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Redescription of *Astiotrema reniferum* (Looss, 1898) Stossich 1904, A Digenean Intestinal Parasite of *Clarias lazera* in Egypt

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Abstract

A redescription is given of *Astiotrema reniferum* (looss, 1898) Stossich, 1904, a digenean intestinal parasite of the catfish *Clarias lazera* inhabiting Nile Delta waters in Egypt. Particular attention has been paid to the anatomy of the general surface, forebody glands, digestive system and reproductive organs. Two types of general surface, forebody gland cell have been recognized in the head region and their possible functions are discussed. The anterior alimentary tract is provided with three types of gland, namely prepharyngeal, pharyngeal and oesophageal glands. Possible functions of these glands have been discussed. The reproductive system is studied in detail and possible functions of some of its internal organs are discussed.

Key words: Redescription, *Astiotrema reniferum*, Trematoda, catfish, Egypt

Introduction

During the course of studying the digenean fauna of the Egyptian freshwater fishes, digeneans belonging to the genera *Astiotrema* Looss, 1900, *Orientocreadium* Tubangui, 1931 and *Eumasenia* Srivastava, 1951 were collected from the alimentary tract of the catfish *Clarias lazera* Cuvier and Valenciennes. One of them, *Astiotrema reniferum* (Looss, 1898) Stossich, 1904 will be described in this paper; the others are being prepared for publication in separate papers. Previous descriptions of the digeneans of the genus *Astiotrema* (See Beverly-Burton 1962; Fischthal and Kuntz, 1963; Imam, 1971; Moravec, 1977) are incomplete since only the shapes and sizes of the parasite and some of its internal organs were described. Therefore, the present study was extended to include a detailed study of the parasite particularly the general surface, forebody glands, the digestive system and the reproductive organs. The term, general surface, forebody glands, is used in this study to describe the gland cells lying in the head region and opening to the exterior through its general (non-specialized) surface and also to differentiate between these cells and others that supply their secretion to specialized organs (lappets and adhesive organs) in the head.

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Materials and Methods

The specimens of the catfish *Clarias lazera* were collected from Manzala Lake and from the Demietta branch of the River Nile, Dakahlia Province, Egypt. They were maintained, until required, in tanks containing circulating river water. In order to collect the parasites from the alimentary tract, fishes were killed by a blow on the head. The alimentary tract was removed and placed in 0.06% saline solution. The parasites were dislodged using a fine needle under a stereomicroscope. For studying the anatomy of the parasite, some living specimens were transferred in a drop of water to a clean slide, carefully flattened with a coverslip and examined with phase-contrast and oil immersion optical equipment. Other specimens were flattened under a coverslip, preserved either in 10% formaldehyde or Bouin's fixative, stained with alum carmine, dehydrated in ethyl alcohol, cleared either in xylene or terpineol, mounted in Canada balsam and examined with oil immersion optical equipment. Some unflattened specimens were preserved in 2.5% buffered glutaraldehyde, post

fixed in 1% osmium tetroxide,, dehydrated in ethyl alcohol and embedded in Spurr resin. Semithin sections of the resin-embedded specimens were cut at 0.5–1 μm and stained in 1% solution of toluidine blue in 1% borax; other sections were stained in 3% basic fuchsin.

Results

Fourty to fifty living specimens have been studied. The average dimensions of ten, flattened adult specimens were as follows: length 2.20 (1.40–2.70) mm and breadth 0.35 (0.32–0.45) mm. The anterior region is nearly rounded while the posterior region is narrow and forms a stalk terminating with two lobes. The oral sucker is subterminal, circular in outline and measures 0.22 (0.15–0.25) mm × 0.21 (0.15–0.24) mm (Fig. 1). The ventral sucker is rounded and lies at about 0.49 mm from the oral sucker. It measures 0.21 (0.17–0.23) mm × 0.20 (0.14–0.23) mm. Living parasites have been seen moving on the bottom of the glass dish in a leech-like manner using the head region in conjunction with the posterior body lobes. Moreover, the head region was seen extending and moving in all directions whenever the posterior body lobes are fixed. The parasite is capable of extending its body twice its normal length.

The general surface, forebody glands:

Two kinds of uninucleated gland cell supply secretion onto the general body surface of the head region, one producing relatively large oval to spherical bodies (A1) and the other producing smaller spherical bodies (A2) (Fig. 1). The G1 cells producing A1 bodies are dorsally located and arranged in two groups in each lateral region of the head. The anterior group comprises 6–8 cells and lies at the level of the posterior region of the pharynx while the posterior group is composed of 9–12 cells and lie at the level of the oesophageal region and intestinal bifurcation. The ducts of the G1 cells are long and narrow and run in an anterior direction where each duct dilates forming relatively large reservoir before opening onto the general body surface in the region of the oral sucker. The ducts opening on the ventral and lateral surfaces are more numerous than those opening on the dorsal surface of the head. The G2 cells producing A2 bodies distribute singly and most of them lie ventrally and extend from the level of the anterior region of the ventral sucker to the level of the oral sucker. There are 80–100 G2 cells in the head region and each cell is pear-shaped and opens immediately onto the ventral surface of the head via a short narrow duct. On some occasions, a few G2 cells opens onto the dorsal surface of the head.

The digestive system

The mouth opens into a buccal cavity which is lined with a relatively thin tegumental layer. In sections, the prepharynx has a branched lumen and its epithelial lining possesses numerous cytoplasmic processes (Fig. 1). The prepharyngeal glands lie dorsally, four cells on each side of the posterior region of the pharynx and produce relatively small, spherical bodies. The pharynx is oval in shape and measures 0.09 (0.08–0.10) mm × 0.11 (0.10–0.11) mm. Its anterior region protrudes forming four lobes, one dorsal, one ventral and two lateral (Fig. 1). The ventral lobe appears larger than the others. There is no evidence of surface processes in the epithelial lining of the pharyngeal lumen. The muscle fibres of the pharynx are arranged in three directions: circular, longitudinal and radial. The circular and longitudinal muscle fibres are located beneath the epithelial lining of the pharyngeal lumen and also surround the outer surface of the pharynx while the radial muscle fibres extend between the inner and outer surfaces of the pharynx. Gland cells producing roughly spherical bodies are found in-between the radial muscle bands. The ducts of these glands could not be traced but it seems likely that they open into the pharyngeal lumen. The oesophagus measures 0.07 (0.05–0.08) mm and oesophageal glands which include 10–12 cells lie ventrally in the median region of the body posterior to the level of the intestinal bifurcation. They produce small spherical bodies and their ducts open into the oesophageal lumen close to its communication with the pharynx. The two intestinal limbs dilate at their posterior region and

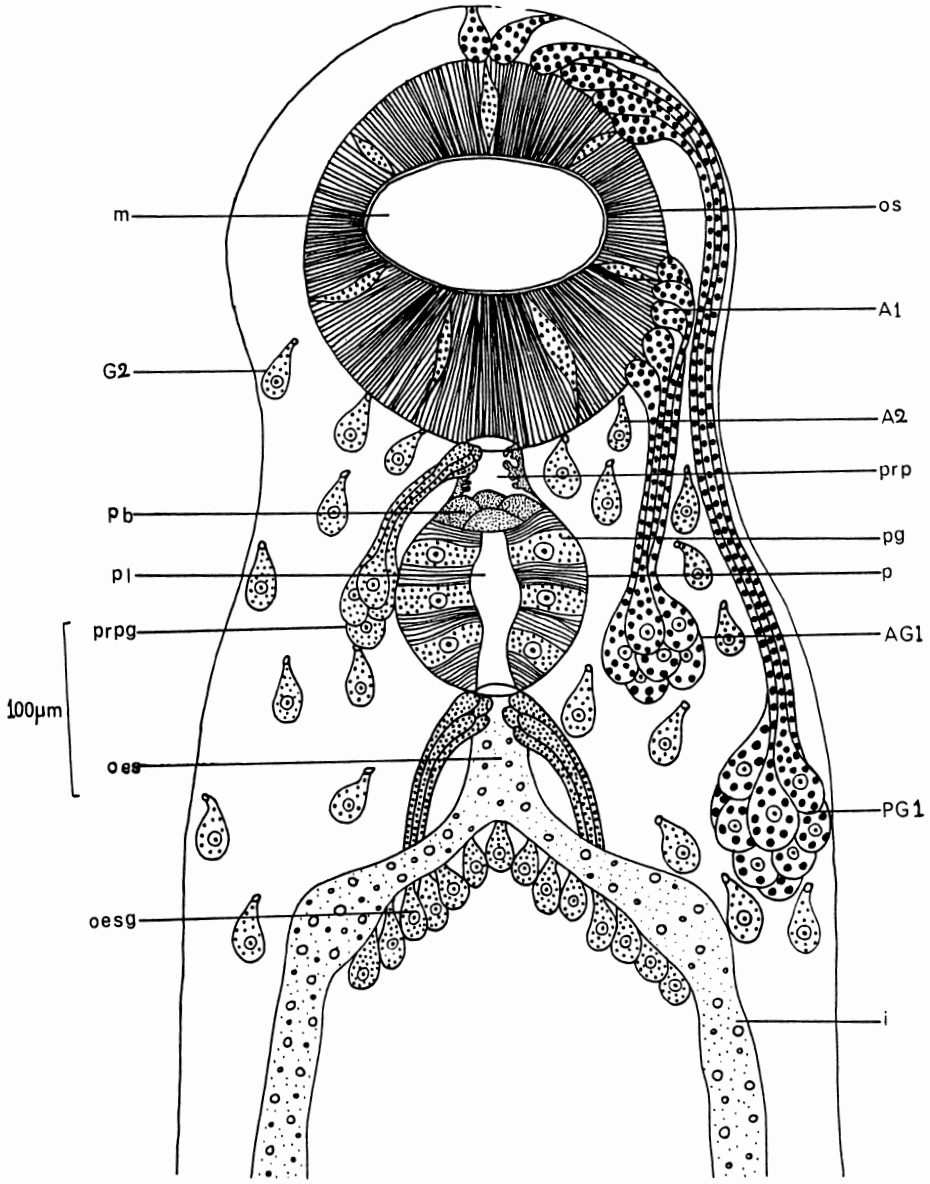

Fig. 1 Schematic drawing of the head region of *A. reniferum,* in a ventral view, showing details of the general surface forebody glands and anterior alimentary tract and associated glands. For the sake of clarity, the G1 cells and prepharyngeal glands of only one side are included. A1, large spherical bodies, A2, small spherical bodies; A G1, anterior group of the G1 cells; G1, gland cell producing large spherical bodies; G2, gland cell producing small spherical bodies; i, intestinal limb; m, mouth opening; oes, oesophagus; oesg, oesophogeal glands; os, oral sucker; p, pharynx; pb, pharyngeal lobes; pl, pharyngeal lumen; PG1, posterior group of the G1 cells; pg, pharyngeal glands; prp, prepharynx; prpg, prepharyngeal glands.

end blindly. However, the left intestinal limb extends more posteriorly than the right limb. The microvillus luminal intestinal epithelium is nucleated and contains small and large granular contents. Similar contents were frequently seen in the lumen of both pharynx and prepharynx. No evidence has been found of blood pigment in the alimentary tract of *A. reniferum*.

The Reproductive System.

The two rounded testes are tandem in position and situated in the posterior half of the body between the intestinal limbs (Fig. 2). The posterior testis is slightly larger than the anterior one and measures 0.18 (0.16–0.25) mm × 0.15 (0.14–0.19) mm while the anterior testis measures 0.16 (0.15–0.24) mm × 0.13 (0.10–0.20) mm. The vasa efferentia penetrate the wall of the cirrus pouch separately and open individually into the posterior region of the vesicula seminalis. The claviform cirrus pouch shows a clear variability in its position; in some specimens, it lies in the left side of the body anterior to the ovary while in others it lies in the right side adjacent to the ventral sucker (Fig. 2). In both cases, the cirrus pouch runs anteriorly along the lateral side of the ventral sucker where it opens into the genital atrium which opens ventrally in the right region of the body adjacent to the anterior extremity of the ventral sucker. The wall of the cirrus pouch is highly muscular. The vesicula seminalis is divided by a conspicuous constriction into two chambers; an anterior small chamber and a posterior extremely larger chamber (Fig. 2). The muscular wall of the anterior chamber is thicker than that of the posterior chamber. Spermatozoa were frequently observed in both chambers, but in few cases, they were absent in the anterior chamber. Spermatozoa were occasionally seen moving from the posterior chamber to the anterior one. The unicellular male accessory glands fill the space between the wall of the cirrus pouch and its internal organs (Fig. 2). Their ducts apparently open into the lumen of the pars prostatica. The elongated cirrus accommodates a narrow ejaculatory duct and its wall is composed of distinctive circular muscle fibres. The anterior region of the cirrus is funnel-shaped when protruding from the opening of the genital atrium. In most specimens examined large masses of unknown material and spermatozoa are seen in the pars prostatica and in the ejaculatory duct.

The ovary is oval to pear-shaped and measures 0.14 (0.10–0.24) mm × 0.13 (0.11–0.20) mm. It lies ventrally on the left side of the body behind the cirrus pouch. The anterior region of the ovary extends as a pointed process from which arises the oviduct (Fig. 2). There is an evidence to suggest the presence of a sphincter between the pointed end of the ovary and the oviduct. The anterior end of the oviduct joins the ducts of the receptaculum seminis and Laurer's canal after receiving a small duct from the vitelline reservoir. At this point of communication, arises the ootype which extends posteriorly as a uterine tube. In living parasites, the epithelial lining of the ootype lumen possesses a large number of ciliary structures which have been seen beating. Living sperms, oocytes and vitelline cells were seen in the ootype lumen. Mehlis' glands which comprise 10–12 cells distribute around the dilated portion of the ootype and produce small, spherical bodies. The receptaculum seminis lies ventrally in the left side of the body just posterior to the ovary and measures 0.12 (09–0.14) mm × 14 (0.10–0.15 mm). There is a conspicuous constriction which may represent a sphincter between the receptaculum seminis and its duct (Fig. 2). Living sperms have been seen moving from the receptaculum seminis into the oviduct. The Laurer's canal is relatively long and opens dorsally in the region overlying the vitelline reservoir. The vitelline follicles extend from the level of the ventral sucker to a short distance behind the posterior testis. They were not observed in-between the intestinal limbs. A transverse vitelline duct, from each side, opens into a dorsally located vitelline reservoir. In mature specimens, the uterus fills the intercaecal space as a convoluted tube which extends backward to the posterior edge of the body. The metraterm is a relatively long tube which runs forwards and opens into the genital atrium. The uterus is filled with operculated, oval-shaped eggs; each measures 0.032 (0.030–0.036) mm in

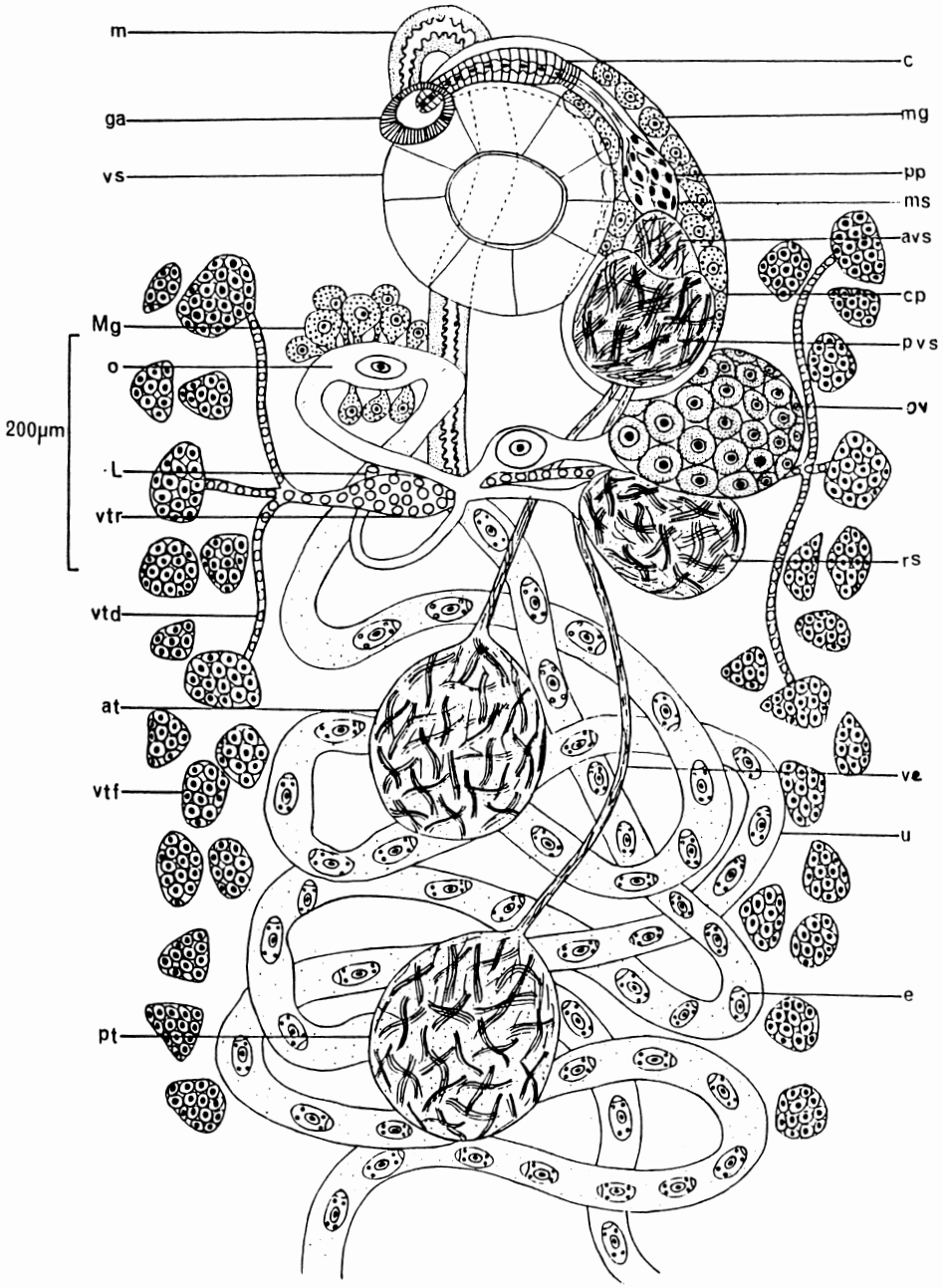

Fig. 2 Schematic drawing showing details of the reproductive system of *A. reniferum* in a ventral view. at, anterior testis; avs, anterior chamber of the vesicula seminalis; c, cirrus; cp, cirrus pouch, e, egg; ga, genital atrium; L, Laurer's canal; m, metraterm; Mg, Mehlis' glands; mg, male accessory glands; ms, masses of unknown material; o, ootype; ov, ovary; pp, pars prostatica; pvs, posterior chamber of the vesicula seminalis; pt, posterior testis; rs, receptaculum seminis; u, uterus; ve, vas efferens; vs, ventral sucker; vtd, vitelline duct; vtf, vitelline follicle; vtr, vitelline reservoir.

length and 0.017 (0.016–0.020) mm in breadth.

Discussion

The present study has revealed that the pattern of the general surface, forebody glands in *A. reniferum* is different from that of the corresponding glands in other digeneans. Two kinds of these glands have been recognized in *A. reniferum,* one producing relatively large, oval to spherical bodies and the other producing smaller spherical bodies. Only one kind of general surface, forebody glands was described in the genera *Haplometra* and *Opithioglyphe* by Halton and Dermott (1967), in *Apatemon gracilis minor* by Erasmus (1969a), in *Diplostomum phoxini* by Erasmus (1969b), in *Aspidogaster conchicola* by Halton and Lyness (1971) and in *Microphallus similis* by Davies (1979).

Although the chemical composition of the secretory bodies produced by the G1 and G2 cells has not yet been determined, it seems likely that the two kinds of gland cell are functionally different since their distribution pattern and the morphological appearance of their secretory bodies are different. The presence of the G1 duct openings in the region around the oral sucker suggests that the secretions of these cells is functionally integrated with the feeding and attachment mechanisms performed by the oral sucker. This is consistent with the observation that the parasite uses the anterior head region in conjunction with the posterior body lobes to move temporarily in a leech-like manner over the bottom of the glass dish. The secretion of the G1 cells may also play a role in the extracellular digestion of the host tissues (see Smyth and Halton, 1983). The forebody surface of *A. reniferum,* particularly the ventral surface, is an important host-parasite interface since it comes into close contact with the host tissues during permanent and temporary attachment as well as during feeding. It is possible that the A2 bodies released into the ventral surface of the worm serve to suppress the immune response of the host. In *Microphallus similis,* Davies (1979) suggested that the cholinesterase secreted over the surface of the forebody serves to compensate for the irritating effect of the toothed spines by neutralizing host acetylcholine and reducing the movement of the villi in the immediate vicinity of the fluke thereby reducing the likelihood of dislodgment and expulsion.

Halton and Stranock (1976) stated that the anterior alimentary tract of the digeneans is generally non glandular. This is not true in *A. reniferum* where the anterior alimentary tract is supplied with prepharyngeal, pharyngeal and oesophageal glands. Only one kind of prepharyngeal gland cell could be identified in *A. reniferum.* Similar prepharyngeal glands of only one type were described by Halton et al. (1974) in the blood feeding monogenean *Diclidophora merlangi.* They suggested that the secretion plays a role in the extracellular digestion of the blood. *A. reniferum* appears to be a tissue feeder since no blood pigments were observed in its alimentary tract. Therefore, the secretion of the prepharyngeal glands of *A. reniferum* may have a role in the extracellular digestion of the host's tissues.

The pharynx of *A. reniferum* contains gland cells dispersed between the radial muscle fibres and producing spherical bodies. It appears that this is the first record of pharyngeal glands in digeneans although they have been frequently described in monogeneans e.g. *Fntobdella soleae, Polystoma malayi* and *Macrogyrodactylus clarii* (See Kearn, 1963; Rohde, 1974; El-Naggar and Serag, 1987, respectively). The chemical composition and possible functions of the pharyngeal gland secretion of *A. reniferum* are not known. However, Kearn (1963) found that the contents of the pharyngeal glands of *E. soleae* have a proteolytic activity and probably serve to digest the epidermis of the host. El-Naggar and Serag (1987) suggested that the pharyngeal gland secretion in *M. clarii* aid in digestion of the host's gill tissues.

Oesophageal glands have been described in a few digeneans e.g. *Microphallus similis, Aporocotyle simplex* and *Schistosoma mansoni* (see Davies, 1979; Thulin, 1980; Bogitsh and Carter, 1979 respectively) and in some monogeneans e.g. *D. merlangi* and *Calicotyle kroyeri* (see Halton *et al.*, 1974; Halton and Stranock, 1976, respectively). The chemical composition

and possible functions of these glands in *A. reniferum* are not known. However, in *C. kroyeri* the oesophageal gland secretion was suggested to contain enzymes necessary for the initial extracellular phase of digestion (see Halton and Stranock, 1976).

Although there are extensive unicellular male accessory glands in *A. reniferum,* they produce only one kind of secretion. In this respect, they resemble the corresponding glands in the digeneans *Fasciola hepatica* and *Aporocotyle simplex* (see Threadgold, 1975 and thulin, 1980, respectively). However, two types of secretion were reported in the so-called prostate glands in the digeneans *Paramphistomum epiclitum* and *Paradistomoides orientalis* (see Kanwar and Kansal, 1980), *Paramphistomum cervi* (see Gupta *et al.*, 1983) and *Quinquserialis quinqueserialis* (see Wittrock, 1986). In many specimens of *A. reniferum* large masses of unknown material are found in the pars prostatica and in the ejaculatory duct. The origin of this material is not known but it seems likely that it represents coalescence of male accessory secretion. Possible functions of the male accessory gland secretions in digeneans include: stimulation of spermatozoa at the time of copulation by providing a fluid medium for their transfer, provision of an additional energy source to the spermatozoa and stimulation of the female reproductive tract during copulation (Threadgold, 1975; Halton and Hardcastle, 1977).

A characteristic feature of the vesicula seminalis of *A. reniferum* is the presence of a conspicuous constriction between the anterior and posterior chambers. It is not known whether the constriction is structurally and functionally a sphincter. However, spermatozoa were frequently observed in the two chambers but in few cases, they were absent in the anterior chamber. This may indicate that the constriction is a sphincter which controls the passage of spermatozoa from the posterior chamber to the anterior one.

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