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MONOGENEAN PARASITES OF SOME MARINE FISHES, MICROHABITAT DISTRIBUTION AND DESCRIPTION OF THREE NEW SPECIES

(With 5 Tables and 5 Plates)

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طفيليات المونوجينيا في بعض الأسماك البحرية ,التوزيع الدقيق ووصف لثلاثة أنواع جديدة

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لقد تم تجميع ١٥٠ سمكة من البحر المتوسط قرب سواحل مدينة بور سعيد. ٥٠ من الدراك و٥٠ من السيجان و ٥٠ من الوقار. هذه الأسماك فحصت فحصا دقيقا لهدفين الأول: الأستعراف على ديدان المونوجينيا التى تصيب خياشيم هذه الأسماك ومعرفة مدى انتشارها وشدة الإصابة بها. والثاني: معرفة التوزيع الدقيق لأحد هذه الديدان الأكثر شيوعا على كل خيشوم وكذلك على أجزاء الخياشيم نفسها. وقد أسفرت الدراسة عن وجود أربعة أنواع من المونوجينيا. نوع جديد (نيوهكموستوما) من الوقار بنسبة ٤ % ونوع جديد (جوتوكوتيلا) من السيجان بنسبة ٦ % ونوعين من الدراك هما (برايميا) بنسبة ٥٨% ونيوثوراكوكوتيل (نوع جديد) بنسبة ١٠ % وكانت النسبة العامة للإصابة ٢٦ %. وقد لوحظ أن أحد الأنواع الموجودة (برايميا) على خياشيم أسماك الدراك تتركز على الخيشوم الأول الخارجي على كلا الجانبين ويقل تواجدها كلما اتجهنا للداخل على الخيشوم الثاني والثالث والرابع مما يدل على أن هناك انعزال طولي لهذه الديدان وقد لوحظ أيضا أن هذه الديدان تتركز على الربع الأمامي العلوي للخياشيم وتقل كلما اتجهنا للخلف مما يدل على أن هناك انعزال عرضي أيضا لهذه الديدان والأسباب الحقيقية لهذه الظاهرة غير معروفة ولكن يرجح أن هذا الانعزال قد يساعد على التكاثر الخلطى لهذه الديدان الخنثى أو بسبب التنافس على الغذاء وحيث أن هذه الديدان ماصة للدماء فربما يكون تركيزها في الخيشوم الأول والأجزاء الأمامية بسبب مواصفات معينة للدم في هذه الأجزاء مثل ارتفاع نسبة الأكسجين. وبالرغم من أن أسماك الدراك كانت مصابة بنوعين من المونوجينيا لكن العدوى المختلطة لم تلاحظ ويرجع ذلك للتنافس بين الأنواع مما يساعد على عدم تكوين الهجن بين الديدان.

SUMMARY

One hundred fifty marine fishes, 50 *Scomberomorus commerson*, 50 *Siganus canaliculatus* and 50 *Epinephelus gigas* were examined for gill monogenean parasites. The total prevalence of monogenean parasites was 26%. The detected monogenea were *Neohexostoma epinepheli* sp. nov. (4%) from *Epinephelus gigas*, *Pricea multae* (58%) and *Neothoracocotyle commersoni* sp. nov. (10%) from *Scomberomorus commerson* and *Gotocotyla sigani* sp. nov. (6%) from *Siganus canaliculatus*. The mean intensity of infestation was high in *Pricea multae* (7.79) followed by *Neothoracocotyle commersoni* (4.4), *Gotocotyla sigani* (2.6) and *Neohexostoma epinepheli* (2). Microhabitat distribution of *Pricea multae* on the gill arches and sectors of *Scomberomorus commerson* revealed that this parasite preferred the first gill arch and the anterior top sectors of gills followed by second, third and fourth. This distribution probably offered a suitable niche for reproduction or a suitable food resource in this blood feeding monogenea. Depending upon the morphological variations of the flukes specially the shape, size and number of terminal anchors and clamps as well as the host species, three new species were identified.

Key words: *Monogenean Parasites, Marine Fishes*

INTRODUCTION

The monogenean Parasites of marine fishes had been reported by many authors in different localities of the world (Sabodash and Semenenko, 1994 from Azov-Black Sea, Ukraine; Badawy, 1995 from Egypt; Bondad-Reantaso *et al*., 1995 from Japan; Kohn and Cohen, 1996 from Brazil; Cruze-e-Silva et al., 1997 from southern Portugal; Fuentes-Zambrano, 1997 from Venezuela; Geets et al., 1997 from Kenya; Wang, 1997 from China; Zhang et al., 1997 & 1999 from South and East China Sea; Moles et al., 1998 from Gulf of Alaska; Luque and Chaves, 1999 from the coast of Rio-de Janeiro and Pan, 1999 from Hainan Island, South China Sea.

The gills of spanish mackerels (*Scomberomorus* sp., fam. Scombridae) were known to be infested with representatives of the two families Gotocotylidae Yamaguti, 1963 and Thoracocotylidae, Price, 1936 (Lebedev, 1986). Hayward and Rohde (1999)[7] reviewed the family Thoracocotylidae and erected a new Subfamily Scomberomorocotylinae.

Also in the same year[2], Hayward and Rohde reviewed the subfamily Thoracocotylinae and described a new species *Pseudothoracocotyla munroi*. During examination of monogenea of *Scomberomorus* spp., numerous specimens of genus *Pricea* which belonging to subfamily Priceinae was collected (Lebedev, 1986 and Murugesh, 1995). Rohde and Hayward (1999) reviewed the subfamily Priceinae and concluded that *Pricea multae* was the only valid species. Little specimens from *Neothoracocotyle* and *Scomberocotyle* which belonging to subfamily Neothoracocotylinae were collected from several species of scombrid fish (Lebedev, 1986 and Hayward and Rohde, 1999[3]).

Ectoparasites of fish are suitable objects to study ecological questions like interspecific competition and microhabitat partition (Euzet, 1972; Lambert and Maillard, 1975; Lebedev, 1978 and Rohde, 1978 & 1993). Ktari (1971) noted that narrow microhabitat favor mating while Rohde (1993) showed that the space is an important factor limiting microhabitat in blood feeding monogenea. El Hafidi et al., (1998) studied the microhabitat distribution of two monogeneans species, *Metamicrocotyla cephalus* and *Microcotyla mugilis* on the gills of *Mugil cephalus* in Morocco. Oliva and Luque (1998) studied the microhabitat selection of the monogenean, *Microcotyle nemadactylus* on gill filaments of *Cheilodactylus variegatus* and concluded that the parasite preferred the first gill arch.

This study aims to identify the gill monogenean parasites of some marine fishes from Mediterranean Sea near Port-Said and to study the microhabitat selection of the predominant one of them.

MATERIALS and METHODS

One hundred fifty fish specimens were collected immediately after fishing from the coastal of Mediterranean Sea at El-Gameel district near Port-Said City. The collected fishes were immediately killed by freezing and identified according to Murno (1967) as fifty *Epinephelus gigas*, fifty *Scomberomorus commerson* and fifty *Siganus canaliculatus*. The gills were dissected and each gill was examined separately for monogenean parasites using dissecting microscope. The distribution of monogenea was mapped on drawing of the gills, which were made by tracing arches and filaments of the gills with pencil. Divisions of gills into longitudinal quarters were made by dividing the line between the base of the filaments and the arches into 4 sectors of equal length and by tracing the filaments arising at the end of each sector. The number, mean

number and percentage of monogenea were calculated for each gill arch and gill sector (Oliva and Luque, 1998).

The collected monogenea were fixed in FAA (Formalin-Ethyl Alcohol-Acetic Acid), stained in Semichon's acetocarmine, dehydrated in ascending ethanol series, cleared in xylene and mounted in Canada balsam (Rohde, 1980). Prevalence and mean intensity of infestation were calculated according to Margolis et al. (1982).

RESULTS

Out of one hundred fifty gills of marine fishes thirty-nine representing three fish species, *Epinephelus gigas*, *Scomberomorus commerson* and *Siganus canaliculatus* were infested with 4 monogenean parasites. The total prevalence of infestation was 26%. *Epinephelus gigas* was infested with *Neohexostoma* (*Octocotyle*) *epinepheli* sp. nov. (4%), *Siganus canaliculatus* was infested with *Gotocotyla sigani* sp. nov. (6%), while *Scomberomorus commerson* was infested with both *Pricea multae* (58%) and *Neothoracocotyle commersoni* sp. nov. (10%). The mean intensity of infestation was high in *Pricea multae* (7.79) followed by *Neothoracocotyle commersoni* (4.4) while it was low in *Neohexostoma epinepheli* (2) and *Gotocotyla sigani* (2.6) (Table, 1).

Table (2) explained the distribution of 226 *Pricea multae* specimens on gill arches and sectors of 29 *Scomberomorus commerson*. The highest number of flukes was present on the first gill arch (93) followed by second (60) and third (43) while the fourth gill arch had the lowest number (30). Also, highest number of the flukes was present on the first (anterior) gill sector (103), followed by the second (56), third (38) and fourth (29). Following to this distribution, the mean number and percentage of parasites were high in the first gill arch and sector followed by second, third and fourth (Plate, 5).

Description of the detected monogenean parasites

Superfamily: Diclidophoroidea
Family : Hexostomatidae, Price 1936
Genus : *Neohexostoma (Octocotyle)* Price 1960
Species : *N. epinepheli* sp. nov. (Plate, 1)
Host : *Epinephelus gigas*
Habitat : Gill filaments

Description : Body was elongate and measured 10-11 x 1.2-1.35 mm with maximum width in the ovarian region. Anteriorly, there was paired oral suckers which was ovoid in shape and measured 49.9-53.8 x 43.3-

49.9μ. The pharynx was present and measured 81 x 47.2μ. The intestine broken up into network extending throughout most of the body. The testes divided into numerous follicles in post-ovarian inter-caecal field. The intestine and testes terminating a considerable distance anterior to opisthohaptor leaving the inter-space entirely free. The common genital atrium was ovoid, unarmed and measured 43.3 x 66.6μ. The ovary was extensively coiled and the eggs were filamented, barrel-shaped, each one measured 202.5 x 67.66μ. The vagina was with two pyramidal structure, armed mid-dorsal and measured 46.6 x 66.66μ for each half. An elongate waist-like constriction was present anterior to opisthohaptor and measured 0.95-1.2 mm. Opisthohaptor was disc-like, clearly set off from the body proper and measured 1-1.2 mm in diameter and supported with 4 pairs of sessile clamps which had been modified into suckers arranged in less vertical manner. Each clamp containing three irregular and dissimilar sclerites. The two lateral sclerites were semilune in shape, bipartite laterally and measured 108-135 x 54-67μ. The other sclerite was x-shaped, superficially located in the middle and measured 121-162 x 67-81 m. The posterior pair of clamps were slightly small (216 x 175.5μ) than those of other pairs (297-337 x 175.5-270μ). Two pairs of anchors were present between the two posterior clamps, attached directly to hind margin of the body. The large one measured 135μ while the small one was 33.3 m in length.

Superfamily: Microcotyloidea
Family : Thoracocotylidae, Price, 1936
Subfamily : Priceinae Chauhan, 1953
Genus : *Pricea*
Species : *P. multae* (Plate, 2)
Host : *Scomberomorus commerson*
Habitat : Gill filaments

Description: The body was cylindrical measuring 4-4.66 x 0.9-1.1 mm. Oral suckers were kidney-shape and each one measured 92-94 x 65-67μ. Pharynx was ovoid in shape measuring 52-54 x 39-41μ. Genital atrium was armed and oval in shape measuring 146-148 x 65-67μ. Intestinal crura branched. Testes were numerous (50-60 in number) post-ovarian and the ovary was compact. Eggs were filamented at each pole and each one measured (214-216 x 92-94μ). Vagina was present forming a sac enclosing U-shaped hook and opening dorsally at intestinal bifurcation. Vitellaria were extending along intestinal crura commencing at level of vaginal pore or just behind it. Y-shape vitelline reservoir was largely or entirely post-ovarian. Opisthohaptor was an elongated curved structure

obliquely attached to posterior axis of the body measuring 1.9-2 x 0.33-0.36 mm with two arms nearly equal. It was symmetrical bearing two rows of numerous pedunculated clamps (113-133 in number). Clamp skeleton consisted of two pairs of lateral arms, three central pieces set on basal piece, a pair of apical transverse bars and several pairs of rib-like thickenings. A single pair of anchor on an oval lappet was present at posterior end of opisthohaptor and measured 54-60μ in length.

Superfamily: Microcotyloidea
Family: Gastrocotylidae
Subfamily: Gotocotylinae
Genus: *Gotocotyla* syn. *Lithidiocotyle* Sporston 1946
Species: *G. sigani* sp. nov. (Plate, 3)
Host: *Siganus canaliculatus*
Habitat: Gill filaments

Description: Body was slender (4-4.2x0.4-0.48 mm). The oral suckers were oval and each measured 56-58 x 33-35μ away from each other. Pharynx was oval and the esophagus was without prominent diverticula. Intestinal crura extended into opisthohaptor. Testes were numerous about 35 in number extending from behind ovary into the base of opisthohaptor. Cirrus was sub-cylindrical, rod-like shaped, covered with spines and measured 405x 67.5μ. Ovary was horse shoe-shaped with distal end directed backward. Receptaculum seminis was large, oval behind distal end of ovary and measured 135 x 94.5v. Genito-intestinal canal was spirally, short and measured 472.5μ. Eggs were filamented and each measured 270 x 67.5μ. Vagina present and connected by median duct with anterior inverted Y-shaped vitelline commissure. Vitellaria were extended in lateral fields from level of intestinal bifurcation to anterior part of opisthohaptor. Opisthohaptor occupied with two symmetrical rows of clamps and a pair of terminal anchors (39.9μ in length). The clamp skeleton (56μ in width) consisted of two sets of sclerites. One set was made up of two large hook-shaped and a slender sigmoid lateral pieces and slender nearly straight middle piece with a pediform tip. The sigmoid lateral and the straight middle joined together at the base. The average number of clamps was about 130.

Superfamily: Microcotyloidea
Family: Gastrocotylidae
Subfamily: Gastrocotylinae
Genus: *Neothoracocotyle*, Hargis 1956
Species: *N. commersoni* sp. nov. (Plate, 4)

Host: *Scomberomorus commerson*
Habitat: Gill filaments
Description: Body was lanceolate measuring 11.3-12 x o.7-1 mm. Anteriorly, there were a paired buccal suckers each measured 81 x 27 m. Pharynx was oval in shape and measured 108 x 54µ. Esophagus was simple and measured 675µ. The intestinal crura were with outer diverticula, not extend into opisthohaptor and not united posteriorly. Testes were numerous. Cirrus was armed and 189 m in length. Genital atrium was armed and postbifurcal. Ovary was looped with distal end directed backward and located median and pretesticular. Eggs were filamented at both poles and each one measured 175.5µ in length. Vagina was single with middorsal opening in front of intestinal bifurcation. Vitellaria were largely co-extensive with intestinal crura. Opisthohaptor was symmetrical or sub-symmetrical with a row of numerous small clamps of uniform structure with accessory sclerites and 7 oblique rib-like thickenings, commencing behind the level of testes and projecting backward beyond body proper. Each clamp measured 56µ in width. The terminal anchor present and measured 49.95µ.

DISCUSSION

The common monogenean parasite on gill filaments of *Scomberomorus commerson* was identified as *Pricea multae* which belong to subfamily Priceinae depending upon the morphological features of opisthohaptor which carried 2 rows of thoracocotylide clamps and a pair of hamuli (anchor) on terminal lappet (Lebedev, 1986) who added that another small hamuli may be present beside the large one. Rohde and Hayward (1999) mentioned that the small hamuli beside the large one present in larval specimen, and the adult one had large hamuli only and concluded that *Pricea multae* was the only valid species in subfamily priceinae.

The detected monogenean parasite on gill filaments of *Epinephelus gigas* was identified as *Neohexostoma*, one of the genera of the family Hexostomatidae depending upon the morphological features of opisthihaptor specially the sucker-like clamps. Because of this species varied from *N. thunninae* (Price, 1961) of *Thynnus thunnina* in dimensions of the whole worm, the arrangement of sucker-like clamps and the position of common genital atrium as well as host species (Table, 4), the name *Neohexostoma epinepheli* sp. nov. was proposed for this species.

The member of gastrocotylid monogenea on gills of *Siganus canaliculatus* was placed in the genus *Gotocotyla* (Sporston, 1946) depending upon the presence of both receptaculum seminis and vagina which differentiate this genus from *Pseudomicrocotyle* (Sandars, 1947). The detected *Gotocotyla* species varied in size, number of clamps and number of testes as well as in host species from the previous detected species (Table, 3), so the name *Gotocotyla sigani* sp. nov. was proposed for this species. While the gastrocotylide member on gills of *Scomberomorus commerson* was placed in genus *Neothoracocotyle* (Hargis, 1956 and Hayward and Rohde, 1999[3]) of subfamily gastrocotylinae depending upon the structure of opisthohaptor specially the clamps, which had accessory sclerites and oblique rib-like thickenings. The size of the worm and the number of terminal anchors as well as the host species differentiate this species from other previous ones (Table, 5). The name *Neothoracocotyle commersoni* sp. nov. was proposed for this species.

Examination of 50 specimens of *Scomberomorus commerson* obtained from the coastal of Mediterranean Sea near Port-Said showed that *Pricea multae* was the common parasite of gill filaments, with prevalence of infestation reached 58%. The distribution of worms along the four gill arches was not homogenous showing preference to the first gill arch suggesting a horizontal partition. These results were in line with that of Rohde (1980 & 1993) for *Kuhnia* sp. The distribution of worms on sectors of gill showing similar pattern and preference to the first sector of gill. These findings were in agreement with those of Oliva and Luque (1998) for *Microcotyle nemadactylus*. *Pricea multae* was found on 4 gill arches and sectors but the distribution was not uniform and clear preference by gill arches and sectors was evident suggesting longitudinal and transverse partition. The factors limited population of blood feeding monogenea such as *Pricea multae* appeared to be space rather than competition, reinforcement of reproductive barrier and/or the need for ensure mate. Also, *Neothoracocotyle commersoni* was found on the gills of *S. commerson* but mixed infestation was unnoticed. Microhabitat segregation is a common process observed when more than one parasitic species could be found in the same microhabitat. This is due to competition, reinforcement of reproductive barrier to avoid production of hybrid (Rohde, 1980).

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LEGEND OF FIGURES

Plate 1: Fig. 1-7: *Neohexostoma epinepheli* sp.nov.

Fig. 1: Whole fluke, bar = 2mm.

Fig. 2: Anterior end, common genital atrium (arrowhead) and toothed vagina (arrow), bar = 150µ.

Fig. 3: Prohaptor, oral suckers (arrows), bar = 40µ.

Fig. 4: High magnification of toothed vagina (V).

Fig. 5: Opisthohaptor showing 8 sucker-like clamps with X-shape central sclerites (arrows), bar = 165µ.

Fig. 6: Posterior portion of opisthohaptor showing unequal terminal hooks (arrows), bar = 35µ.

Fig. 7: Circular opisthohaptor which separated from the rest of the fluke (arrowhead), bar = 160µ.

Plate 2: Fig. 1-6: *Pricea multae*

Fig. 1: Prohaptor showing two oral suckers (arrows), Pharynx (arrow-head) and armed common genital atrium (C), bar = 46µ.

Fig. 2: Anterior portion showing filamented eggs (arrowheads), bar = 420µ.

Fig. 3: Posterior portion of fluke showing opisthohaptor with clamps (arrowhead) and testes (T), bar = 0.6 mm.

Fig. 4: Whole fluke, bar = 1.2 mm.

Fig. 5: Posterior portion of opisthohaptor showing terminal hooks (arrowhead) and clamps (C), bar = 23µ.

Fig. 6: High magnification of vagina (V), bar = 20µ.

Plate 3: Fig. 1-7: *Gotocotyla sigani* sp. nov.

Fig. 1: Whole fluke, bar = 0.75 mm.

Fig. 2: Anterior portion showing vagina (V) and genito-intestinal canal (G), bar = 182µ.

Fig. 3: Prohaptor with oral suckers (arrows), bar = 22.5µ.

Fig. 4: Anterior portion showing common genital atrium (arrowhead), bar = 84µ.

Fig. 5: Posterior portion of opisthohaptor showing terminal hooks (arrowhead), bar = 18µ.
Fig. 6 & 7: High magnification of clamps, bar = 9.5µ.
Plate 4: Fig. 1-6: *Neothoracocotyle commersoni* sp. nov.
Fig. 1: Whole fluke, bar = 1.84 mm.
Fig. 2: Prohaptor with oral suckers (arrows) and pharynx (arrowhead), bar = 92.5µ.
Fig. 3: Armed common genital atrium, bar = 28µ.
Fig. 4 & 5: High magnification of clamps, bar = 9.8µ.
Fig. 6: Posterior portion of opisthohaptor showing terminal anchor, bar = 17µ.
Plate 5: Fig. 1-4: Distribution of *Pricea multae* on gill sectors, (arrowheads).

Table (1): Prevalence and intensities of infestation of monogenean parasites in marine fishes

Species of monogenea	Host	Habitat	Prevalence		Intensity	
				%		χ
Neohexostoma epinepheli sp. n.	*Epinephelus gigas*	Gills	2/50	4	4/2	2.00
Pricea multae	*Scomberomorus commerson*	Gills	29/50	58	226/29	7.79
Neothoracocotyle commersoni sp.n.	*Scomberomorus commerson*	Gills	5/50	10	22/5	4.40
Gotocotyla sigani sp. nov.	*Siganus canaliculatus*	Gills	3/50	6	8/3	2.60
Total			39/150	26		

Table (2): Number (No.), mean number (x) and percentage of *Pricea multae* in each gill arch and sector of infested *Scomberomorus commerson*.

Gill sector		Gill Arch				Total
		1	2	3	4	
1	No.	45	31	17	10	103
	X	1.55	1.06	0.58	0.34	3.55
	%	19.91	13.71	7.52	4.42	45.57
2	No.	21	13	15	7	56
	X	0.72	0.44	0.51	0.24	1.93
	%	9.29	5.75	6.63	3.09	24.77
3	No.	15	10	8	5	38
	X	0.51	0.34	0.27	0.17	1.3
	%	6.63	4.42	3.53	2.21	16.81
4	No.	12	6	3	8	29
	X	0.4	0.2	0.10	0.27	1.0
	%	5.3-	2.65	1.32	3.53	12.28
Total	No.	93	60	43	30	226
	X	3.2	2.06	1.48	1.03	7.79
	%	41.15	26.54	19.02	13.27	100

Table (3): Comparison between the previous detected *Gotocotyla* spp. and the present record.

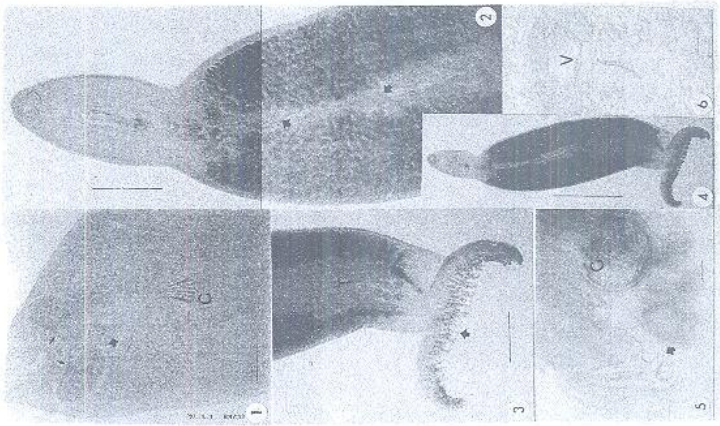
Species of Monogenea	Host	Size	Number of Clamps	Number of testes
Gotocotyla sawara Ishii 1936	*Sawara nipponica*	10-12 x 0.75-0.85 mm	140 on each side	-
G. acanthophallus MacCallum & MacCallum 1913	*Roccus lineatus*	7 x 0.67mm	43	60
G. acanthura Meserve 1938	*Brama rayi*	10 x 1 mm	-	-
The present record *G. siganl* sp. n.	*Siganus canaliculatus*	4.2 x 0.4 mm	130	35

Table (4): Comparison between *Neohexostoma thunninae* and the present record

Point of difference	*N. thunninae* Price 1961	*N. epinepheli* sp. nov.
Host	*Thynnus thunnina*	*Epinephelus gigas*
Habitat	Gill filaments	Gill filaments
Length	8-9 mm	10-11 mm
Width	2 mm	1.2-1.35 mm
Anterior end	Blunt	Tapered
Opisthohaptor	Elongated	Disc-like
Common genital atrium	Just postbifurcal	Away from intestinal bifurcation
Sucker-like clamps	Arranged in vertical manner	Arranged in less vertical manner

Table (5): Comparison between the previous detected *Neothoracocotyle* spp. and the present record.

Species	Host	Size	Terminal anchor
N. coryphaenae Hargis, 1956	*Corypheana hippurus*	12 x 0.9 mm	2 pairs
N. acanthcybii Hargis, 1956	*Acanthocybium solandri*	6.28- 11.1 mm	2 pairs
Present record	*S. commerson*	11.312x0.7-1mm	1 pair



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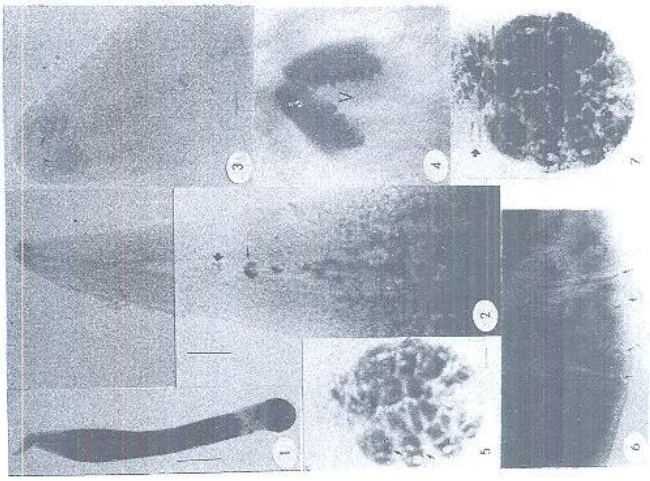
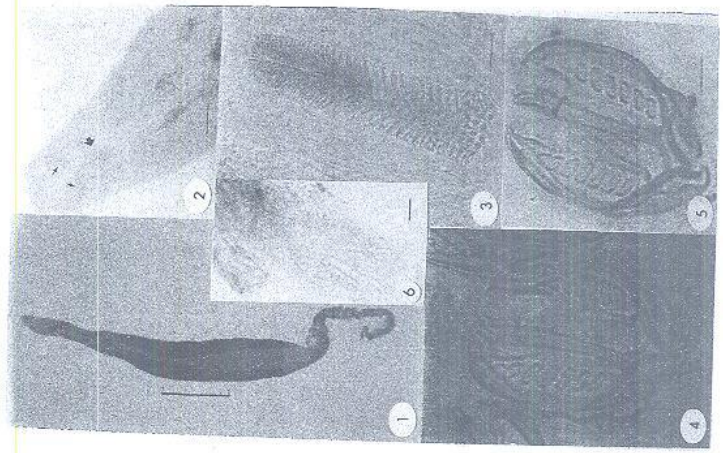


Plate (1)



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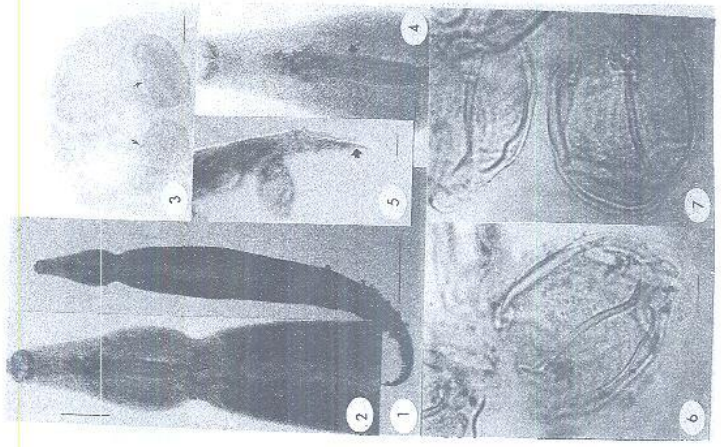

Plate (3)

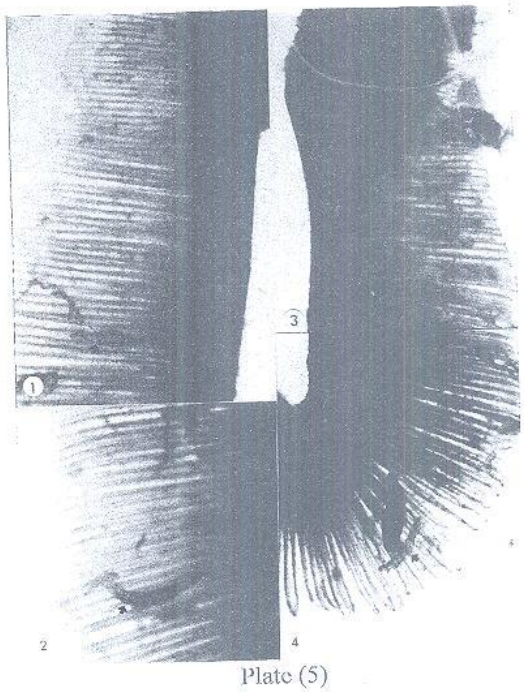

Plate (5)