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REDESCRIPTION OF *PSEUDOLEPIDAPEDON BALISTIS* MANTER, 1940 AND A BIOLOGICAL VARIANT (TREMATODA: ACANTHOCOLPIDAE) FROM THE RED SEA FISHS; *BALISTOIDES VIRIDESCENS* AND *RHINECANTHUS ASSASI.*

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Abstract

During a survey of Red Sea fish parasites, two trematodes belonging to the genus *Pseudolepidapedon*Yamaguti, 1938 were encountered: *P. balistis*Manter, 1940 was found in the small intestine of the fish *Balistoidesviridescens* and was redescribed for the first time from Egypt; adding many detailed morphological and ultrastructural characters. The second trematode was found in the small intestine of the fish *Rhinecanthusassasi* and found to represent a biological variant of the previous species as it differs from it in its generally smaller dimensions, shape of suckers and pharynx and the testes. SEM details of the first species were described for the first time illustrating the differences in the spination and papillae on different parts of the body; which may be of taxonomic importance in recognizing future different species of the genus.

Keywords: *Pseudolepidepadonbalistis, Balistoidesviridescens,* biological variant, *Rhinecanthusassasi,* Red Sea fish

Introduction

Studies on the helminth parasites of the Red Sea fishes tend to be limited to short reports describing new taxa. Previous stud-ies on trematodes of the subclass Digenea in the region included the pioneer works of Nagaty between the 1930s and 1970s, Parukhin and Abdel-Aal in 1970s, Saoud and Ramadan in the 1970s; 1980s, Overst-reet in 1980s and Shalaby and Hassanaine since the 1990s (Reda*et al,* 2005).

The present study deals with two newly described trematodes belonging to family *Pseudolepidapedon*Yamaguti, 1938: AcanthocolpidaeLuhe 1906; from the Red Sea fishes.

Acanthocolpids are parasites of marine fishes, occasionally of sea snakes. They are characterized by spinous tegument, the lack of external seminal vesicle and the presence of a uterine terminal receptacle (Jones *et al,* 2005) and the concept of *Pseudolepidapedon*Yarnaguti, 1938 is based mainly on the type-species, *P. paralichthydis*Yamaguti, 1938

Materials and Methods

Fishes were caught from Sharm El-Naga (Safaga, Red Sea) and kept on ice until examined under a dissecting microscope. They were identified by keys of Lieske and Meyers (1994) and Randall *et al.* (1990). The entire alimentary canal and the surrounding peritoneal cavity were examined. Encountered worms were collected alive, washed in saline, fixed in cold AFA (alcohol-formalin-acetic acid) under slight cover glass pressure (Garcia andAsh, 1979), washed in distilled water, rinsed in 70% ethanol, stained with alum carmine, destained in diluted HCl, dehydrated in ascending concentrations of ethanol, cleared in clove oil, and mounted in DPX. Prevalence and worm burden were estimated according to definitions given by Bush *et al.* (1997). The measurements were expressed in micrometers. Drawings were prepared by the Camera Lucida (PZO 01852 10x). Ultra-

structure of the parasite was studied by Scanning Electron Microscope (SEM).

Results

1) *Pseudolepidapedon balistis* Manter, 1940 (Fig.1). Family: AcanthocolpidaeLühe, 1906. Subfamily: PseudolepidapedinaeYamaguti, 1971,
Genus:*Pseudolepidapedon*Yamaguti,1938Species: *Pseudolepidapedon balistis*Manter, 1940,
Prevalence: 2/2 = 100 %; worm burden: 4-7 worms per infected fish.
Fish host:*Balistoidesviridescens*.
Description (based on 5 mature worms; measurements (Tabs. 1, 2& 3): The living specimens were slightly pink in color and with sluggish movement. Body was club-shaped to pyriform completely spined. Posterior end broadly rounded, anterior end more tapering. Greatest width was near posterior end under testicular level. Tegument was armed with simple spines of two types; the first type was two rows of large oral spines. The second type was shorter and surrounding almost the whole body. Oral sucker was ovoidal to oblate, sub-terminal, with sub-terminal oral aperture. Prepharynx long and dilated at its posterior end to fit in wide pharynx which was barrel-shaped and the anterior third provided with circular muscles forming a prepharyngeal ring. Esophagus was very short. The intestinal bifurcation was in mid-fore body. Ceca were flask-shaped, wide, diverging laterally and terminating at level of posterior end blindly. Acetabulum was pre-equatorial, situated at level of intestinal bifurcation and clearly larger than oral sucker. Genital pore was median, immedia-tely anterior to acetabulum. The seminal receptacle was absent. Laurer's canal not observed and the uterine seminal receptacle was somewhat clear. Testes were two in number, ovoid, smooth or with slightly irregular contour, tandem or slightly diago-nal, close together, intercecal, in posterior half of body. Ovary was semi-ovoid, un-lobed, pretesticular, slightly to the right, just median to right cecum and contiguous with anterior testis. Uterus was pre-ovarian, inter-caecal and to the left of ovary, extending to left side of ventral sucker. Ootype was com-plex and anterior to the ovary. Eggs were elongate translucent, oval, thin-shelled and operculated. Vitellinefolicles were large, extend from intestinal bifurcation to post-eriorbodyend, dorsal, ventral, and lateral to ceca, confluent posterior to testes and dorsally between acetabulum and pharynx. They were extending over the esophagus and may cover the lower edge of the pharynx. Excretory vesicle was I-shaped with terminal aperture.
Ultrastructure: SEM examination showed that the body was surroundby different-sized spines throughout its length (Plate1,A) with characteristically two rows of elonga-ted finger-like crown-shaped circle of spines on the top of the oral sucker; surrounding the mouth opening (Plate1,B). These spines possess rough surfaces with many transverse furrows and formed of shorter row anteriorly and a longer one posteriorly (Plate1,C).
Magnification of oral sucker papillae showed that they were equal-sized and sessile; distributed randomly around the muscular portion (Plate1,D). Oral sucker was formed of a layer of transverse muscle fibers surrounded by circular fibers (Plate1,E). Anterior part of the body was character-ized by dense dome-like spines (Plate1,F) followed by dense irregularly tapering spines with broad ends on the middle part of the body (Plate1,G). Multi-pointed short spines with 3-4 finger-like termination were condensed on the posterior third of the body (Plate1,H) while the hind part of the body was equipped with fewer scattered spines with more prominent finger-like termina-tions (Plate1,I).
2) *Pseudolepidapedon balistis* (Biological Variant;Fig. 2).
Prevalence; 2/13 (15.4 %); worm burden: 2 per infected fish.
Fish host: *Rhinecanthusassasi*
Description: Living specimens were fleshy with the slight pink color and with sluggish

movement.Body was oblong, closelycylindrical, and plump. Maximum breadth was at level of ovary and the anterior testis, body tapering anteriorly and rounded at its posterior extremity. Tegument was thick, beset throughout its length with well developed, scale-like spines of two types; the first type was large oral spines, the second type was the body spines which were shorter and arranged in rows which were crowded and most numerous at the anterior extremity and become gradually distant at the posterior extremity with somewhat few numbers. A pair of inconspicuous eye-spots was present. Oral sucker was finger-bowl-shaped to oblate, sub-terminal, with sub-terminal oral aperture. Prepharynx was long, entirely below the oralsucker and dilated at its posterior end to fit in wide pharynx. Pharynx was ovoidal; not provided with prepharyngeal ring. Esophagus was very short and wide. The intestinal bifurcation was in mid-fore body. Ceca were flask-shaped, simple, gradually widened posteriorly, diverging laterally and terminating close to each other at posterior extremity. Acetabulum with sphincter around aperture, pre-equatorial at anterior part of middle ofthebody, situated at level of intestinal bifurcation and was clearly larger than the oral. Genital pore was median immediately anterior to the acetabulum and between the upper part of the ventral sucker and the intestinal bifurcation. The seminal receptacle was absent, Laurer's canal was not clearly observed. Cirrus sac elongate, more or less divided by a narrowed region into a fairly wide anterior portion lying along the left anterior border of acetabulum and a claviform posterior portion extending to ovary and overlapping that organ slightly dorsally. Ovary was ovoidal, unlobed, pretesticular, slightly to the right, (just postequatorial) just median to right cecum and contiguous to anterior border of anterior testis (antero-dextral). The two testes were ovoidal, with slightly irregular contour, tandem or diagonal, close together, intercecal, in posterior half of body and the opposite nearby edges of each testis may overlap with each other. The anterior testis was wider, smaller and kidney-shaped while the posterior testis was longer, larger and ovoid. Vitelline follicles were large, extremely crowded, extending from intestinal bifurcation to posterior end of body, dorsal, ventral, and lateral to ceca, confluent posterior to testes and anteriorly between acetabulum and pharynx. Also, they extend over the esophagus and may cover the lower edge of the pharynx. Excretory pore was terminal. Excretory vesicle was I-shaped (tubular), extending below the posterior testis, with terminal pore. Uterus was large saccular, preovarian, fully engorged with mature eggs, intercaecal and to the left of ovary, extending to left of ventral sucker. Eggs were elongate oval, thin-shelled, operculated and translucent. Ootype was complex and anterior to the ovary. Cirrus sac was long and slender, curving and extending from below ventral sucker to above of acetabulum.

Discussion

1) *Pseudolepidapedon balistis* Manter,1940:
The present authors reckon that there are 11 marine species satisfying the present criteria used by Bray (2005) to define the genus, including one of the *Pseudolepidapedon* species described by Yamaguti (1938).The dimensions, measurements and ratios of the present specimens compared with those of *Pseudolepidapedonbalistis* of the other previous works showing that the investigated samples were similar morphometrically to*Pseudolepidapedonbalistis,* mainly in the ratio of the suckers, esophagus length, body spines length and egg dimensions.
On the other hand, the measurements of the current worms having a slight higher values than those of the previous works especially; body length, prepharynx length, pharynx dimensions, the obvious large size of ventral sucker, ovary width, anterior testis length and post-testicular distance. Thedifferences may be biological due to the difference in hosts or methods of study.

Manter (1940) stated in his original description that there were two eye spots but current specimens were lacking these structures. Besides, he said that acetabulum was only slightly larger than oral sucker while the present measurements showed that the acetabulum was obviously larger than oral sucker. Also, Manter (1940) stated the presence of seminal receptacle while the subsequent re-description of *P. balistis* made by Caballero and Caballero (1952), Bravo-Hollis (1956), Manter (1963), Mago and Chinchilla (2002) and the current research indicated the absence of seminal receptacle.

Revising the previous works on that parasite indicated that its ultrastructure was described for the first time illustrating many details which may be of great taxonomic importance to be used in differentiating different species of this genus. Moreover, *Pseudolepidapedonbalistis* was recorded in Egypt for the first time, thereby increasing the geographic range of the species and was recorded in the fish*Balistoidesviridescens*as a new host record for the parasite. This raises the question; why the present samples have a slightly high scale of measurements, dimensions and ratios than that of the other previous descriptions of this parasite.. The answer can be traced to the parasite that has a higher ability to adjust and adapt within this host or other. On the other hand, the present host could be the original definitive host for this parasite.

2) *Pseudolepidapedon balistis* (Biological variant): By applying Bray keys (Bray, 2005) to identify the specimens, and found that this parasite belongs to *Pseudolepidapedon* (Yamaguti, 1938) and the comparisons among the current specimens and the available other species of this genus, it was found that the main morphological features of the current biological variant were very similar to those of the present re-description of *Pseudolepidapedon balistis* with the following differences in the current specimens: In spite of being generally smaller in measurements, they have a bigger prepharynx-length, pharynx width and ventral sucker to ovary length. The presence of two eye spots around the esophagus which indicates the young age of specimens. The body was semi cylindrical and its maximum width was at the ovarian level. Shape of the oral sucker and pharynx were different. Also, specimens had an elongated saccular uterus fully engorged with more condensed mature eggs indicating a high rate of fecundity in apparently young worms. The anterior testis was clearly wider than the posterior. The eggsize was characteristically smaller.

Conclusion

The worms were found in another final host (*Rhinecanthusassasi*). However, the present differences are not enough to create a new variety of the redescribed species and we preferred to describe it as a biological variant; with the fish *Rhinecanthusassasi* a new host record for the parasite.

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Table 1: Dimensions, measurements and ratios of *Pseudolepidapedon balistis*.

Dimension & Measurements (µm)	Min.	Max.	Mean	Ratios	Min.	Max.	Mean
Length	2479	3200	2840	Width %*	31	39	35
Width (Widest region)	968	1006	987	Fore body %*	32	34	33
Width at level of:				Hind body length %*	51	53	52
• Under oral sucker directly	375	453	414	Sucker length ratio	1:2.34	1:2.99	1:2.67
• Ventral sucker	772	807	790	Sucker width ratio	1:1.21	1:1.21	1:1.21
• Ovary	925	951	938	Oral sucker length%*	5	5	5
• Anterior testis	951	981	966	Ventral sucker length%*	13	16	15
• Posterior testis	946	955	951	Oral sucker length/ Width ratio	1:1.81	1:2.17	1:1.99
Fore body length	848	1019	934	Ventral sucker length/ Width ratio	1:0.88	1:0.95	1:0.92
Hind body length	1274	1702	1488	Sucker total length%*	18	21	20
Oral sucker length	132	175	154	Oral sucker length/ Pharynx length ratio	1:2.13	1:2.77	1:2.45
Oral sucker width	286	317	302	Ventral sucker length/ Pharynx length ratio	1:0.91	1:0.92	1:0.92
Prepharynx length	162	230	196	Prepharynx length%*	7	7	7
Pharynx length	365	372	369	Pharynx length%*	12	15	14
Pharynx width	231	325	278	Anterior testis length%*	10	13	12
Esophagus length	16	27	22	Anterior testis length/ Width ratio	1:0.98	1:0.76	1:0.85
Precaecal sacs	0	0	0	Posterior testis length%*	13	14	14
Intestinal bifurcation to ventral sucker	46	162	104	Posterior testis length/ Width ratio	1:0.71	1:0.81	1:0.77
Genital pore to ventral sucker	0	3	12	Ovary length%*	6	7	7
Ventral sucker length	395	409	402	Ovary length/ Width ratio	1:1.31	1:1.73	1:1.34
Ventral sucker width	347	387	367	Ventral sucker to ovary %*	8	10	9
Ventral sucker to ovary	188	313	251	Distance between testes%*	0.3	0.3	0.3
Ovary length	173	203	188	Post-testicular distance %*	13	14	14
Ovary width	229	266	248	Prepharynx distance%*	12	12	12
Ovary to anterior testis	3	9	6	Prebifurcation distance %*	26	28	27
Anterior testis length	249	412	331	Oral to ventral sucker distance%*	37	37	37
Anterior testis width	243	318	281	Previtelline distance %*	22	24	23
Distance between testes	7	10	9	Pre-genital pore distance %*	29	29	29
Posterior testis length	311	455	383	Preovarian distance %*	53	54	54
Posterior testis width	221	369	295	Post-uterine distance %*	42	42	42
Post-testicular distance	344	456	400	Intestinal caeca length%*	68	72	70
Post-vitelline region length	18	33	26				
Egg length	68	81	75				
Egg width	41	50	46				
Prepharyngeal distance	305	372	339				
Prebifurcal distance	682	785	7334				
Oral to ventral sucker distance	928	1194	1061				
Pre-genital pore distance	723	914	819				
Previtelline distance	538	763	651				
Preovarian distance	1302	1737	1520				
Post-uterine distance	1044	1349	1197				
Intestinal caeca length	1683	2306	1995				

* = percentage of body-length, µm= Micron

Table 2: Numbers, rows and dimensions of body spines on different regions of current specimens

Spines	Number			Spine length (µm)			Spine width (µm)		
	min.	Max.	mean	min.	Max.	mean	min.	Max.	Mean
Oral spine numbers									
• 1st row	31	33	32	24	30	27	13	16	15
• 2nd row	32	33	32	27	31	29	13	15	14
Other body spines rows:■									
• At anterior region	40	43	42	6	8	7	7	9	8
• At median region	39	42	41	10	13	12	6	7	7
• At posterior region	22	26	24	4	7	6	3	5	4

Table 3: Comparison of the dimensions and measurements of current specimen and previously described forms. Dimensions are in mm.

Source (Reference)	Manter (1940)	Manter (1963)	Mago& Chinchilla (2002)	Current research
Host Name	*Balistes 'Verres*	*Balistesaculeatus; B. capriscus& B. verres*	*Balistesvetula*	*Balistoidesviridescens*
Host Number	1	NotMentioned	2	2
Host Locality	James Island, Galapagos	Bermuda Islands, Puerto Vallarta, Jalisco and Red Sea..	Mochima Bay	(Sharm EL-Naga) Safaga-Red sea
Parasite Number	2	Not mentioned	4	7
Parasite Location (habitat)	Intestine	Intestine	Intestine	Small Intestine (3rd part of Ileum)
Dimension and Measurements (mm)				
Length	1.957-2.794	1.35-4.61	0.995-1.575(1.785)	2.479-3.200 (2.840)
Width (Widest region)	0.945-1.093	0.440-1.093	0.560-0.595(0.560)	0.968-1.006 (0.987)
Fore body length	0.712-1.161	Not mentioned	Not mentioned	0.848-1.019 (0.934)
Oral sucker length	0.275-0.307	0.110-0.275	0.144-0.225(0.183)	0.132-0.175 (0.154)
Oral sucker width		0.200-0.307	0.180-0.243(0.212)	0.286-0.317 (0.302)
Prepharynx length	0.142-0.315	Not mentioned	Not mentioned	0.162-0.230 (0.196)
Pharynx length	0.232-0.277	0.090-0.277	0.135-0.180(0.156)	0.365-0.372 (0.369)
Pharynx width	0.225-0.262	0.110-0.262	0.135-0.189(0.159)	0.231-0.325 (0.278)
Esophagus length	very short	short	0.018-0.027(0.024)	0.016-0.027 (0.022)
Ventral sucker length	0.300-0.360	0.210-0.300	0.243-0.306(0.261)	0.395-0.409 (0.402)
Ventral sucker width		0.274-0.360	0.243-0.315(0.273)	0.347-0.387 (0.367)
Ovary length	Not mentioned	0.148-0.210	0.099-0.144(0.120)	0.173-0.203 (0.188)
Ovary width	Not mentioned	0.114-0.210	0.135-0.171(0.150)	0.229-0.266 (0.248)
Anterior testis length	Not mentioned	0.170-0.210	0.198-0.306(0.255)	0.249-0.412 (0.331)
Anterior testis width	Not mentioned	0.230-0.323	0.225-0.342(0.276)	0.243-0.318 (0.281)
Posterior testis length	Not mentioned	0.150-0.220	0.234-0.324(0.291)	0.311-0.455 (0.383)
Posterior testis width	Not mentioned	0.230-0.334	0.189-0.315(0.255)	0.221-0.369 (0.295)
Post-testicular distance	0.285-0.465	Not mentioned	Not mentioned	0.344-0.456 (0.400)
Egg length	0.060-0.070	0.060-0.080	0.043-0.080	0.068-0.081 (0.075)
Egg width	0.031-0.0 37	0.031-0.050	0.018-0.053	0.041-0.050 (0.046)
Body spines length	Not mentioned	0.015	0.010-0.130 (0.012)	0.010-0.013 (0.012)
Body spines width	Not mentioned	0.004	0.003-0.005(0. 004)	0.006-0.007 (0.007)
Sucker length ratio	1:1.09-1.17	1:1.29	1:1.20-1.69(1.40)	1:2.34-2.99 (2.67)
Sucker width ratio		1:1.15-2.20	1:1.17-1.45(1.34)	1:1.21

Measurements and dimensions are in millimeters, mm= millimeter

Table 4: Dimensions, measurements and ratios of *Pseudolepidapedon balistis* (biological variant).

Dimension and measurements (µm)	Min.	Max.	Mean	Ratios	Min.	Max.	Mean
Length	2348	2355	2352	Width %*	24	25	24.5
Width (Widest region)	585	593	589	Fore body %*	36.6	36.7	36.7
Width at level of:				Hind body length %*	51.8	52	51.9
• Under oral sucker directly	160	163	162	Sucker length ratio	1:2	1:2.1	1:2.1
• Pharynx	303	309	306	Sucker width ratio	1:1.1	1:1.9	1:1.5
• Ventral sucker	439	445	443	Oral sucker length%*	4.9	5	5
• Ovary	584	589	587	Ventral sucker length%*	10	10.1	10.1
• Anterior testis	574	579	577	Oral sucker length/ Width ratio	1:1.5	1:1.6	1:1.6
• Posterior testis	534	537	536	Ventral sucker length/ Width ratio	1:0.88	1:0.88	1:0.88
Fore body length	860	865	863	Sucker total length%*	14.9	15.1	15
Hind body length	1217	1225	1221	Oral sucker length/ Pharynx length ratio	1:2	1:2	1:2
Oral sucker length	114	117	116	Ventral sucker length/ pharynx length ratio	1:1	1:1	1:1
Oral sucker width	173	188	181	Prepharynx length%*	11.3	11.5	11.4
Prepharynx length	265	271	268	Pharynx length%*	9.8	10	9.9
Pharynx length	230	236	233	Anterior testis length%*	10.2	10.4	10.3
Pharynx width	173	180	177	Anterior testis length/ Width ratio	1:1.16	1:1.18	1:1.17
Esophagus length	27	34	31	Posterior testis length%*	12.8	13.1	13
Precaecal sacs	0	0	0	Posterior testis length/ Width ratio	1:0.80	1:0.81	1:0.81
Intestinal bifurcation to ventral sucker	83	88	86	Ovary length%*	6.5	6.6	6.6
Genital pore to ventral sucker	0	5	3	Ovary length/ Width ratio	1:1.25	1:1.27	1:1.26
Ventral sucker length	235	239	237	Ventral sucker to ovary %*	14.3	14.4	14.4
Ventral sucker width	206	211	209	Distance between testes%*	0.2	0.3	0.3
Ventral sucker to ovary	335	338	337	Post-testicular distance %*	12	12.1	12.1
Ovary length	152	155	154	Prepharynx distance%*	15.8	16	15.9
Ovary width	191	195	193	Prebifurcation distance %*	25.6	25.7	25.7
Ovary to anterior testis	3	5	4	Oral to ventral sucker distance%*	34.5	34.7	34.6
Anterior testis length	239	244	242	Previtelline distance %*	24.2	28.4	26.3
Anterior testis width	279	284	282	Pre-genital pore distance %*	29.2	29.3	29.3
Distance between testes	4	7	6	Preovarian distance %*	43.8	44	43.9
Posterior testis length	300	309	305	Post-uterine distance %*	37.9	38	40
Posterior testis width	244	247	246	Intestinal caeca length%*	67.5	67.5	67.5
Post-testicular distance	281	285	283				
Post-vitelline region length	9	12	11				
Egg length	74	88	81				
Egg width	44	52	48				
Prepharyngeal distance	372	377	375				
Prebifurcal distance	601	605	603				
Oral to ventral sucker distance	810	817	814				
Pre-genital pore distance	685	691	688				
Previtelline distance	661	668	665				
Preovarian distance	1029	1036	1033				
Post-uterine distance	889	894	8912				
Intestinal caeca length	1584	1589	1587				
* = percentage of body-length, µm= Micron							

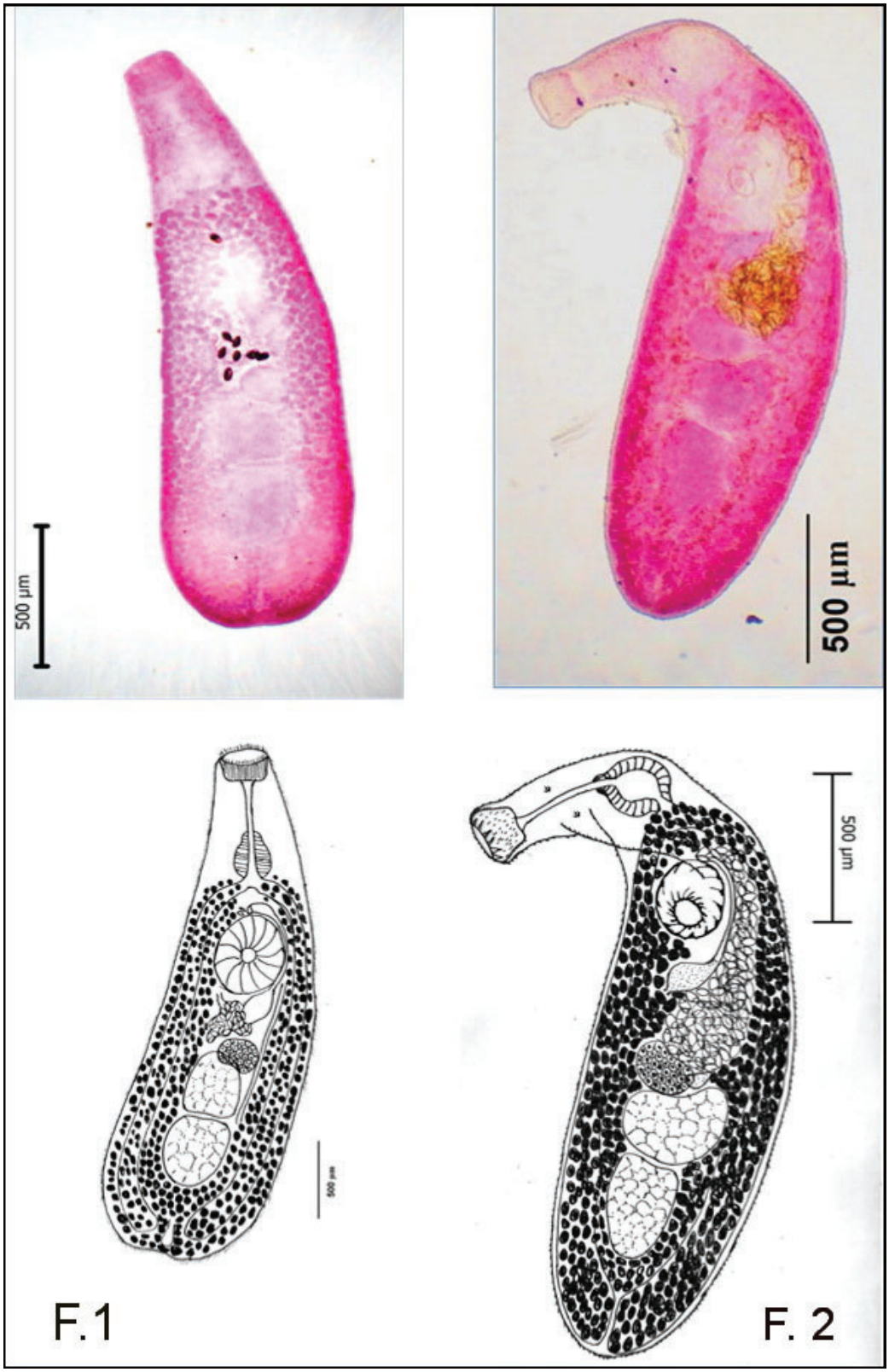

Fig. 1: Ventral view of adult*Pseudolepidapedon balistis* Manter, 1940
Fig. 2: Ventral view of adult*Pseudolepidapedon balistis* (biological variant)

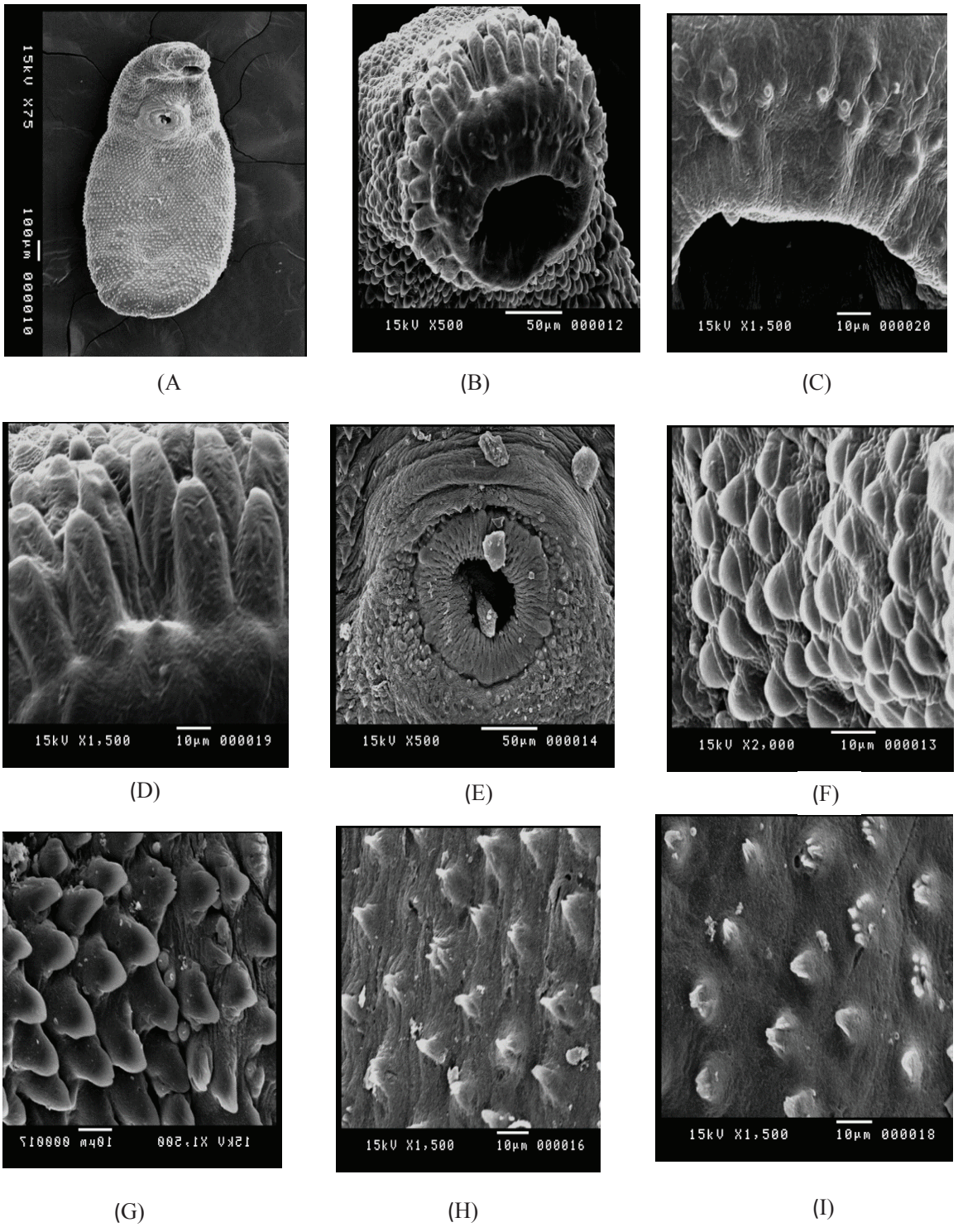

Plate 1: Scanning electron microscope of *Pseudolepidapedon balistis*