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Protochondracanthus alatus infesting Gills of Some Marine Fish Species

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Abstract: The distribution of parasitic copepod, *Protochondracanthus alatus* (Heller, 1865) was investigated in 22 host species, only 2 species, *Psettodeserumei* and *Epinepheluschlorostigma* were found susceptible to infestation with parasitic copepods in their gills, with prevalence 29.17% and 25% respectively. The isolated parasitic copepod, *P.alatus*was identified by using light and electron microscopes. The infested fishes did not show any clinical abnormalities but the gross pathological lesions were observed in the gill filaments. The overall percent of *P.alatus* infestation among the infested fish species reached 7.63%. The infestation rates showed seasonal variations, peaked in summer (32.58%), followed by the spring (28.18%), winter (20.54%) and autumn (19.45%). The histopathological alterations showed advanced stages of sever hyperplasia of secondary gill lamellae with infiltration of inflammatory cells, hypertrophy of mucous cells lining epithelial cell layer of secondary gill filaments as well as congestion of branchial blood vessels accompanied with telangiectasis.

Key words: Flatfish · *Psettodeserumei* · *Epinepheluschlorostigma* · *Protochondracanthusalatus* · Arabian Gulf

INTRODUCTION

Copepods play a dual role in the life of fish. They serve as the first intermediate hosts of numerous cestodes such as Trypanorhyncha and Protocephalids. Many of nematodes like Drancunculids (*Philometra* sp.) and Camallanids are transmitted by them. Acanthocephalans also use copepods as first intermediate hosts. The fungus Ichthyosporidium can presumably also be transmitted by copepods [1].

Copepods may be free-living, symbiotic, or internal or external parasites on almost every phylum of animals in water. Special organs of attachment are developed in the parasitic forms. Some of copepods that affect wild fish are of commercial significance as they affect host survival or cause unsightly changes in the flesh, others cause ongoing problems in aquaculture [2-4] and are, therefore, in certain countries actually classified as a notifiable disease.

Copepods have been reported from a great range of depths [5]. Few families of parasitic copepods have successfully exploited demersal fishes as hosts. The most commonly encountered parasite families from demersal fishes are the Chondracanthidae within the order Poecilostomatoida.

Chondracanthidae is one of the major families of Copepoda, comprising more than 150 species. Flatfishes are the most preferred hosts of chondracanthids [6]. *Protochondracanthus alatus* was recorded from the Indian Ocean regions, Singapore, India, Kerala and Bombay, Sri Lanka, Pakistan andTaiwan [7, 8]. *Protochondracanthusalatus* is host specific to the bigmouth flounder (*Psettodeserumei*).

Investigations of parasitic copepods from the North-West Indian Ocean along the Arabian Gulf coast are scarce. From the coastal of Kuwait, Ho and Kim [9] reported Irodeskuwaitensis (Copepoda, Taeniacanthidae) from the goatfish, *Upeneussulpureus* Cuvier and

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Valenciennes. Morales-Serna *et al*. [10] recorded that in Santa María La Reforma lagoon (northwestern coast of México) five parasitic copepod species were observed, including Acantholochuszairae, Caligusserratus, Lepeophtheirus simplex, *Pseudochondra canthusdiceraus* and *Parabrachiella* species, Ho and Kim [11] reported that three species of caligid copepods (Siphonostomatoida) belonging to genus Hermilius isolated from marine catfish, *Arius thalassinus*.

Thus present study was designed to investigate *Protochondracanthus alatus* which infest gills of some marine fish from the Arabian Gulf on the Saudi coast, to detect the clinical signs, prevalence and seasonal variation of infestation and morphological characters of parasitic copepod using light and scan electron microscope (SEM) and histopathological alterations of gills of naturally infested marine fish with *Protochondracanthus alatus*.

MATERIAL AND METHODS

Fish Collection: At different times between March 2012 and February, 2013, a total of 380 fish belonging to 22 species freshly collected from Qatef wholesale market, Eastern Province of Saudi Arabia. Individual species were separated, packed in separate plastic bags, in ice and transported to the laboratory of the Fisheries Research Center, Al-Qatef, Saudi Arabia. All collected fish were examined for infestation with parasitic copepods. Fish specimens were measured to the nearest cm of total length. Prevalence, seasonal infestation and correlation between fish size and infestation, clinical and postmortemlesions were recorded.

Clinical and Postmortem examination: The investigated fish examined externally for any abnormal clinical sings on the skin, fins and internally, for the dissected gills according to Noga [12].

Parasitological Examination: Examination of fishes for the parasitic copepods was carried out while the fish were fresh.Gills, skin, fins and branchial cavities were investigated macroscopically using magnified lens and also with the help of a stereo-microscope under 6X magnification for the presence of copepods.

The copepods parasites removed from the fish hosts were preserved immediately in 4% formalin for microscopical examination of the parasites, the preserved specimens were rinsed in water, transferred and kept in 70% alcohol for 24 hours before clearing in 85% lactic acid [13].

Preparation of copepods samples for scan electron microscope (SEM): Some collected parasites were prepared for scanning electron microscopy (SEM) by fixation specimens in 3% glutaraldehyde in Sorensen's Phosphate Buffer, (pH 7.2), overnight. Wash specimens in 3 changes of buffer 5 min/eachthen in H_20 5 min. Immerse specimens in 2% OsO_4 for 2 hr, after that wash in 3 changes of H_20 5 min/each. Dehydrate using a series of ethanol. Critical Point Dry, 2 hr. mount specimen on a stub with silver paint, then coat with gold and lastly viewed in Hitachi S-1300 Scanning Electron Microscope (SEM).

Histopathological studies: Sections were taken from the affected gills of diseased fish and fixed in 10% formal saline for twenty four hours. Washing was done in tap water then; serial dilutions of alcohol (methyl, ethyl and absolute ethyl) were used for dehydration. Specimens were cleared in xylene embedded in paraffin. Paraffin wax tissue blocks were prepared for sectioning at 5-7 microns thickness by sledge microtome. The obtained tissue sections were collected on glass slides, deparafinized, stained by hematoxylin and eosin and examination was done using the light electric microscope [14].

RESULTS

Clinical and Post Mortem Lesions: Naturally infested fish with parasitic copepod showed no any clinical abnormalities and appeared to be apparently healthy. The gross pathological lesions in the gill filaments of the infested flatfish, *Psettodeserumei* as well as *Epinepheluschlorostigma* (Fig.1, A) showed gill filaments frayed, slimy, nibbled and pale with characteristic marbling appearance,(Fig.1, B) showed thepresence of parasitic copepod which characterized by the double long threads of the ovisacs with its whitish color. Excessive accumulation of mucous in gill filaments was observed in some fishes.

Identification of Isolated Copepods: The collected parasitic copepod was identified as *Protochondracanthus alatus* which characterized by the body of the adult female is elongated and depressed, head oval, longer than wide composed of cephalosome only and protruded posterolaterally into a small process. Neck region isa little narrower than the head, bearing a pair of tripartite, lateral processes on both sides. Trunk segments completely fused. Anterior part of trunkis bearing a pair of long, lateral processes in front and another pair of short posterior processes at end and a vermiform process at midposterior end. Genito-abdominal somitesareshort,

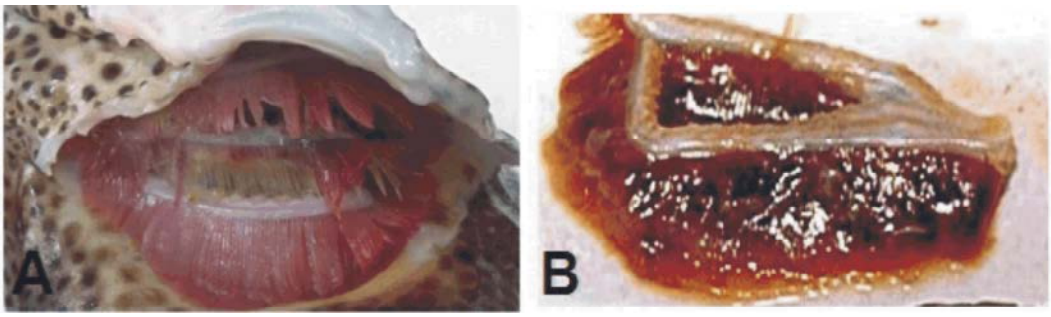

Fig. 1: *Epinepheluschlorostigma* (A) gills infested with *Protochondracanthus alatus*, the tips of the gills are frayed, slimy, nibbled and pale with characteristic marbling appearance (arrows) (B) gill of *Psettodeserumei*havingthick mucus on fishinfestedwith parasitic copepod *Protochondracanthus alatus* appear as white dots (arrows).

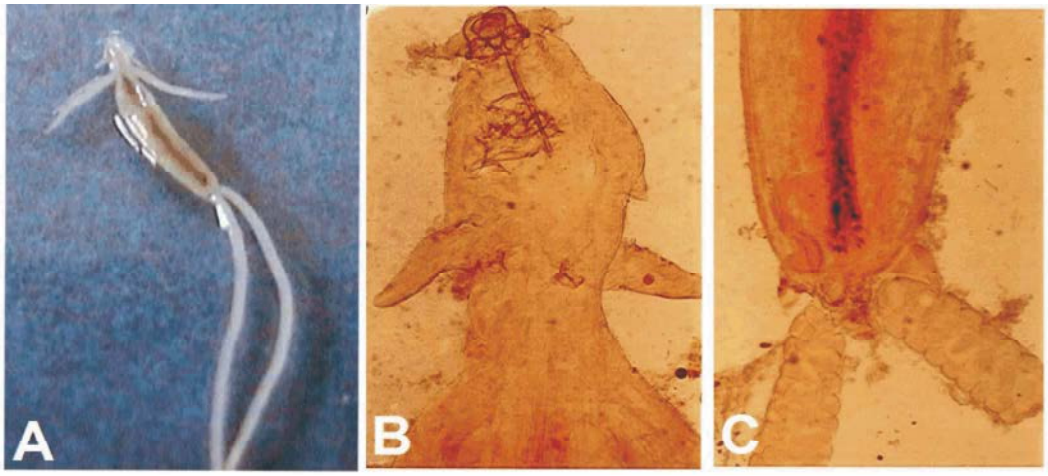

Fig. 2: (A) Fresh isolated preparation of *P.alatus*; adult female. (B) Head and neck region of *P.alatus*. X10. (C) 2:Large egg sacs in the posterior part of *P.alatus*. X10.

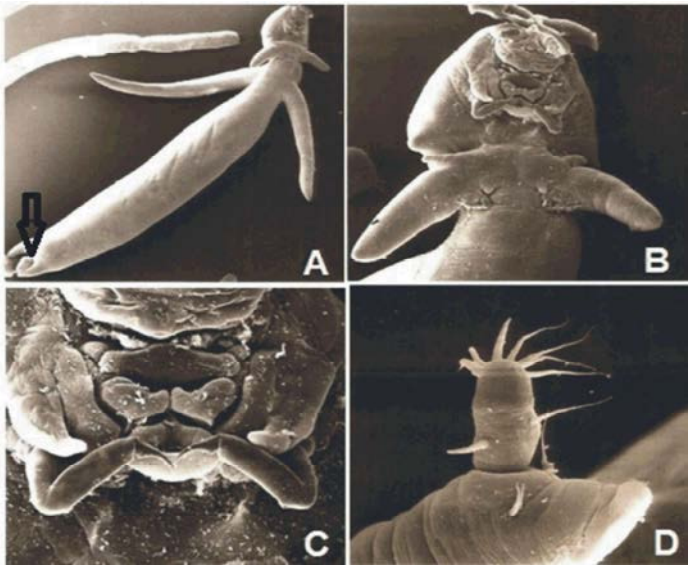

Fig. 3: SEM of *Protochondracanthus alatus;* (A) adult female habitus, (ventral view) (Notice that black arrow pointed to copulation between male and female) (B) head and neck region, (ventral view) (C) magnified mouth parts (D) tip of antennule.

Table 1: showing occurrence of *Protochondracanthusalatus* in local fish as well as some imported marine fish

Species	Length (cm)	No. of examined fish.	No. of Infested fish	%
Acanthopagrusberda	30 - 52	6	0	0
Acanthopagrusbifasciatus	29 - 33	6	0	0
Arius thalassinus	46	1		
Cephalopholishemistiktos	25 - 37	18	0	0
Cheimeriusnufar	27 - 41	4	0	0
Psettodeserumei	39 - 74	48	14	29.17
Epinephelustauvina	47 - 91	25	0	0
Halichoeresstigmaticus	28 - 35	8	0	0
Johinus maculates	36 - 60	27	0	0
Lethrinusnebulosus	31 - 65	30	0	0
Lutjanusehrenbergi	21 - 35	24	0	0
Lutjanusmalabaricus	28 - 48	21	0	0
Nematalosanasus	11 - 14	6	0	0
Parastromateusniger	32 - 36	6	0	0
Plectorhinchusgaterinus	30 - 45	6	0	0
Plectorhinchuspictus	35 - 50	8	0	0
Pomadasysargenteus	37 - 50	7	0	0
Epinepheluschlorostigma	58 - 85	60	15	25
Scomberomoruscommerson	75 - 115	21	0	0
Seriolinanigrofasciata	33 - 52	9	0	0
Siganuscanaliculatus	24 - 35	20	0	0
Tylosuruscrocodilus	68 - 98	19	0	0
Total	380	29	7.63	

Table 2: Showing seasonal variations of *Protochondracanthusalatus*infestation.

	winter			spring			summer			autumn		
Fish sp. Seas.	No. Exam.	No. Infect.	%	No. Exam.	No. Infect.	%	No. Exam.	No. Infect.	%	No. Exam.	No. Infect.	%
Epinepheluschlorostigma	8	1	12.5	18	5	27.78	22	7	31.82	12	2	16.67
Psettodeserumei	7	2	28.57	14	4	28.57	18	6	33.33	9	2	22.22
Total	15	3	20.54	32	9	28.18	40	13	32.58	21	4	19.45

wider than long, carrying a pair of conical caudal rami. Egg sac islonger than the body. Body of male, *Protochondracanthus alatus* is pyriform, with a head larger than the rest of the body. First two pedigers separated, but remaining pedigers fused with genito-abdominal somites. Genito-abdominal appeared with a pair of conical caudal rami. (Fig. 2A, B and C) and SEM (Fig.3 A,B,C and D).

Prevalence and Seasonal Variation of protochondracanthosis: At different times between March, 2012 and February, 2013, Different marine fish were collected from Qatef wholesale market, Eastern Province of Kingdom of Saudi Arabia.Out of 22 fish species, only 2 species, *Psettodeserumei* and *Epinepheluschlorostigma* were found susceptible to infestation with parasitic copepods in their gills. The overall percentage of *Protochondracanthus alatus* infestation among examined fish species reached 7.63%. However, the infestation percentages in *Psettodeserumei* and *Epinepheluschlorostigma* reached 29.17% and 25% respectively, while in the remainder of the examined fish species were 0 % Table 1.

There is a relationship was found between the size of the fish and its infestation with *Protochondracanthus alatus*, the infestation detected in *Psettodeserumei* and *Epinepheluschlorostigma* at more than 56 cm and72 cm respectively.

Regarding the seasonal variations of *Protochondracanthus alatus*, infestation peaked during the summer season (32.58%), followed by the spring season (28.18%), winter (20.54%) and autumn (19.45%) with some variations among the two infested fish species; *Psettodeserumei* and *Epinepheluschlorostigma* Table 2.

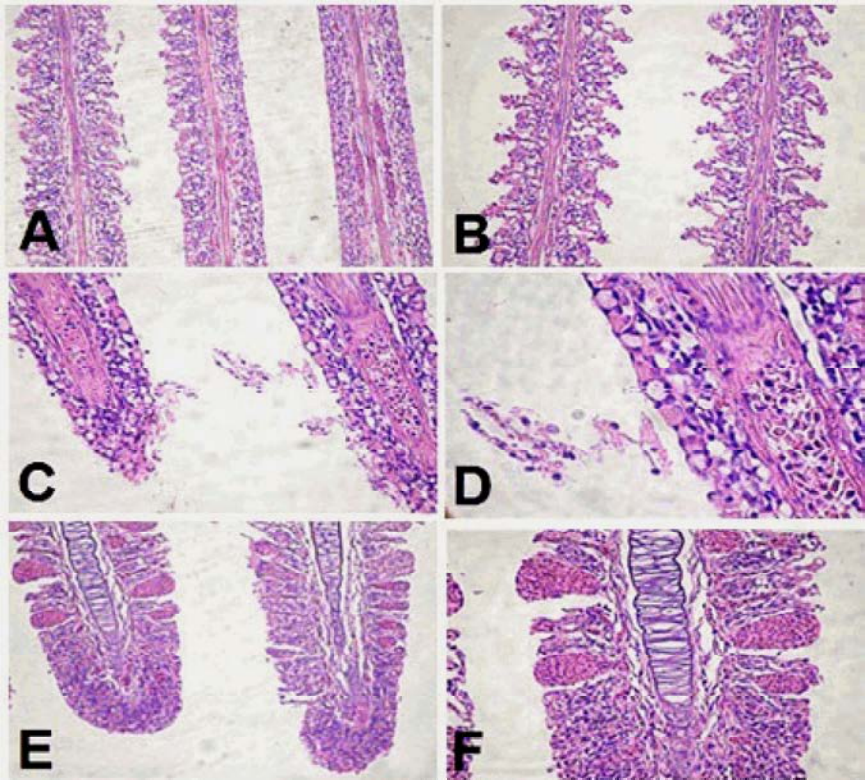

Fig. 4: Showing primary gill filaments suffered from (AandB) different and advanced stages of sever hyperplasia of secondary gill lamellae with infiltration of inflammatory cells,(C) hyperplasia and hypotrophy of mucous cells lining epithelial layer of primary gill filaments, (D) magnified hypertrophied mucous cells with congestion of branchial blood vessels (EandF) sever hyperplasia of secondary gill filaments with infiltration of inflammatory cells and telangiectatic secondary lamella.

Histopathological studies: Results revealed that the histopathological study of naturally infested fish with *Protochondracanthus alatus* displayed that, primary gill filaments suffered from advanced stages of sever hyperplasia of secondary gill lamellae with infiltration of inflammatory cells, also hyperplasia and hypotrophy of mucous cells lining epithelial cell layer of secondary gill filaments, with congestion of branchial blood vessels accompanied with (marbling appearance) with telangiectasis on the apical parts of primary lamellae Fig.4.

DISCUSSION

Copepods are the most important crustaceans in the world's oceans in that they are the predominant first-level consumers upon which the rest of the aquatic food chain is based. They play a major role in the science of parasitology. They not only act as intermediate hosts for a multitude of other parasites but are, in many cases, obligatory parasites themselves. Parasitic copepods belonging to order, Poecilostomatoida, are frequently reported from demersal fishes [5]. Information on parasitic copepods infestation along the coasts of the Arabian Gulf is limited. *Protochondracanthus alatus* that were recovered in the present study were also found in the gills of marine fish species at other localities. Irodeskuwaitensis (Copepoda, Taeniacanthidae) were isolated from the goatfish, *Upeneussulphureus* [9] as well as three species of caligid copepods (Siphonostomatoida) belonging to genus Hermilius were also reported from marine catfish, *Arius thalassinus* at the coast of Kuwait [11].

Depending on morphological characters which confirmed using light microscope and Scan Electron Microscope (SEM), the recovered copepode was identified as *Protochondracanthus alatus* [5]. The presence of *Protochondracanthus alatus* (Crustacea, Copepoda, Chondracanthidae) in present study represent the first record in the investigated locality Al-Qatef, Saudi Arabia.

Regarding the clinical sings and post-mortem lesions, present study revealed that infested fish with copepods *Protochondracanthus alatus* showed noclear visible abnormal external clinical signs, but in post-mortem examination, large number of the parasite, *Protochondracanthus alatus* observed on the gill filaments of the infected flatfish, *Psettodeserumei* as well as *Epinepheluschlorostigma*. The parasite appeared as long threads, white dots on the gill filaments. In heavily infested fishesthe tips of the gills was gray, frayed covered with excessive thick mucus and marbling or mosaic appearance may be appeared, the results nearly agree with that obtained by Andrews *et al*. and Fajer-Ávila *et al*. [3, 4].

The isolated copepods parasites may harm the fish in different ways. The crustaceans can be harmful by its presence; there is a degree of association between parasite and host (host specificity), duration of contact, relative size and feeding habits of the parasites as well as the hard body causing pressure atrophy of the soft tissues of the fish. The attachment of such parasites may lead to mechanical damage to the host. Finally, they cause great damage by feeding (depending on the type of food). Thus, all degrees of damage may vary in severity depending on the age and size of both fish and parasite as well as on the site of the parasite's activity [15].

Regarding the seasonal variation of copepods infestation in the investigated marine fish, thepresent study revealed that, the higher prevalence of infection was recorded at summer season (32.58%) while the lowest prevalence was recorded at autumn (19.45%), these may be due to that under overstocking conditions, parasite populations quickly build up and may cause heavy mortality in the stocked fish especially, in high water temperature where their life cycles become faster for these reason the prevalence was higher at summer season. The obtained results nearly agree with those of Boxshall and Marcelo *et al*. [5, 16] and Morales *et al*. [10] who reported that theseasonal pattern was only observed for*L. simplex*, with higher infection levels in the warmest month than in the coldest month. They added that the studyindicated that the intensity of *L. simplex* was positively correlated with water temperature.

Present study revealed that there are some investigated marine fish susceptible while, another not susceptible, to infection *Epinepheluschlorostigma* species and *Psettodeserumei* species only infested out of 22 species of marine fishes, *Epinepheluschlorostigma* was lower in infestation rate with *P. alatus* (16.66%) than *Psettodeserumei* (22.22%) there is degrees of association between parasite and host what is called (host specificity), so there is different degrees of associations between the copepod parasite and host for that reason some species was susceptible while another was not [3, 4, 15].

Regarding the prevalence the disease in relation to size of the infested host,present study revealed that the prevalence was increased with the increase of host size these meaning that there positive coloration between prevalence rate and size of the host these results coincide with the results ofMarcelo*et al.*[16] who reported thatprevalence is positively and significantly correlated with size only for the following parasites: *Ceratomyxa*sp., *L. edwardssi*, *Chondracanthuspsetti*, *Cainocreadium*sp., Opecoelidae (Metacercariae), *C. australis*, *P. decipiens* and *Anisakis*species and Etchegoin *et al*. [17] reported that all of the 995 copepods of the genus Chondracanthus collected from hake showed identical morphology and were identified as *Chondracanthusaustralis*, This species was detected on fish longer than 15 cm of total length and showed an increase in both prevalence and mean intensity in relation to the increase in the size of the host.

Regarding the histopathological examination, present study revealed that naturally infested fish with *Protochondracanthus alatus* displayed that, the primary gill filaments suffered from excessive advanced stages of sever hyperplasia of secondary gill lamellae with inflammatory cellsinfiltration, also hyperplasia and hypertrophy of mucous cells lining epithelial cell layer of secondary gill filaments, with congestion of branchial blood vessels accompanied by sever inflammation and necrosis of secondary gill filaments (marbling appearance) with telangiectasis on the apical parts of some primary lamellae, these results nearly agree with the results obtained by Basilio and Pablo [18] and Andrews *et al*. [3] who reported that the chondracanthid copepod, *Chondracanthusgoldsmidi*is an ectoparasite of gills, inner opercula and nasal cavities of cultured striped trumpeter, *Latrislineata* (Forster) *C. goldsmidi* was associated with extensive epithelial hyperplasia and necrosis. Pathological changes were most pronounced near the parasite's attachment site, with papilloma-like growths surrounding the entire parasite resulting in deformation of the filament. The number of mucous cells

increased near the parasite attachment sites on both the opercula and gills and Covello *et al*. [19] who reported that gill arch at the site of parasite attachment showing epithelial and mucous cell hyperplasia.

From present study it was concluded thatsome investigated marine fish susceptible and another not susceptible to infection with Protochondracanthosis, *Epinepheluschlorostigma* and *Psettodeserumei* species only infested out of the examined 22 species. *Epinepheluschlorostigma* was lower in infestation rate. In heavily infested fishesthe tips of the gills was gray, frayed, slimy with presence of numerous white dots, with congestion. Prevalence of Protochondracanthosis was increased in warmer seasons mainly summer with larger fish.

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