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ECHINOCHASMUS, NEW SPECIES (TREMATODA: ECHINOSTOMATIDAE) FROM EGYPT

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Abstract

In this study a new species of *Echinochasmus* (Dietz, 1909) is recorded for the first time in Egypt. Life cycle of one of gymnocephalous cercariae procured from *Melanoides tuberculata* snail was successfully completed in the laboratory. A total of 407 *Melanoides tuberculata* snails were collected from Mansouriya Canal, Giza Governorate. They were individually exposed to artificial light to determine natural infection with trematode larvae. Seven snails were found infected with gymnocephalous cercariae (infection index of 1.71). These cercariae were used to infect *Gambusia affinis* fish as second intermediate host. The infected gills were given to clean laboratory bred *Rattus norvegicus* as experimental final host. Adult worms were obtained ten days post-infection from rats' intestine identified as *Echinochasmus* after accurate comparison with standard keys. The diagnostic morphology of developmental stages were given.

Keywords: *Melanoides tuberculata, Echinochasmus*, morphology, experimental infection.

Introduction

Eleven different types of cercariae procured from *Melanoides tuberculata* (Müller, 1774) were discovered from Egypt (Yousif *et al*, 2010). They belong to four main type groups, namely pleurolophocercous cercariae (4 types), xiphidiocercariae (2 types), gymnocephalous cercariae (3 types) and furcocercous cercariae (2 types). To identify these trematodes the life cycle should be completed. By completion the life cycle of one of the gymnocephalous cercaria it was found to be very close to *Echinochasmus* (family: Echinostomatidae) Dietz, 1909. Echinostomatidae is a large family of flukes of birds and mammals. It is distinguished by small size, elongated spinose with a reniform collar, armed with a single or double row of spines surrounding the dorsal and lateral margins of the oral sucker (Soliman, 1996). Out of the flukes of Echinostomatidae, genus *Echinochasmus* Dietz, 1909 has vitellaria extending to posterior margin of ventral sucker, head crown armed with collar spines dorsally interrupted (Yamaguti, 1958).

The number of collar spines is different for each species. The species which has 24 collar spines are *Echinochasmus japonicus* is known to utilize *Parafossarulus striatulus* snail in Korea (Choi *et al*, 2006) and Vietnam (Besprozvannykh *et al*, 2013); *E. liliputanus* use *Bellamya aeruginosa* snail in China (Xiao *et al*, 2000); *E. bagulai* use *Natica marochiensis* snail in India (Ramalingam,1960); *E. belocephalus* in Europe use *Bithynia tentaculata* (Besprozvannykh, 2009) *E. macrocaudatus* has 22 spines and *E. leopoldinae* has 20 use *Pyrgophorus coronnatus* snail in Mexico (Ditrich *et al*, 1996; Scholz *et al*, 1996). *Melanoides tuberculata* was recorded as first intermediate host for 2 species, *E. japonicus* in China (Cheng and Fang, 1989) and *Echinochasmus milvi* in Iran (Farahnak *et al*, 2005); This trematodes found naturally in the gills of many fish species as second intermediate host in various countries such as *E. mohiuddini* has 24 spines (Dharejo *et al*, 2007), 26 spines in *E. passeri* (Dharejo *et al*, 2010) *E. mazharuddini* has 28 spines (Ujan *et al*, 2014) in Pakistan. *E. liliputanus* was recorded in Egypt as intestinal fluke in dogs (El Gayar, 2007) and *E. aspinuosa* new species in herons in Giza and Sharkia Governorate. (Yassin *et al*, 2011)

Tanabe (1926) commented the possibility of human infection of *E. japonicus*. In

Korea, Seo *et al*. (1985) recorded 4 human cases of natural infection, while Chai and Lee (2002) studied on food-borne intestinal trematodiasis reported *E. japonicus* from human. Sayasone *et al*. (2009) recorded 3 cases of the same species in Lao PDR. Human natural infection with *Echinochasmus liliputanus* was found for the first time from the inhabitants of Hexian country of Anhui Province in China through drinking unboiled water containing *E.liliputanus* cercariae or eating raw fish containing the metacercaria (Xiao *et al*, 1992; 1995; 2005). Therefore, medical attention has been paid to this fluke.

The aim of the present work was completion of the life cycle of gymnocephalous cercaria procured from *Melanoides tuberculata* for proper identification of the trematode.

Material and Methods

A total of 407 of the prosobranchial, *M. tuberculata* snails Müller (Thiaridae) (Fig. 1) and a total 125 fresh water *Gambusia affinis* fish were collected from Al Mansouriya main canal and its branches, Giza Governorate from May to July 2015 using a dip nets and sieved through 0.25 inch mesh screening. Four albino rats, *Rattus norvegicus*, 4-6 weeks old (180-200g) were obtained from the Animal House at Theodor Bilharz Research Institute (TBRI). The snails and fish were transported to the laboratory where they distributed in several separate aquaria provided with dechlorinated tap water. The aquaria were maintained in air condition room under a water temperature 24±1°C. Snails were fed on blue green algae (*Nostoc muscorum*) from Schistosome Biological Supply Centre (SBSC), at (TBRI). The aquaria containing fish were aerated and provided with fish food "tetramin". The snails were examined twice weekly for cercarial shedding by putting them individually in wells containing 3ml dechlorinated water each and exposed to artificial light (60 W) at 30°C for 2 hr. The procured cercariae belonging to the same type were collected by a pipette using a steromicroscope to complete the life cycle of the parasite. Some of them were stained by natural stain (Nile Blue Sulphate and Neutral Red) for examination. Some of the infected snails with this type of cercariae were crushed and rediae obtained.

To confirm the conspecificity of cercariae with metacercariae from naturally infected fish, infection-free a *Gambusia affinis* fish were kept in small aquaria (5 liters) and 30-65 cercariae /fish were added to water. After exposure (24hrs) they were maintained in large aquaria (24x36x18cm) at 22-25°C fed tetramin. They were examined successively for presence of metacercariae in the gills which were detached. The fish's gills were examined 3, 6, 9, 12 & 18 days post-infection. The cercariae, rediae and metacercariae stages were pressed between two slides and cover to examine.

Rattus norvegicus rats (definitive hosts) were feed the infected gills with metacercariae and reared in the Animal House at TBRI. On the 10^{th} day post infection they were dissected and their small intestines were opened longitudinally in a petri dish containing phosphate buffer saline (pH 7.40) and examined for worms using a stereomicroscope. They were washed in distilled water, and preserved in 70 % ethanol. Whole-amount preparations were produced by staining specimens with borax carmine, dehydrating the worms in a graded ethanol series and clearing in xylene, followed by mounting in Canada balsam under a coverslip on a glass slide. All measurements are given in micrometers (µm) mean for 10 specimens of the total body size and several structures such as oral sucker, acetabulum, pharynx, ovary and testes_(Tab. 1). They were photo by digital camera Canon A640 and drawn with camera Lucida. Adult worms were washed well with phosphate buffer saline and fixed in 2.5% glutaraldehyde and processed for further examination by Scanning Electron Microscope SEM (inspect S, FEI Company, Holland), according to a standard method (Shaw and Erasmus 1987).

Results

Morphological description of larval stages and adult worm

Cercaria (Fig. 2a&b): Seven *Melanoides tuberculata* from 407 were found infected with gymnocephalous cercaria (infection index of 1.71%). Body of cercaria is oval 392x178µm (300-500x130-230). Oral sucker and acetabulum are circular, oral sucker is smaller than acetabulum 40µm (38-42), acetabulum lies almost in the middle of the body 53 µm (41-62). Head crown and collar spines, characteristic of adult worms, are not recognizable around the oral sucker. The gut includes a prepharynx 23µm (21-25) in mean length. A muscular pharynx 36x19 µm (29-40x13-23) follow by a long oesophagus 68µm. Bifurcation of gut extends posteriorly to the end of the body.

Excretory vesicle is small kidney shaped, located at the posterior end of the body. Two excretory ducts extend along both sides of cercaria and drain in the excretory vesicle. They contain characteristic large granules and in the tail. Tail is almost equal length of the body 374x43µm (354-395x39-47) ends with a small spine.

Cercaria has a slow motion; while it swims in circles and its tail lashes on both sides

Redia (Fig. 3): It is 786 µm (600-890) in length and 140 µm in width (110-180). The mouth lies at the anterior end of the body, the pharynx is well developed subterminal 73x84µm (60-86x78-93) leading to a short oesophagus which leads to a wide intestine which extends till the middle of the redia. The germ cells are scattered at the posterior end. A locomotary process is found near the end of the redia.

Metacercariae (Fig. 4): Metacercariae were found only in the gill lamellae at day 9 post infection. They were fully encysted. They are oval, measuring 108 x 93µm (98-120 X 66-112). The outer wall is thick and tough while inner is thin. Oral sucker is sub-terminal circular 31µm (25-37) and acetabulum is larger than oral one 45.5µm (35-56) at the beginning of 2 3 of the body. The excretory vesicle widely elongated posterior to the acetabulum, received two main longitudinal ducts, each with 9-12 differently-sized excretory granules near the middle of the post-acetabular space.

The morphology of adult worms (Fig. 5a-e, Tab. 1):

Body elongated, slightly tapered at both side 995x309 µm (850-1200x270-340) (Fig. 5a&b). Anterior 1/4 of dorsal surface and ventral surface of the body covered with large scale-like tegumental spines. Oral sucker is small and subterminal 51x69 µm (46-56x 64-72). Acetabulum is almost sepherical situated at the end of 1/3 of anterior body measures 100x 108 µm (98-102x100-120).

The collar reniform 133x311µm (100-160x280-340) with 24 spines (Fig. 5c) interrupted dorsally and ventrally. Three in both side arranged in one row dorsally, 14 µm in length. They are in different direction. The lateral spines are five arranged in one row 12µm, first one of this group is the smallest spine 5µm in length. In the ventral, four are alternating with one another the outer spines are longer than the inner one, inner is (8 µm) and outer row is (10 µm). Prepharynx is short 33µm (30-35µm) and pharynx is circular or elliptical, strongly muscular 53x44 µm (45-60-x40-50). Oesphagus is long 105x30 µm (85-120x24-38), widened anteriorly, forming funnel shape. Intestinal caecae are long, straight, extending close to posterior end 69x20 µm (60-80x18-22). Testes tandem, entire unlobed nearly spherical, anterior testis larger than posterior one 122x138 µm (100-140x110-170) and posterior one is 99x94 µm (90-120x84-140). Cirrus sac is large thick-walled situated dorsally behind oesphageal bifurcation and slightly overlapping anteriorly acetabulum 100x82 µm (85-115x72-92). Seminal vesicle is large 67x55µm (63-72x51-60) and cirrus short 16x30 µm (10-21x27-34). Genital pore situated posterior to caecal bifurcation. Ovary is oval in shape 122x64µm (110-134x50-76 µm) situated to the right of the median line

of body post-lateral of acetabulum. Vitelline follicles extend from middle level of acetabulum and extend to caeca up to the hinder border of the posterior testis. Vitelline reservoir is large, triangular close to anterior testis. Excretory vesicle Y shaped and branch at the end.

Discussion

In the present study, a new species of *Echinochasmus* was recorded during parasitological survey in Egypt. Cercaria was recovered from *Melanoides tuberculata*, the 1st intermediate host (Yousif *et al*, 2010). The life cycle was successfully completed in the laboratory through *Gambusia affinis* and *Rattus norvegicus* as experimental second and final intermediate hosts respectively for the first time in Egypt. The identification of the obtained worm was made depending on showing the main characters of *Echinochasmus*. It has vitellaria extending to posterior margin of ventral sucker, head crown armed with collar spines dorsally interrupted (Dietz, 1909). Number of collar spines is different for each species. The present species, *Echinochasmus* has 24 collar spines. Other than *Echinochasmus* n.sp, in Egypt was recorded (El-Gayar, 2007), *E. liliputanus* infected dogs in Ismailia with 24 collar spines and *E. aspinuosa*, in heron in Giza and Sharkia Governorate with 22 collar spines (Yassin *et al*, 2011).

The worldwide distribution of this genus is due to fact that members use different snails for each species as 1st intermediate host. Genus *Echinochasmus* included a large number of species with 24 collar spines, *E. japonicus* Tanabe, 1926, in Lao PDR, Korea and Vietnam (Sayasone *et al*, 2009; Choi *et al*, 2006, 2013); *E. liliputanus* (Looss, 1896) Odhner, 1911 in China (Xiao *et al*, 1992); *E. bagulai* Verma, 1935 (Ramalingam, 1960) in India; *E. mohiuddini* Dharejo *et al*, 2007 in Pakistan and *E. beleocephalus* (Linstow, 1873) in Europe (Skrjabin and Baschkirova, 1956; Besprozvannykh *et al*, 2009). However, they have different measurements and other items of this parasite from different species as well as of specimens of the present study.

In the present work, cercaria is characters by the ventral sucker lies almost in middle of body, tail is almost equal length with its body ends with a small spine. Cercaria of *E. japonicus*'s tail is shorter than its body (Choi *et al*, 2006) and in *E. bagulai* tail is 1/3 of body length (Ramalingam, 1960). Tail of the latter 2 species is without spine.

Adult worms were recovered from intestine of *Rattus norvegicus*, which experimentally infected is differ from the most closely related species. It is larger than *E. japonicus* almost double. Tegumental spine is scale-shaped cover anterior 1/4 of dorsal side of worm, collar spine has characteristic size and arrangement, the acetabulum is situated at end of first third of the body length and excretory vesicle is Y-shaped and branch at the end. Funnel shape of oesophagus is characteristic for the present work. Anterior testis is larger than posterior one and this is different from the other *Echinochasmus* species. As compare with, *E. japonicus* the tegumental spine is spade-shaped and covers the anterior half of dorsal surface, 4 spines of oral spines were in different direction and the acetabulum is in the beginning of the anterior half of body (Lee *et al*, 1987). *E. liliputanus* most of body surface covered with spines except collar, acetabulum and body posterior aspect (Thaddeus *et al*, 2007). *E. aspinuosa* new species in Egypt has 22 collar spines (Yassin *et al*, 2011).

Conclusion

The perusal the literature would reveal that the present species has considerable differences between the cercariae and adults of this species and other *Echinochasmus* species possessing 24 collar spines (*E. japonicus, E. liliputanus, E. bagulai, E. mohiuddini* & *E. beleocephalus*). The former taxon is considered to represent a new species.

Echinochasmus is undoubtedly an important zoonotic parasite in Egypt. The presence of metacercariae in the fish's gills may affect its life span, and medical importance

since it is recorded as intestinal fluke of man as mentioned before on other species. It could also infect man by contaminated water with cercaria (Xiao *et al*, 1992; 1995; 2005).

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References

Besprozvannykh, V, 2009: Life cycle of trematodes *Echinochasmus japonicus* and *E. belocephalus* (Echinostomatidae) in conditions of Primorsky Region. Parazitol. 43, 3:248-58.

Besprozvannykh, VV, Ngo, HD, Ha, NV, Hung, NM; Rozhhovan, KV, *et al,* 2013: Description of digenean parasites from three snail species, *Bithynia fuchsiana* (Morelet), *Parafossarulus striatulus* Benson and *Melanoides tuberculata* Müller, in North Vietnam. Helminthol. 50, 3:190-204.

Chai, JY, Lee, SH, 2002: Food born intestinal trematode infections in the Republic of Korea. Parasitol. Int. 51:129-54.

Cheng, YZ, Fang, YY, 1989: The discovery of *Melanoides tuberculata* as the first intermediate host of *Echinochasmus japonicus*. Zhongguo Ji Sheng Chong Xue Yu Ji Sheng Chong Bing Za Zhi.7, 1:47-8.

Choi, M, Kim, S, Chung, J, Jang, H, Eom, J, *et al,* 2006: Morphological observations of *Echinochasmus japonicus* cercariae and the in vitro maintenance of its life cycle from cercariae to adults. J. Parasitol. 92, 2:236-41.

Dharejo, A, Bilqees, F, Khan, M, 2007: *Echinochasmus mohiuddini*, new species (Trematoda: Echinostomatidae) from Paddy Bird *Ardeola grayii* (Ardeidae) of Hyderabad, Sindh, Pakistan. Pakistan J. Zool. 39, 5:285-8.

Dharejo, A, Birmani, N, Khan, M, 2010: *Echinochasmus passeri*, new species (Digenea: Echinostomatidae) from Gallbladder of House Sparrow, *Passer domesticus* (Aves: passeridae) of Hyderabad, Sindh, Pakistan. Proc. Parasitol. 50:139-45,

Ditrich, O, Scholz, T, Vazquez, J, 1996: life-cycle of *Ecinochasmus macrocaudatus* n. sp. (Trematoda: Echinostomatidae). Syst. Parasitol. 33:225-35.

El-Gayar, A, 2007: Studies on some trematode parasites of stray dogs in Egypt with a key to the identification of intestinal trematodes of dogs. Vet. Parasitol. 144:360-5

Farahnak A, Setodeh, S, Mobedi, I, 2005: A Faunistic survey of cercariae isolated from *Melanoides tuberculata* and their role in transmission diseases. Arch. Razi Ins. 59:113-9

Lee, S, Sohn, W, Hong, S, 1987: Scanning electron microscopic findings of *Echinochasmus japonicus* tegument. Kor. J. Parasitol. 25, 1:51-8.

Ramalingam, K, 1960: The morphology and life history of *Echinochasmus bagulai* Verma, 1935 (Trematoda: Echinostomatidae) with ecological observations on its larval forms. J. Mar. Boil. Assoc. India 2, 1:35-50.

Sayasone, S, Tesana S, Utzinger, J, Hatz, C, Akkhavong, K, *et al,* 2009: Rare human infection with the trematodeEchinochasmus japonicusin Lao PDR. Parasitol. Int. 58:106-9.

Schloz, T, Ditrich, O, Vazquez, A, 1996: *Echinochasmus leopoldinae* n. sp. (Trematoda: -Echinostomatidae) and data on its life-cycle. Syst. Parasitol. 33:157-65.

Seo, BS, Lee SH, Chai, JY, Hong, SJ, 1985: Studies on intestinal trematodes in Korea XX. Four cases of natural human infection by *Echinochasmus japonicus*. Korean J. Parsitol. 23, 2: 214-20

Shaw, MK, Eramus, DA, 1987: *Schistosoma mansoni* structural damage and tegumental repair after in vivo treatment with Praziquantel. Parasitol. 94:243-54.

Skrjabin, KI, Baschkirova, E, 1956: Family Echinostomatidae Dietz, 1909. In: Skrjabin K. I. (Ed.) [Trematodes of animals and man: Fundamentals of Trematodogy. Moscow: Akad. Nauk Publishing.

Soliman, G, 1996: Invertebrate Zoology: TheMideastern Invertebrate Fauna. Part I, Publisher: The Palm Press. First Edition,.

Tanabe, H, 1926: Studies on trematodes with fresh water fishes as their intermediate host III: On a new species *Echinochasmus japonicus* (n.s.). Nippon Byori Gakkai Kaishi. 16: 295-6.

Thaddeus, K. Graczy, K, Fried, B, 2007: Advances in Parasitology vol. 64:119-120.

Verma, SC, 1935: Studies on the Indian species of the genus *Echinochasmus.* Part I. and on an allied new genus *Episthochchasmus.* Proc. Ind. Acad. Sci. 1:837-56.

Ujan, H, Birmani, N, Shaikh, A, 2014: *Echinochasmus mazharuddini* n. sp. (Digenea: Echi-

nostomatidae) from the Bank Myna Acridotheres ginginianus L. (Passeriformes: Sturnidae) in Sindh province, Pakistan. J. Entomol. Zool. Stud. 2, 6:226-32.

Xiao, X, Lu, D, Wang, T, Gao, J, Zhu, C, *et al*, 1995: Studies on mode of human infection with *Echinochasmus liliputanus*. Zhongguo Ji Sheng Chong Xue Yu Ji Sheng Chong Bing Za Zhi. 13, 3:197-9.

Xiao, X, Wong, T, Lu, D, Gao, J, Xu, L, *et al*, 1992: The first record of human natural infection of *Echinochasmus liliputanus.* Chinese J. Parasitol. Parasit. Dis. 10:132-5

Xiao, X, Li, CT, Fang, GR, Wang, TP, Lu, DB, *et al*, 2000: Studies on biology of *Echinochasmus liliputanus* cercariae. Chin. J. Parasit. Dis.Control 13:199-204.

Xiao, X, Wong, T, Zheng X, Shen, G, Tian, Z, 2005: In vivo and in vitro encystment of *Echinochasmus liliputanus* cercariae and biological activity of the metacercariae. J. Parasitol. 91: 492-8.

Yamaguti, S, 1958: Systema Helminthum. Vol. I, Part I &II: The Digenetic Trematodes of Vertebrates International Science Publishers Inc., New York and London.

Yassin, M, El-Attar, N, Samn, A, 2011: *Echinochasmus aspinuosa* as a new record species in Egyptian heron. J. Egypt. Soc. Parasitol. 41, 1: 215-20.

Yamaguti, S, 1971: Synopsis of Digenetic Trematodes of Vertebrates. Parts I, II Tokyo: Keigaku Publishing Co.

Yousif, F, Abdalla, I, El Bardicy, S, Sleem, S, Ayoub, M, 2010: Morphology of new eleven cercariae procured from *Melanoides tuberculata* snails in Egypt. Aust. J. Bas. Appl. Sci. 4, 6:1482-94.

Table 1: Comparative characteristics of various species of genus *Echinochasmus* Dietz, 1909 in µm

Species	Present species	*E. japonicus* Tanabe, 1926	*E. liliputanus* (Looss, 1896)	*E. bagulai* (Verma, 1935)	*E. mohiuddini Dharejo etal., 2007*	*E. beleocephalus* (Linstow,1873)
1st IH*	*Melanoides tuberculata*	*Parafossarulus striatulus*	*Bellamya aeruginosa*	*Natica marochiensis*	-	*Bithynia tentaculata& Boreoelona ussuriensis*
2nd IH*	*Gambusia affinis*	*Pseudorasbora parva*	*Carassius auratus* Gold fish	Bivalve *Katelysia opima*	-	-
Body spines	1/4 dorsal body and all ventral side -Scales shaped	-1/2 dorsal body and all ventral side -spade shaped	Whole body except for the collar,acetabulum and the posterior aspect of the body	-	-	-
Oral spine	3 dorsal spines in different direction	4 dorsal spines in different direction				
Body length	850-1200	283-403	, 1519-2056	800-1460	780-1613	550 – 620
Body width	270-340	183-328	466.4-564	340-480	215-416	130 – 170
Collar spines	24	24 one row	24	24 arranged in 2 rows	24 single row	24
Oral sucker	46-56x64-72	35-43	107-148x102-148	45-64x	32-83x39-83	34-39x39-42
acetabulum	98-102x100-120	58-80	205-241x205-236	160-210	106-227x90-189	59-70x62-73
Pharynx	45-60x40-50	33-38x25-28	97-127	61-82x47-82	45-98x30-71	28-40x34-39
Esophagus	85-120x24-38	0.23	117-205	70-142	37-106	67-130
Cirrus sac	85-115x72-92	50	211.5-248.5 x 112.8-194.8	115-190x35-55	30-128x41-75	62-81x34-48
Ovary	110-134x50-76	28-50	72-92x76-97	45-75x36-61	30-90x26-106	34-42x31-50
Anterior testis	100-140x110-140	23-38x90-123	133-199x199-256	61-121x134-206	79-227x90-212	50-70x59-80
Posterior testis	90-120x84-140	25-50x78-113	179-251x164-246	76-182x107-200	109-280x90-204	50-70x59-80
Infected by	MC** in *Gambusia affinis* fish' gills	MC** in *Pseudorasbora parva* fish's gills	C*** in drinking un-boiled water& metacercariae in gold fish gills			

* IH: intermediate host MC**: Metacercariae, C***: Cercariae

Abbreviations

act. =Acetabulum, ant. te. = anterior testis, br. p.= birth pore, c.= cercaria, cd. ex. d. =caudal execratory duct, cir. =cirrus, cy. g.= cystogenous gland, ex. d.= execratory duct, ex.g.= execratory granule, ex. v.= execratory vesicle, g. b.= germ ball, int.= intestine, int. ce.= intestinal caecum, l.p. = locomotary process, m. o.= mouth opening, o. s.= oral sucker, o. sp.= collar spines, oes. = oesphagus, ov. = ovary, p.p.= prepharynx, post. Te. = posterior testis, s.v.= seminal vesicle, sp.= spine, t.= Tail and vit.= vitellaria

Explanation of figures

Fig. 1: Photo of *Melanoides tuberculata*., Fig. 2: Cercaria of *Echinochasmus* n.sp. a: camera lucida drawing. b: photo, Fig. 3: Redia of *Echinochasmus* n.sp.: camera Lucida drawing. Fig. 4: Metacercaria of *Echinochasmus* n. sp. photo, Fig. 5: Adult of *Echinochasmus* n. sp.: a-camera Lucida drawing. b- photo c-photo of collar spines d- SEM of adult worm. e- SEM oral sucker of adult worm showing collar spines and tegumental spines.