ilie M.S., Imre M., Dărbăuş Gh., (2013), Zoonotic *Cryptosporidium parvum* in Romanian newborn lambs (*Ovis aries*). *Vet Parasitol*, 191(1-2):119-122
10. Imre K., Morariu S., Ilie M.S., Imre M., Ferrari N., Genchi C., Dărbăuş Gh., (2012), Serological survey of *Neospora caninum* infection in cattle herds from western Romania. *J Parasitol*, 98(3):683-685
11. Imre K., Dărbăuş Gh., Tîrziu E., Morariu S., Imre

- M., Plutzer J., Boldea M.V., Morar A., (2019), *Sarcocystis* spp. in Romanian slaughtered cattle: molecular characterization and epidemiological significance of findings. *BioMed Res Int*, 2019: 4123154
12. Indre D., Dărăbuș Gh., Oprescu I., Morariu S., Mederle N., Ilie M.S., Măndiță D.N., (2009), The identification of gastrointestinal nematodes species in sheep in five localities from Timiș County. *Lucrări Științifice Medicină Veterinară Timișoara*, 42(1):94-100
 13. Martinez-Ibeas A.M., Munita M.P., Lawlor K., Sekiya M., Mulcahy G., Sayers R., (2016), Rumen fluke in Irish sheep: prevalence, risk factors and molecular identification of two paramphistome species. *BMC Vet Res*, 12(1):143
 14. Morariu S., Bulacu A., Dărăbuș Gh., Mederle N., Ilie M.S., Badea C., Cioban Gh., Imre M., (2016), Preliminary research on the prevalence of *Toxoplasma gondii* infection in wisents (*Bison bonasus*) from Romanian Armenis-Plopu reserve. *Lucrări Șt. Medicină Veterinară Timișoara*, 49(3): 113-116
 15. Morariu S., Bulacu A., Dărăbuș Gh., Mederle N., Mihăilă C., Nechiti R., Brîncoveanu A., Badea C., (2014), Prevalence of internal parasites in wisents (*Bison bonasus bonasus*) from two Romanian reservations - preliminary results. *Lucrări Științifice Medicină Veterinară Iași*, 57(3-4):170-173
 16. Naranjo-Lucena A., Munita Corbalán M.P., Martínez-Ibeas A.M., McGrath G., Murray G., Casey M., Good B., Sayers R., Mulcahy G., Zintl A., (2018), Spatial patterns of *Fasciola hepatica* and *Calicophoron daubneyi* infections in ruminants in Ireland and modelling of *C. daubneyi* infection. *Parasite Vector*, 11:531
 17. Nechiti R., Dărăbuș Gh., Morariu S., (2017), Preliminary research regarding the prevalence of digestive and respiratory parasitosis in meat cattle from the Hârtibaci Valley, Sibiu County. *Lucrări Științifice Medicină Veterinară Iași*, 60(3):392-396
 18. Panyarachun B., Ngamniyom A., Sobhon P., Anuracpreeda P., (2013), Morphology and histology of the adult *Paramphistomum gracile* Fischoeder, 1901. *J Vet Sci*, 14(4):425-432
 19. Panyarachun B., Sobhon P., Yotsawan T., Chotwiwatthanakun C., Anupunpisit V., Anuracpreeda P., (2010), *Paramphistomum cervi*: surface topography of the tegument of adult fluke. *Exp Parasitol*, 125:95-99
 20. Rondelaud D., Titi A., Vignoles P., Mekroud A., Dreyfuss G., (2013), Consequence of temperature changes on cercarial emergence from *Galba truncatula* infected with *Fasciola hepatica* or *Paramphistomum daubneyi*. *Parasite*, 20:10
 21. Saowakon N., Lorsuwanarat N., Changklungmoa N., Wanichanon C., Sobhon P., (2013), *Paramphistomum cervi*: The in vitro effect of plumbagin on motility, survival and tegument structure. *Exp Parasitol*, 133(2):179-186
 22. Sîrbu C., Badea C., Imre M., Suici T., Dărăbuș Gh., Oprescu I., Ilie M.S., Morariu S., (2017), Endoparasitic screening in sheep and cattle from Bethausen, Timiș. *Lucrări Științifice Medicină Veterinară Timișoara*, 50 (2):269-274
 23. Sîrbu C.B., Imre K., Dărăbuș Gh., Suici T., Mateș B., Morariu S., (2020), Prevalence of gastrointestinal parasitic infections in cattle and sheep in two regions of Romania. *Turk J Vet Anim Sci* (in press)
 24. Sobhon P., Upatham E.S., (1990), Snail hosts, life-cycle, and tegumental structure of oriental schistosomes. UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases, 57-88
 25. Sorescu I.D., Dărăbuș Gh., Ilie M.S., Morariu S., Oprescu I., Mederle N., Imre K., Hotea I., Indre D., Balint A., Imre M., (2011), Identification of *Giardia* spp. parasitism in goat kids. *Revista Romana de Medicina Veterinara*, 21(4):131-135
 26. Titi A., Mekroud A., Chibat Mel H., Boucheikh choukh M., Zein-Eddine R., Djuikwo-Teukeng F.F., Vignoles P., Rondelaud D., Dreyfuss G., (2014), Ruminal paramphistomosis in cattle from northeastern Algeria: prevalence, parasite burdens and species identification. *Parasite*, 21:50
 27. Zintl A., Garcia-Campos A., Trudgett A., Chryssafidis A.L., Talavera-Arce S., Fu Y., Egan S., Lawlor A., Negredo C., Brennan G., Hanna R.E., De Waal T., Mulcahy G., (2014), Bovine paramphistomes in Ireland. *Vet Parasitol*, 204:199-208.