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Comparative histochemical study of four enzyme activities on some organs of male *Schistosoma mansoni* and *S. haematobium*

Eman A. El-Shabasy[1*], Enayat S. Reda[1], Ashraf E. Said[2] and Mai A. Saleh[1]

Abstract

Background: Enzymes are the biological catalysts which accelerate the various cellular reactions. In general, the enzymes alkaline phosphatase (ALPase), acid phosphatase (ACPase), 5′-nucleotidase (5′-Nu) and glucose-6-phosphatase (G-6-Pase) have important roles in metabolism. In the present study, a comparative account of the localization of activity of four enzymes acid phosphatase, alkaline phosphatase, glucose-6-phosphatase and 5′-nucleotidase was carried out in the organs and tissues of important human digeneans *Schistosoma mansoni* and *Schistosoma haematobium* from infected hamster (*Cricentus auratus*) in Egypt.

Material and methods: Histochemical methods were used on whole parasites to study the distribution of these enzymes. The following organs and tissues were studied: oral sucker, oesophagus, oesphageal gland, ventral sucker, ventral sucker rim, gynaecophoral canal edge, intestine, tubercles, muscles, tegumental folds, testes and excretory pore.

Results: Variable observations in the different organs and tissues were recorded. In *S. mansoni*, glucose-6-phosphatase was detected with the highest activity while alkaline phosphatase showed the lowest activity in most organs and tissues. In *S. haematobium*, acid phosphatase showed the highest activity and alkaline phosphatase showed the lowest activity in most organs and tissues. Although all enzymes showed clear activity in the oral sucker for both species, only glucose-6-phosphatase gave a positive reaction in the ventral sucker rim and acid phosphatase in the excretory pore of *S. haematobium*. On the other hand, all enzymes showed no activity in the muscles and excretory pore in *S. mansoni*.

Conclusion: ACPase activity was observed in most organs of *S. haematobium*. Also, G-6-Pase was detected in the ventral sucker rim and 5′-Nu was detected in tubercles. Meanwhile, ACPase and 5′-Nu were observed in the muscle fibers, testes, excretory pore and tegumental folds. On other hand, G-6-Pase is the most active enzyme in most organs of *S. mansoni* except the muscle fibers.

Keywords: Acid phosphatase, Alkaline phosphatase, Glucose-6-phosphatase and 5′-nucleotidase, *Schistosoma mansoni*, *S. haematobium*, Histochemical study

* Correspondence: emanahmah@gmail.com
[1]Department of Zoology, Faculty of Science, Mansoura University, Mansoura, Egypt
Full list of author information is available at the end of the article

Background

Enzymes are biological catalysts that accelerate the various cellular reactions (Pardeshi & Hiware, 2010). In general, the enzymes alkaline phosphatase (ALPase), acid phosphatase (ACPase), adenosine triphosphatase (ATPase), 5′-nucleotidase (5′-Nu) and glucose-6-phosphatase (G-6-Pase) have important roles in metabolism.

ALPase is a hydrolase enzyme responsible for dephosphorylation of many types of molecules, including nucleotides, proteins and alkaloids. It is most effective in an alkaline environment. ACPase is a type of enzyme used to free attached phosphate groups from other molecules during digestion. It is stored in lysosomes and has an acid pH optimum. A nucleotidase is a hydrolytic enzyme that catalyses the hydrolysis of a nucleotide to its original constituents. 5′-Nu is involved in various functions, such as cell-cell communication, nucleic acid repair, signal transduction and membrane transport. G-6-Pase is an enzyme that hydrolyses of glucose 6-phosphate.

In recent years, these enzymes have been studied in parasites. Alkaline phosphatase has been reported in the fluid of sterile and fertile *Echinococcus granulosus* cysts reported (Vatankhah, Assmar, Vatankhah, & Shokrgozar, 2003) and the activities and properties of mitochondrial and microsomal glucose-6-phosphatases in free-living turbellarian *Phagocata sibirica* and cestodes *Bothriocephalus scorpii* (Burenina, 2009). The localization of both alkaline and acid phosphatases in various organs of *Orientocreadium striatusae* was reported (Pardeshi & Hiware, 2010). The activity of five enzymes in *Macrogyrodactylus clarii* and *Gyrodactylus rysavyi* was studied (Arafa, Abd El-Hady, & El-Abbassy, 2012; Abd El-Hady, Arafa, El-Naggar, & El-Abbassy, 2012 respectively). Localization, characteristics and activity of enzymes in *Schistosoma mansoni* were examined by many authors (Cesair, 1974; Dusanic, 1959; Halton, 1967; Nimo-Smith & Standan, 1963; Pujol & Cesari, 1990). Humiczewska (1975, 1996, 2002) studied the activity of enzymes and some specific and non-specific phosphatases in different stages of *Fasciola hepatica*. Wang, Dai, Hongjun and Liang (2011) studied enzyme activity in *Oncomelania hupensis* the intermediate host of *Schistosoma japonicum*.

Methods

Demonstration of alkaline phosphatase activity according to El-Aaser and Hassanein (1975)

Living, flattened specimens (about 10 worms) were fixed in a mixture of 95% methanol and 4% formalin for about 30 min, then washed in distilled water, incubated in the stock solution (Tris-buffer (150 ml, 0.2 M), β-glycerophosphate (25 ml, 0.05 M), distilled water (50 ml) and tartaric acid (6 ml, 1%)) at pH 9.5 for 60 min. Specimens were transferred to a mixture of proper media (45 ml), lead nitrate (5 ml, 1%) and magnesium chloride (0.5 ml, 0.05 M) for about 90 min and then washed in distilled H_2O. Fresh yellow ammonium sulphide (1%) was prepared, and parasites were stained in it for 2–3 min and then washed in distilled H_2O. Finally, parasites were mounted in neutral glycerin jelly and examined by bright field microscopy.

Some specimens were prepared to be used as controls by incubation without the substrate (β-glycerophosphate) and examined as described.

Demonstration of acid phosphatase activity according to Tice and Barnett (1962)

Living, flattened specimens (about 10 worms) were used. They were fixed in a mixture of methyl alcohol (25 ml), acetone (20 ml) and formalin (5 ml) for about 45–

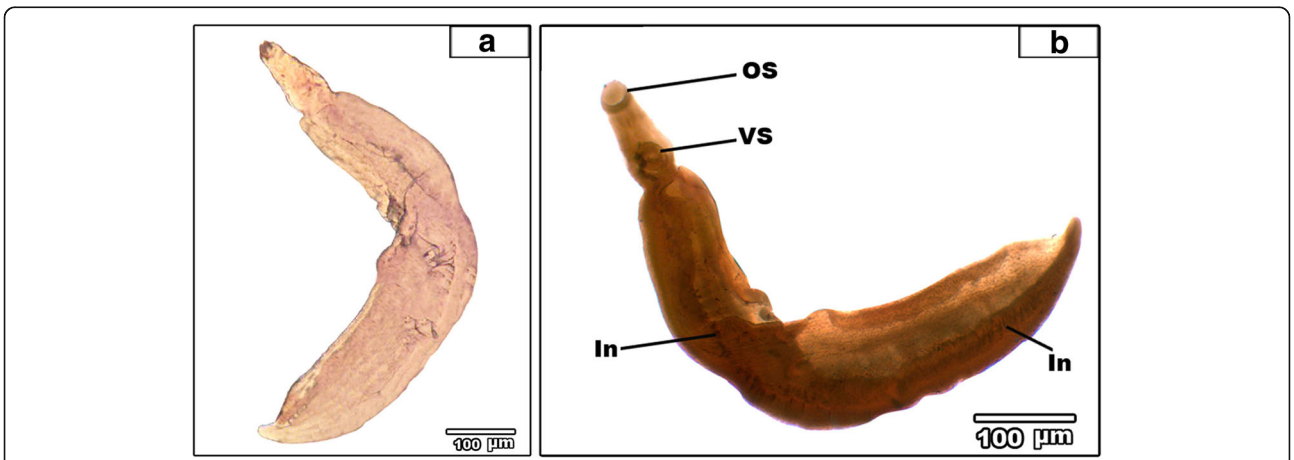

Fig. 1 Light micrographs of control and enzymes activity of male *S. mansoni*. **a** Control whole mount for acid phosphatase (ACPase) activity. **b** Whole mount stained for acid phosphatase activity showing a positive reaction in the intestine (In), oral sucker (OS) and ventral sucker (VS)

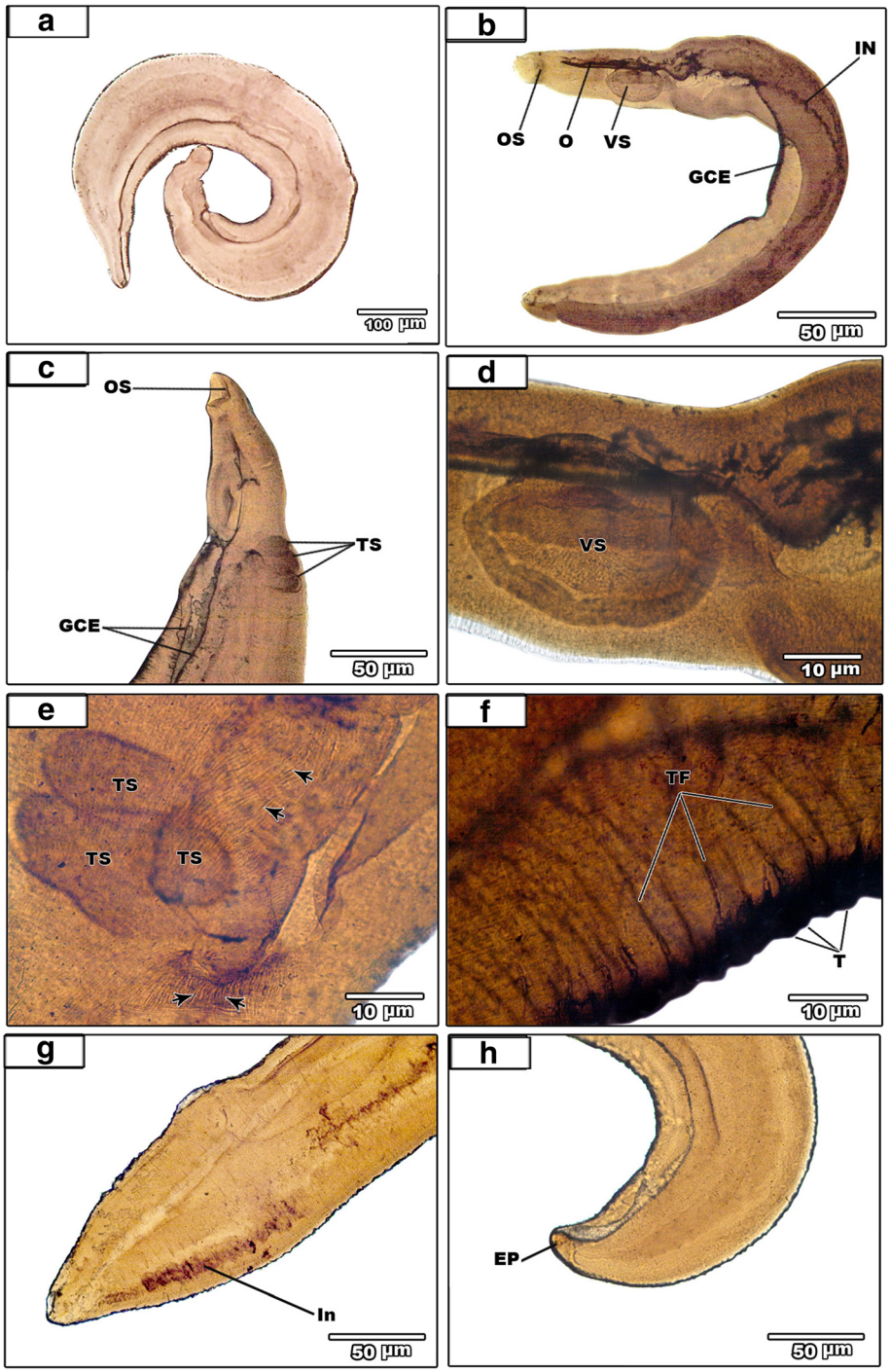

Fig. 2 Light micrographs of control and enzymes activity of male *S. haematobium*. **a** Control whole mount for acid phosphatase (ACPase) activity. **b** Whole mount stained for acid phosphatase activity showing a positive reaction in the gynaecophoral canal edge (GCE), intestine (In), oesphagus (OE), oral sucker (OS) and ventral sucker (VS). **c** Anterior region showing the gynaecophoral canal edge (GCE), oral sucker (OS) and testes (Ts). **d** The ventral sucker (VS). **e** The region below the ventral sucker showing the muscle fibers (head arrow) and testes (Ts). **f** Magnification of the body surface showing the tegumental folds (TF). **g** Posterior region showing the intestine (In). **h** Posterior region showing the excretory pore (EP)

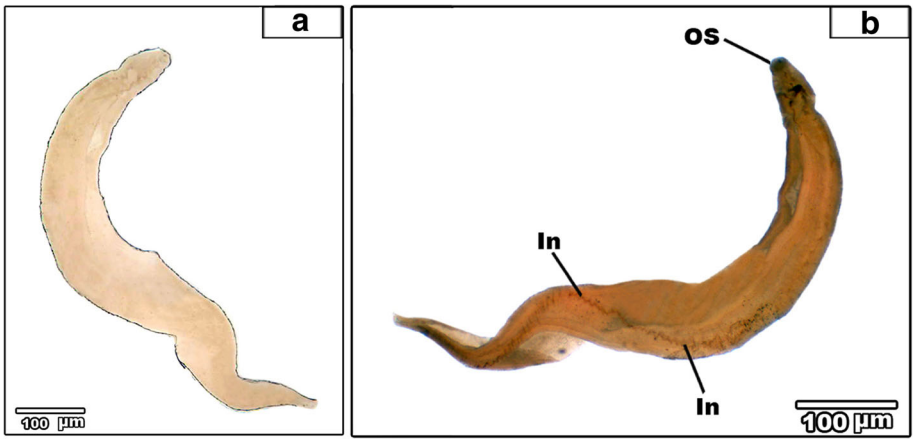

Fig. 3 Light micrographs of control and enzymes activity of male *S. mansoni*. **a** Control whole mount for alkaline phosphatase (ALPase) activity. **b** Whole mount stained for alkaline phosphatase activity showing a positive reaction in the intestine (In) and oral sucker (OS)

60 min. Then, distilled H_2O was used to wash them. They were incubated in acetate buffer (20 ml, 0.05 M), lead nitrate (3 ml, 1%) and β-glycerophosphate (2 ml, 0.05 M) for about 120 min and then washed again. Fresh yellow ammonium sulphide (1%) was prepared, and worms were stained (2–3 min) and then washed (distilled H_2O). Finally, worms were mounted in neutral glycerin jelly and examined.

Some specimens were prepared to be used as controls by incubation without the substrate (β-glycerophosphate) and examined.

Demonstration of 5′-nucleotidase activity according to Wachstein and Meisel (1957)

Living, flattened specimens (about 10 worms) were fixed in glutaraldehyde (2.5%, pH 7.4) for 5–7 min at 4 °C.

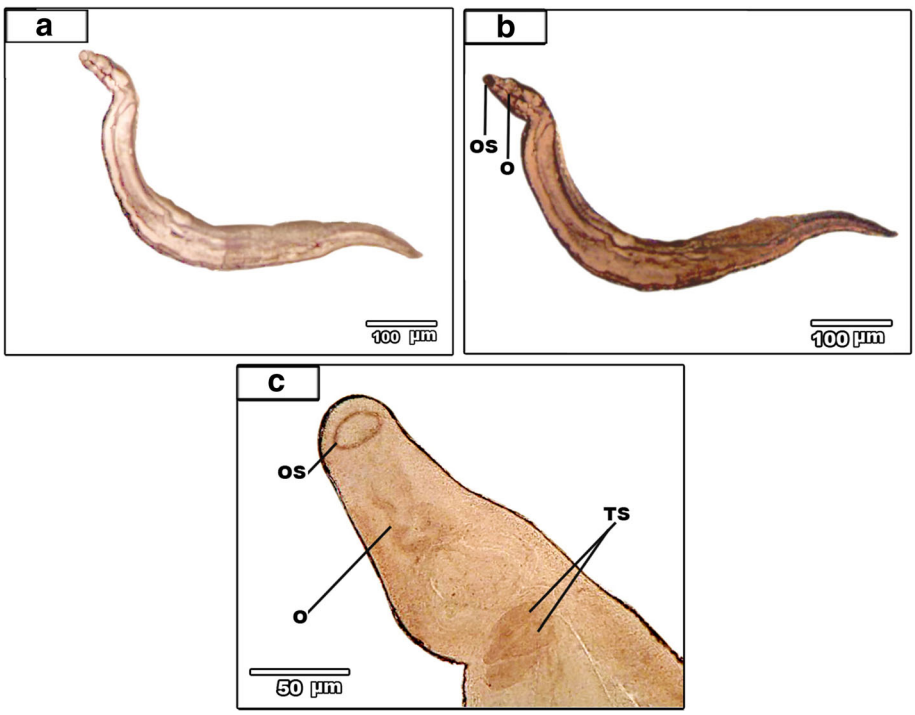

Fig. 4 Light micrographs of control and enzymes activity of male *S. haematobium*. **a** Control whole mount for alkaline phosphatase (ALPase) activity. **b** Whole mount stained for alkaline phosphatase activity showing a positive reaction in the oesphagus (OE) and oral sucker (OS). **c** Anterior region showing a positive reaction in the oesophagus (OE), oral sucker (OS) and testes (Ts)

Then, the worms were washed in distilled H_2O. They were incubated in Tris-maleate (12 ml, 0.02 M), distilled H_2O (8.5 ml), lead nitrate (3.5 ml, 1%), magnesium chloride (1 ml, 0.1 M) and adenosine 5′-monophosphate disodium salt (2.5 ml, 0.05 M) for 60 min at 37 °C and washed again. Fresh yellow ammonium sulphide (1%) was prepared, and worms were stained (2–3 min) then washed. Finally, parasites were mounted in neutral glycerin jelly and examined.

Some specimens were prepared to be used as controls by incubation without the substrate (adenosine 5′-monophosphate disodium salt) and examined.

Demonstration of glucose-6-phosphatase activity according to Tice and Barnett (1962)

Living, flattened specimens (about 10 worms) were fixed in glutaraldehyde (2.5%, pH 7.4 at 4 °C) for 5–7 min and washed in distilled H_2O. The worms were incubated for 60 min at 37 °C in acetate buffer (20 ml, 0.1 M), lead nitrate (3 ml, 1%) and glucose-6-phosphate disodium salt (2 ml, 0.05 M). Freshly yellow ammonium sulphide (1%) was prepared, and worms were stained (2–3 min) and then washed. Finally, they were mounted in neutral glycerin jelly and examined.

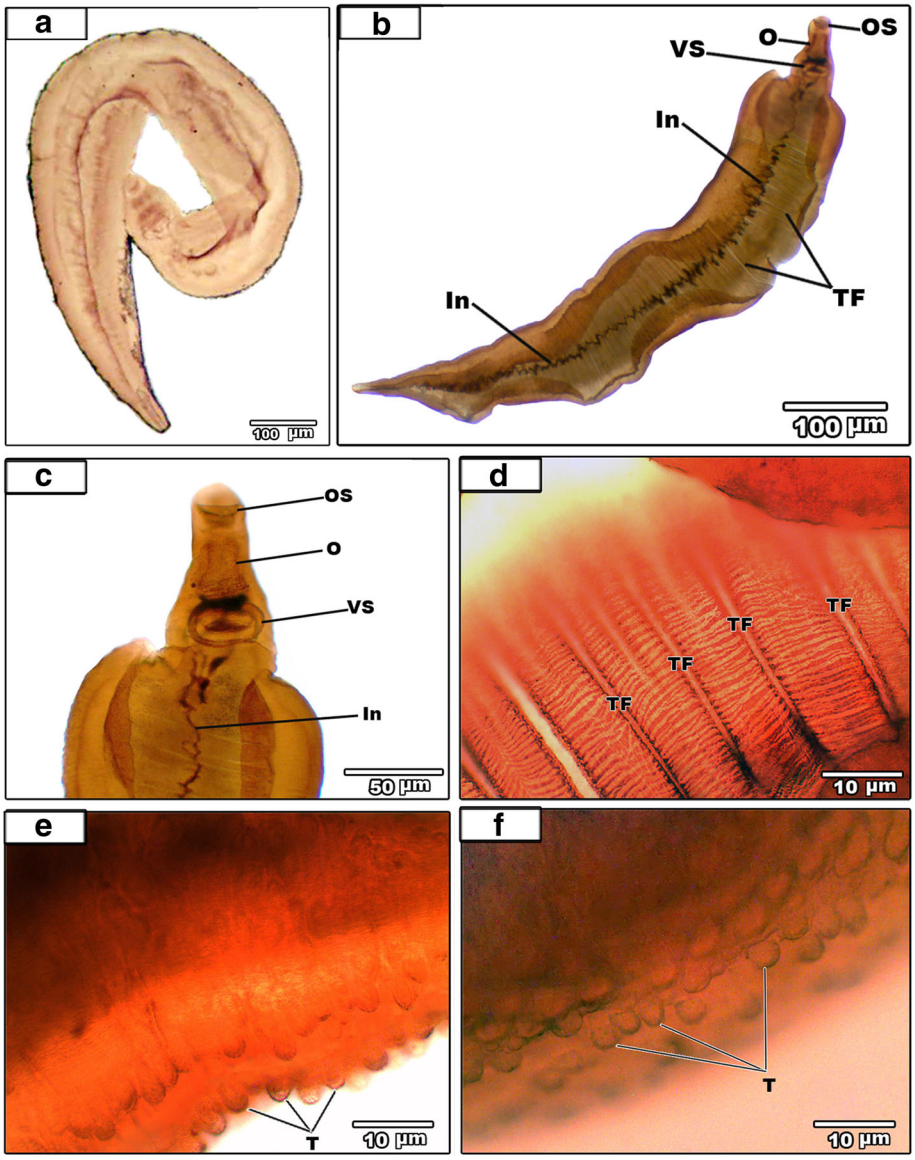

Fig. 5 Light micrographs of control and enzymes activity of male *S. mansoni*. **a** Control whole mount for glucose-6-phosphatase (G-6-Pase) activity. **b** Whole mount stained for glucose-6-phosphatase activity showing a positive reaction in the intestine (In), oesophagus (OE), oral sucker (OS), tegumental folds (TF) and ventral sucker (VS). **c** Anterior region of male showing a positive reaction in the intestine (In), oesophagus (OE), oral sucker (OS) and ventral sucker (VS). **d** Magnification of the tegumental folds (TF). **e** Magnification of dorsal surface showing the tubercles (T). **f** Magnification of dorsal surface showing the tubercles (T)

Some specimens were prepared to be used as controls by incubation without the substrate (glucose-6-phosphate disodium salt) and examined. All examination was performed using a bright field microscope.

Intensity of enzyme activity was estimated according to the density of colour obtained.

Results

In this study, histochemical techniques were employed to whole-mount fluke to show the distribution of four enzymes, namely, acid phosphatase (ACPase), alkaline phosphatase (ALPase), 5′-nucleotidase (5′-Nu) and glucose-6-phosphatase (G-6-Pase). The following organs and tissues were studied: oral sucker (OS), oesophagus (OE), oesophageal gland (OG), ventral sucker rim (VSR), ventral sucker (VS), gynaecophoral canal edge (GCE), intestine (In), tubercles (T), muscles, tegument folds (TF), testes (Ts) and excretory pore (EP).

ACPase activity

In *S. mansoni*, ACPase activity was detected in the oral sucker, ventral sucker and intestine but not in the oesophagus, oesophagus gland, ventral sucker rim, gynaecophoral canal edge, tubercles, muscles, tegument folds, testes and excretory pore (Fig. 1b). In *Schistosoma haematobium*, ACPase activity was detected in the oral sucker, oesophagus, ventral sucker, gynaecophoral canal edge, intestine, tubercles, muscles, tegument folds, testes and excretory pore only (Fig. 2b–h).

ALPase activity

In *S. mansoni*, a positive reaction for ALPase was observed just in the oral sucker and intestine (Fig. 3b), whereas in *S. haematobium*, ALPase activity was observed in the oral sucker, oesophagus, oesophagus gland and testes only (Fig. 4b, c).

G-6-Pase activity

In *S. mansoni*, positive reactions to G-6-Pase were detected in the oral sucker, oesophagus, ventral sucker, intestine, tubercles and tegument folds (Fig. 5b–f). In contrast, in *S. haematobium*, the activity of G-6-Pase was observed in the oral sucker, ventral sucker rim, ventral sucker and gynaecophoral canal edge (Fig. 6b, c).

5′-Nu activity

In *S. mansoni*, the activity of 5′-Nu was observed in a few organs, namely, the oral sucker, ventral sucker and intestine (Fig. 7b, c), whereas in *S. haematobium*, 5′-Nu activity was detected in the oral sucker, oesophagus, gynaecophoral canal edge, intestine, tubercles, muscles and tegumental folds (Fig. 8b–d).

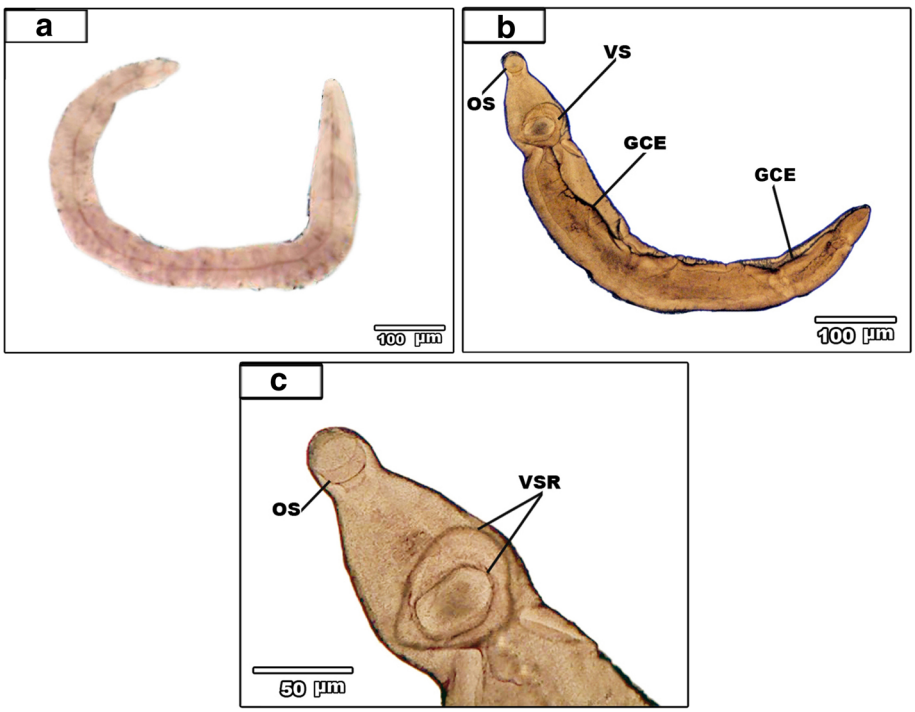

Fig. 6 Light micrographs of control and enzymes activity of male *S. haematobium*. **a** Control whole mount for glucose-6-phosphatase (G-6-Pase) activity. **b** Whole mount stained for glucose-6-phosphatase activity. GCE gynaecophoral canal edge, OS oral sucker, VS ventral sucker. **c** Anterior region showing a positive reaction in the oral sucker (OS) and ventral sucker rim (VSR)

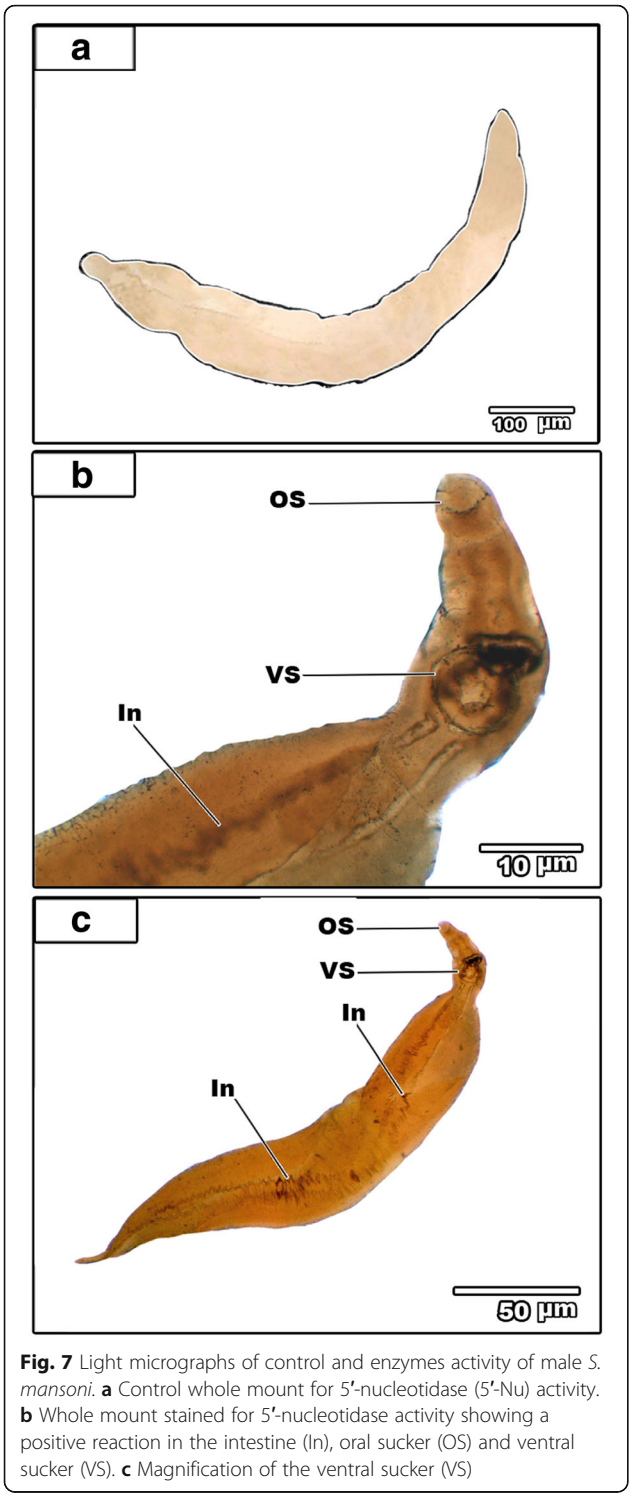

Fig. 7 Light micrographs of control and enzymes activity of male *S. mansoni*. **a** Control whole mount for 5′-nucleotidase (5′-Nu) activity. **b** Whole mount stained for 5′-nucleotidase activity showing a positive reaction in the intestine (In), oral sucker (OS) and ventral sucker (VS). **c** Magnification of the ventral sucker (VS)

Intensity of enzyme activities (shown in Table 1)

All enzymes gave a positive reaction in the oral sucker. On the other hand, all enzymes showed no activity in the muscles and excretory pore of *S. mansoni* males. Only acid phosphatase gave a marked activity in these tissues of *S. haematobium* males. The rim of the ventral sucker of *S. haematobium* showed a positive reaction for G-6-Pase only. In *S. mansoni*, G-6-Pase showed a strong reaction in most organs, while in *S. haematobium*, ACPase showed a strong activity in most organs and other enzymes showed a moderate activity.

Acid and alkaline phosphatase was present in different internal organs and tissues. The largest abundance of ALPase appeared in the oral sucker (moderate reaction) and intestine (weak activity) of *S. mansoni* male, while it was located with extensive intensity in many organs of *S. haematobium* male. Moreover, ALPase of *S. mansoni* male was recorded in the oral sucker and intestine with moderate and positive reaction, respectively, while it was observed with high activity in the oral sucker and moderate concentration in the oesophagus and testes (positive reaction) of *S. haematobium* male.

Discussion

In the present study, a comparative account of the distribution and activities of four enzymes, glucose-6-phosphatase (G-6-Pase), 5′-nucleotidase (5′-Nu,) alkaline phosphatase (ALPase) and acid phosphatase (ACPase), in different organs of *Schistosoma mansoni* and *S. haematobium* is summarized in Table 1. These enzymes showed variable appearance in their activity in various tissues and organs in both species.

Enzyme localization activities were surveyed using enzyme-histochemical techniques. The results showed that the ALPase and ACPase are highly active in the oral sucker in both *Schistosoma* species, while these enzymes are highly active in the oesophagus and testes of *S. haematobium* only and in the intestine of *S. mansoni*. ACPase is present in the ventral sucker in both *Schistosoma* species. In addition, ACPase activities are localized in most tested organs (oral sucker, oesophagus, ventral sucker, gynaecophoral canal edge, intestine, muscles, tegumental folds, testes and excretory pore) of *S. haematobium*. The enzyme activities of G-6-Pase are present in the oral sucker and ventral sucker in both *Schistosoma* species. The intestine, tubercles and tegumental folds contain this enzyme in *S. mansoni* only, but in contrast, *S. haematobium* contain this enzyme in the ventral sucker rim and gynaecophoral canal edge. 5′-Nu is widely distributed in the oral sucker and intestine in both *Schistosoma* species, whereas the same enzyme was present in most organs of *S. haematobium*. 5′-Nu showed a strong enzyme activity in the *S. haematobium* intestine and *S. mansoni* ventral sucker.

Many histochemical studies have been carried out to detect the localization of enzymes in Platyhelminthes (Abd El-Hady et al., 2012; Arafa et al., 2012; Pardeshi &

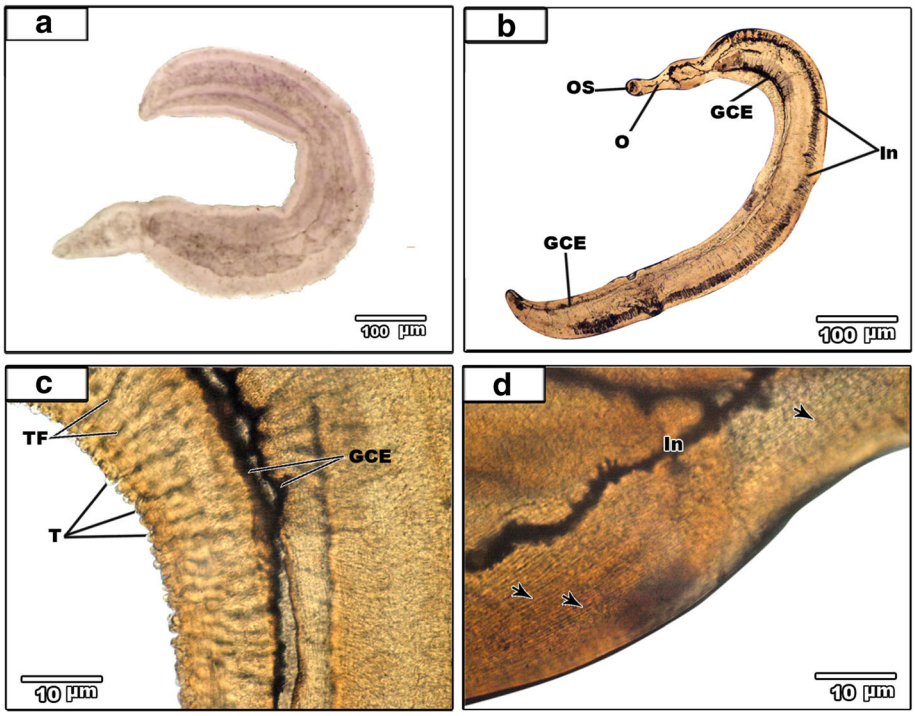

Fig. 8 Light micrographs of control and enzyme activity of male *S. haematobium*. **a** Control whole mount for 5′-nucleotidase activity. **b** Whole mount stained for 5′-nucleotidase activity. Note the positive reaction in the gynaecophoral canal edge (GCE), intestine (In), oesphagus (OE) and oral sucker (OS). **c** Magnification of the body showing a positive reaction in the gynaecophoral canal edge (GCE) and tegumental folds (TF). **d** Magnification of the body showing a positive reaction in the intestine (In) and muscles fibers (head arrow)

Hiware, 2010). Few studies have been done on digeneans (Haseeb, Eveland, & Fried, 1984; Humiczewska, 1975; Nimo-Smith & Standan, 1963; Pujol & Cesari, 1990; Wang et al., 2011). Most of these studies have revealed the distribution of acid phosphatase and alkaline phosphatase (Cesair, 1974; Dusanic, 1959; Halton, 1967; Humiczewska, 2002; Nimo-Smith & Standan, 1963).

Generally, the concentration of both acid and alkaline phosphatase in males of *S. haematobium* was higher than that in males of *S. mansoni.* Acid phosphatase activity gave a positive reaction in the oral sucker and intestine and a moderate activity in the ventral sucker of *S. mansoni* male, whereas in males of *S. haematobium*, the oral sucker, ventral sucker, tegumental folds and

Table 1 Showing the histochemical reactions of male *Schistosoma mansoni* and *S. haematobium*

Parameters	*S. mansoni*				*S. haematobium*			
	ACP	ALP	G-6-p	5-N	ACP	ALP	G-6-p	5-N
Oral sucker (OS)	+	++	+	+	+	+++	+	++
Oesophagues (O)	–	–	+	–	+++	++	–	+
Ventral sucker rim (VSR)	–	–	–	–	–	–	++	–
Ventral sucker (VS)	++	–	++	+++	+	–	+	–
Gynaecophoral canal edge (EGC)	–	–	–	–	++	–	++	++
Intestine (In)	+	+	++	+	++	–	–	+++
Tubercles (T)	–	–	++	–	–	–	–	+
Muscles	–	–	–	–	++	–	–	+
Tegumental folds (TF)	–	–	++	–	+	–	–	++
Testes (Ts)	–	–	–	–	++	+	–	–
Excretory pore (EP)	–	–	–	–	+	–	–	–

*(+ positive reaction, ++ moderate activity, +++ high activity & – negative reaction)

excretory pore showed a positive reaction, and the edge of gynaecophoral canal, intestine, muscles and testes gave a moderate activity while the oesophagus showed a strong activity.

In *S. mansoni*, Giboda and Zdarska (1994) studied the activity of ALPase and they discovered its antigenicity. Also, ALPase acts as a sensitive indicator of viability of the developing embryos of *S. mansoni* as lack of ALPase activity in the eggs is a first sign of their death. ACPase, ALPase and 5′-Nu were studied in the sporocyst of *Fasciola hepatica* by Humiczewska (2002), who reported that the activity of these enzymes indicated that they have roles in the metabolism of the sporocyst. Vatankhah et al. (2003) studied the activity of ALPase in fertile and sterile *Echinococcus granulosus* cysts, and they found that the activity of ALP in fertile cyst is higher than sterile cyst, so they suggested that ALPase is valuable for use as an antigen.

In the present study, the activity of these enzymes in the oral sucker, ventral sucker, intestine, oesophagus, muscles, gynaecophoral canal edge, tubercles, tegument folds and excretory pore of two species indicated that these organs and tissues are active sites for metabolism of nutrients and metabolites. Both of ACPase and ALPase may be a mark for viability and fertility of *S. mansoni* and *S. haematobium*. Also, both enzymes could be used as important antigens for *Schistosoma* sp., as reported by Fonseca, Carvalho, Alves, & De Melo, 2012 who mentioned that some tegument proteins of *Schistosoma* have been evaluated as vaccine antigens in immunization protocols.

Variation in the ACPase, G-6-Pase and 5′-Nu concentration (density of colour) could explain the more rapid movement of *S. mansoni* than of *S. haematobium* as they were presented in the suckers and tegumental folds of *S. mansoni* more than that of *S. haematobium*, coupling of males and females of *S. haematobium* for a long time after recovering from mice as they were founded in the muscles and gynaecophoral canal edge of *S. haematobium* only.

G-6-Pase is considered to be a multifunctional enzyme which is a characteristic constituent of the endoplasmic reticulum. The enzyme splits glucose 6-phosphate into glucose and inorganic orthophosphate. Biochemists use it as a marker of the endoplasmic reticulum. The present work showed that G-6-Pase is more highly active in *S. mansoni* than in *S. haematobium*. This is in agreement with Burenina (2009) who studied the activity and properties of G-6-Pase in *Phagocata sibirica* (free-living turbellarian) and *Bothriocephalus scorpii* (cestode). Moreover, Moore and Halton (1975) reported that G-6-Pase is not present in the larval stages of *Fasciola hepatica* (redia and cercariae). This may be due to the ability of adult Platyhelminthes to release glucose from glycogen and gluconeogenic precursors (Burenina, 2009). This could explain the stronger attachment of *S. mansoni* than of *S. haematobium* to host tissues.

5′-Nucleotidase enzyme activity was observed in most organs and tissues of *S. haematobium* males, whereas in *S. mansoni* males, the activity was detected in the oral sucker, ventral sucker and intestine. This result was confirmed by Humiczewska (2002), who reported that 5′-nucleotidase is associated with the breakdown and transportation of nucleic acids. This enzyme belongs to the plasmalemma hydrolases, which facilitate membrane penetration by nucleotides and polynucleotides. Also, Uusitalo and Karnovsky (1981) reported that 5′-nucleotidase plays an important role in the migration of the cells as it might be involved in the production of adenosine. In addition, Uusitalo (1981) suggested that the enzyme has a role in intracellular regulatory mechanisms, possibly by producing nucleosides that may in turn affect cyclic nucleotide levels. 5′-Nucleotidase may play a role in neurotransmission (Schubert, Reddington, & Kreutzberg, 1979).

The present results suggest that protein synthesis in *S. haematobium* males may be higher than that in *S. mansoni* males. This may be a reflection of the difference in body dimensions between the two species; *S. haematobium* male is larger than *S. mansoni* male. The presence of high levels of enzymes involved in metabolism in the intestine and excretory pore of both species of *Schistosoma* reflects the biological role of these organisms in digestion and excretion.

The results of this histochemical study provide further evidence for the existence of these enzymes in different organs of *S. mansoni* and *S. haematobium*. These enzymes showed variable appearance in their activity in various tissues and organs in both species. It must be remembered that the interpretation of the reasons for the differences in the amount and distribution of the enzymes as well as the functional aspects in each instance must remain speculative in the present state of our knowledge.

Conclusions

ACPase activity was observed in most organs of *S. haematobium*. Also, G-6-pase was detected in the ventral sucker rim and 5′-Nu was detected in the tubercles. Meanwhile, ACPase and 5′-Nu were observed in the muscle fibers, testes, excretory pore and tegumental folds. On the other hand, G-6-Pase is the most active enzyme in most organs of *S. mansoni* except in the muscle fibers.

Abbreviations

EP: Excretory pore; GCE: Gynaecophoral canal edge; Head arrow: Muscle fibers; In: Intestine; OE: Oesophagus; OS: Oral sucker; T: Tubercles; TF: Tegumental folds; Ts: Testes; VS: Ventral sucker; VSR: Ventral sucker rim

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Authors' contributions
Prof. ESR carried out the photographing of the specimens, participated in the design of the study and reviewed the manuscript. Prof. AES carried out the final editing and revision of the manuscript. MAS performed the staining process of worms and participated in the photographing. Dr. EAE-S conceived of the study; participated in its design, getting the worms, staining process and coordination; and helped to draft the manuscript. All authors read and approved the final manuscript.

Ethics approval
All animals in this study were approved by the local ethical committee with code number MZ170012.

Consent for publication
Not applicable

Competing interests
The authors declare that they have no competing interests.

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Author details
[1]Department of Zoology, Faculty of Science, Mansoura University, Mansoura, Egypt. [2]Department of Zoology, Faculty of Science, Damietta University, Damietta, Egypt.

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