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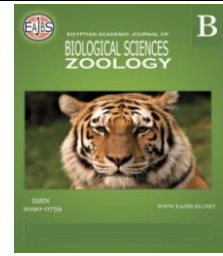


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External and Internal Changes Induced by Parasitic *Sacculina leptodiae* (Sacculinidae) on the Xanthid Crab, *Leptodius exaratus* (Xanthidae: Brachyura) from the Red Sea and Gulfs of Suez and Aqaba, Egypt

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ABSTRACT

The externae of parasitic sacculind, *Sacculina leptodiae* (Sacculinidae: Rhizocephala: Cirripedia) on the xanthid crab, *Leptodius exaratus* (Xanthidae: Brachyura) were recorded during this study. A total of 691 individuals (400 males and 291 females) of this crab were collected from the intertidal coasts of the Egyptian Red Sea, Gulf of Suez and Gulf of Aqaba, of them 38 (23 males and 15 females) were infected with this parasite. The overall infection rate recorded 5.50% for all populations and was slightly higher in males (5.75%) than in females (5.15%). It showed seasonal, spatial and sex variations, recorded the highest rate (7.91%) at Gulf of Suez, declined sharply to 3.85 % at Gulf of Aqaba, and 2.43 % at all populations of the Red Sea, recorded the minimum rate of 0.57% at the southern populations. Autumn has the highest rate (10.34 %) at Hurghada (northern Red Sea), followed by summer with 9.34 % and 7.69 % at the Gulf of Suez and Ras Mohammed (Northern Red Sea), respectively, declined to 2.94 % in summer at Gulf of Aqaba, but increased again to 5.56% during spring. A total of 41 externae were recorded on the infected individuals, comprised 35 individuals with single externa (92.11 %), and only three with double externe (7.89 %). The highest number of externae was 13 (31.70 %) occurred on the 6th abdominal segment, followed by 11 (26.82 %) on the 5th segments, declined to 1-5 on the other segments except the first. The size of externae varied from 1.0 to 10.4 mm in breadth, averaged 5.19 ± 2.76 mm in males and 5.63 ± 3.17 mm in females. The rootlets of internaenae of the parasite invaded ovaries, testes, hepatopancreas, and all spaces within the crab body cavities. The disappearance or destruction of testes in infected males accompanied by remarkable broadness and segmentation of abdomens fringed with dense and length setae lead to "Parasitic castration", compared with a hyperfeminization in infected females due to the destruction of undeveloped ovaries and increasing abdominal setae dense and length.

INTRODUCTION

The xanthid crab, *Leptodius exaratus* (H. Milne Edwards, 1934) belongs to family Xanthidae, within the superfamily Xanthoidea. Males are generally larger than females, and characterized morphologically in mature individuals by narrow and tapering abdomen, consists of 7 segments of them 3-5 are fused together. Their abdomens have only two pairs of uniramous pleopods (the first is the longest). In females, the abdomen is broad, with 7 distinct free segments, occupied by 4 pairs of biramous pleopods on segments 2-5. Both immature males and females have triangular abdomens, characterized with obvious locking mechanisms, remain at maturity in males, but disappeared in females. The genital openings are located on coxae of the 5th legs in males, while are found on the 6th thoracic sternites in females (Barnard, 1950; Serene, 1984; El-Sayed, 2004; Ng *et al.*, 2008; Ahmed, 2020).

The individuals of *Leptodius exaratus* are very common along the coasts of the Red Sea, Gulfs of Suez and Aqaba and Indo-Pacific regions (Ng *et al.*, 2008; Garth and Kim, 1983; Ahmed, 2020). It occurs in the intertidal and shallow subtidal of the mixed sandy and rocky areas, constructing its burrows underneath small stones, gravel, or rock boulders of different sizes. The reproductive cycle of this species is definite and extends from spring to late summer and early autumn, with maximum values during June-July. Ovigerous females were detected during the period from April to September and early October (Fouda, 2000; El-Sayed, 2004).

On the other hand, members of order Rhizocephala, which are among the most familiar orders of class Cirripedia, are highly specialized parasites of decapod malacostracans (Schmidt & Roberts; 1977; Buttler, 1980 and El-Sayed *et al.*, 1997, 1998; Alazaly, 2017; Nour El Deen *et al.*, 2019). Rhizocephala comprises three specialized parasitic families. Sacculinidae is specialized primarily parasites of brachyurans crabs (Guerin-Ganivet 1911; Boschma, 1933a,b, 1936, 1937, 1947, 1948, 1955a,b). The infective stage is a cypris larva, which settles on the cuticle of the base of a hair and enters the crab as a tiny cellular mass. Inside the host's body, the tiny cellular mass takes its position at the base of the hindgut caecum. After that, it develops gradually as a mass of ramifying rootlets which serve a nutritional function and penetrates through the body of the crab, even to the tips of the legs (Hartnoll, 1962, 1967, 2002; Schmidt & Roberts, 1977; Warner, 1977; El-Sayed *et al.*, 1997, 1998; Alazaly, 2017).

The effects of *Sacculina leptodiae* on xanthid crab, *Leptodius exaratus* were investigated by Guerin-Ganivet (1911), Boschma (1936, 1948), Siddiqui and Ahmed (1993), Moazzam and Moazzam (2004) along the Indo-Pacific regions including the Indian Ocean, Arabian Sea and Red Sea. Along the Egyptian Red Sea and its associated gulfs (Suez and Aqaba), El-Sayed *et al.* (1997, 1998) treated the effects of *Sacculina* sp. on the xanthid crab *L. exaratus*. Alazaly (2017) treated occurrences and effects of this parasite on *L. exaratus* at Hurghada (Red Sea). The rate of infection and morphological modifications, in addition, histological demonstrations were given and discussed by those studies.

Therefore, this study aims at through light on the rates of infection and effects of parasitic *Sacculina leptodiae* on the external morphology (secondary sexual dimorphism) and internal changes of the xanthid crab, *Leptodius exaratus* collected from the Egyptian coasts of the Red Sea, Gulf of Suez and Gulf of Aqaba.

MATERIALS AND METHODS

A total of 691 individuals (400 males and 291 females) of the intertidal xanthid crab, *Leptodius exaratus* were examined during this study. The majority specimens of this study (392) were collected from different sites at the northern regions of the Gulf of Suez

comprised: Suez City, Ain Sokhna, Adabia, Km, 32, Km, 40 and Km 85 and Porto Sokhna) at the northwestern coasts of the Gulf of Suez, and Ras Matarma (20 km south Ras Sudr) at the northeastern coasts of the Gulf. In addition, 247 comprised 40 specimens collected from Hurghada and 33 from Ras Mohammed Protected Area at the northern Red Sea, and 174 specimens collected from 20 sites extending along the Red Sea coast from South Safaga to Shalatein. Other 52 specimens of crab were collected from Protected Areas along Gulf of Aqaba (Nabq, Abu Galoum and Taba Protected Areas). All sites of the collection are indicating in Figure (1).

The majority of specimens were collected from the Suez Gulf during the period from April 2014 to February 2018, in addition to Ras Mohammed, in September 2017, Hurghada in February 2018 and Tab in June, 2015. However, the specimens of the Red Sea and Gulf of Aqaba were collected previously during the period from April 1994 to April, 1996 and from July 19934 to May 1995, respectively, and were preserved among the Reference Collection of Al-Azhar University.

The individuals of the host crab were collected by hand from the rocky and mixed sandy –rock intertidal and shallow subtidal zones during low tide or using snorkeling during high tide. The collected individuals were preserved immediately in a 10% seawater formalin solution. All available information on the color of life individuals, carapace case, and occurrence of parasite or its scars on the abdomen of crabs, as well as presence or absence of ovigerous females were recorded.

At the laboratory, the collected crabs were sorted and sexed into mature and immature individuals and identified according to Serene (1984) and compared with those deposited in the Reference Collection of Al-Azhar University and that described by Ahmed (2020). The carapace length (Cl), carapace width or breadth (Cw), length of propodal right chela (Rchl), the height of right chela (Rchh), 6th abdominal segment width (Abw) in females, abdominal setae length for both normal and infected sexes, and right pleopodal length (Pl.) for all normal and infected males were measured by vernier caliper with an accuracy of 0.01 mm and recorded to the nearest 0.1 mm.

Extrenae were cleaned, then examined using a Stereo- binocular microscope (OPIKA microscope, SZM-1, SN: 402748, Italy) and identified according to the description given by Boschma (1937, 1947, 1848, 1955a ,b,1969) and Alazaly, (2017). The number, position and color of externae on the crab's abdomen were recorded. The size of the externae was measured to the nearest 0.1 mm using a caliper vernier.

The dimensions of externa comprised:

Exerna breadth: Is the maximum distance between the two lateral far points on the lateral sides of the externa;

Externa thickness: It includes the maximum distance between the anterior and posterior points on the externa.

Externa highest: It is the maximum distance between the upper and lower points on the externa.

The parasitized crabs (males and females) were dissected to follow up the roots of parasites within different organs, particularly between gonads and hepatopancreas branches and compared with those in normal crabs.

All measurements were treated statistically using Excel statistical program, where an average \pm SD, T-test, and X^2 - values were calculated.



Fig.1: Map shows sites of the collection during this study.

RESULTS

I- Parasitizing of *Leptodius exaratus* with *Sacculina leptodiae*:

A- Morphology of the Host Crab, *Leptodius exaratus*:

All morphometric measurements for both normal and infected crabs are given in Table (1). The individuals of *L. exaratus* have a xanthoid-shaped carapace (Plates I-III), being broader than long, with a ratio of 1.51:1 (breadth: length). The carapace has well-defined regions, appear projected and separated by deep grooves, with surface varied from granular to smooth and sometimes being finely punctate. The anterolateral margins of the carapace are convex, characterized by four-pointed lobes; the first is the smallest than others; while the postero-lateral margins are concave and smooth on their outline. The front is slightly convex, without a marginal crest, has a median fissure but does not extend beyond supra-orbital angles. The front is separated from supraorbital angles by a deep notch. Chelipeds are stout, nearly unequal to equal; chelae and fingers have spoon-shaped extremities. Superior margins of palm with roguer outline; cutting margins with obtuse teeth. The male abdomen has seven segments, divides into 5 segments, 3-5 are fused together into a single unit; sometimes their sutures are visible. First male pleopods are slender, with a straight tip, but without hairs (Plates III a,d). Females have seven free abdominal segments; segments from the 2nd to 5th are equipped with biramous pleopods (Plates I h & II h).

Color: Variables according to habitats, varied from yellowish, whitish to faint with scattered brown spots of different sizes. Fingers and thumbs are dark brown or black with white extremes.

Habitat: This species usually lives under small stones in mixed sandy and rocky areas at the water line.

Status: It is common to frequent and was found in a considerable number in the upper intertidal rocky and sandy areas.

Distribution: Red Sea, Gulf of Suez and Gulf of Aqaba.

Table (1): The morphological ratios in normal (uninfected) *L. exaratus* crabs and those infected with *S. leptodiae* collected from the Gulfs of Suez and Aqaba and Red Sea, Egypt

| Items Characters | | Normal | | Infected | |
|---------------------|---|------------|------------|------------|------------|
| | | Males | Females | Males | Females |
| CW/ RCL | X | 1.51±0.20 | 1.66±0.17 | 1.54±0.22 | 1.72± 0.04 |
| | R | 0.89-2.05 | 1.00-1.96 | 1.06-2.18 | 1.66-1.79 |
| CW / RCH | X | 3.22±0.48 | 3.67±0.37 | 3.39±0.61 | 3.35±0.45 |
| | R | 2.20-4.20 | 2.18-4.29 | 2.41-4.77 | 2.13-3.90 |
| CW/AbdW | X | 9.70±1.26 | 4.28±0.53 | 6.81±2.07 | 3.62±0.47 |
| | R | 7.70-15.20 | 2.88-5.60- | 3.00-9.50 | 2.28-4.22 |
| CL / FPL | X | 2.30±0.30 | - | 2.32±0.35 | - |
| | R | 1.80-4.00 | - | 1.76-2.95 | - |
| Abd.SL mm | X | - | 1.59±1.72 | 0.58±0.23 | 1.98± 0.43 |
| | R | - | 0.8-1.7 | 0.2-1.0 | 1.30 2.9 |
| CW (mm) | X | 17.40±5.77 | 13.68±3.44 | 19.3±6.10 | 18.0±3.10 |
| | R | 5.50-30.60 | 7.80-21.90 | 10.8-30.4 | 11.2-23.40 |
| CL (mm) | X | 11.90±3.67 | 9.44±2.30 | 13.2±0.42 | 12.5+ 1.90 |
| | R | 4.00-19.60 | 5.30-14.80 | 6.6- 20.30 | 8.6- 16.20 |

B- Incidence and Rate of Infection of *Sacculina leptodiae*:

Results in Table (2) show the incidence or the rate of infection among different populations of *L. exaratus* of the parasitic, *S. leptodiae*. Out of 691 individuals (400 males and 291 females) of *L. exaratus*, 38 individuals (23 males and 15 females) were infected with *S. leptodiae* (Plates, I-III) of the 31 (81.58 % of all) were recorded at Gulf of Suez, compared with 3 (7.89 %) at Gulf of Aqaba and 4 (10.63 %) at the Red Sea. The infection rate recorded 5.50% between all populations at the different study areas, and was relatively higher in males (5.75%), than in females (5.15%), and showed seasonal and spatial variations at the studied sites as following:

-Spatial Fluctuations in Infection Rates:

The present results indicated that Gulf of Suez has the highest number of individuals and a higher infection rate (Table, 2). The overall rate of infection for the Suez Gulf population recorded 7.91 %, but females had a slightly higher rate (8.33 %), than males (7.59 %) without significant difference ($P > 0.05$). The rate of infection declined sharply to 3.85 % and 2.43 % at the entire Gulf of Aqaba and Red Sea, respectively. However, within the Red Sea, the rate of infection was greatly fluctuated and recorded 7.5 % at Hurghada and was significantly higher ($P < 0.01$) in females (20.0%) than in males (3.33%). It declined to 6.06% at Ras Mohammed and was restricted only within males (14.7%) and reached the minimum rate of infection (0.57%) at the southern Red Sea where only one female was infected and represented 0.98 %. At the Gulf of Aqaba, all infected individuals were males (6.67 %), and no infection was recorded at Nabq Protected Area, but the infection was restricted at Taba and Abu Galoum Protected Area only (Table, 2).

- Seasonal Fluctuations in Infection Rates:

Results in Table (2) show that the highest number of infected individuals of *L. exaratus* with *S. leptodiae* was recorded at Gulf of Suez. During summer, 24 infected individuals were recorded, giving the highest infection rate of 9.34 %. It declined to 8.43 % in autumn, while no infection was recorded during spring and winter.

At the Red Sea, the occurrence of externae of *S. leptodiae* was recorded during summer and autumn only and recorded 10.34 %, 7.67 % and 2.04 % during autumn, summer

and summer at Hurghada, Ras Mohammed and the southern Red Sea, respectively. It was the high in females at Hurghada during autumn (40 %) than males (4.17 %), while the reverse was recorded during summer at Ras Mohammed and recorded 16.67 % in males and no infection was detected in females. At Gulf of Aqaba, the infection rates recorded 5.56% and 2.94 % during spring and summer at Gulf of Aqaba.

Table 2: Prevalence of parasitic *Sacculina leptodiae* on *Leptodius exaratus* from the Egyptian coasts of the Red Sea and Gulfs of Suez and Aqaba (seasons are pooled).

| Areas & Seasons | | Sex& ratios | | Examined crabs | | | infected crabs | | Prevalence % of infection | | |
|------------------------|----------------------|-------------|-----|----------------|-----|-----|----------------|-------|---------------------------|-------|------|
| | | No. | ♂♂ | ♀♀ | ♂♂ | ♀♀ | ♂♂ | ♀♀ | ♂♂&♀♀ | | |
| Red Sea | Hurghada | Au | 29 | 24 | 5 | 1 | 2 | 4.17 | 40.0 | 10.34 | |
| | | Win. | 11 | 6 | 5 | 0 | 0 | 0 | 0 | 0 | |
| | | Total | 40 | 30 | 10 | 1 | 2 | 3.33 | 20.0 | 7.50 | |
| | Ras Mohammed | Su. | 26 | 12 | 14 | 2 | 0 | 16.67 | 0 | 7.69 | |
| | | Sp. | 7 | 2 | 5 | 0 | 0 | 0 | 0 | 0 | |
| | | Total | 33 | 14 | 19 | 2 | 0 | 14.29 | 0 | 6.06 | |
| | Southern Red Sea | Sp. | 121 | 76 | 45 | 0 | 0 | 0 | 0 | 0 | |
| | | Su | 49 | 24 | 25 | 1 | 0 | 4.17 | 0 | 2.04 | |
| | | Au. | 4 | 2 | 2 | 0 | 0 | 0 | 0 | 0 | |
| | | Total | 174 | 102 | 72 | 1 | 0 | 0.98 | 0 | 0.57 | |
| | Total Red Sea | | | 247 | 146 | 101 | 4 | 2 | 2.74 | 1.98 | 2.43 |
| | Gulf of Aqaba | Sp. | 18 | 13 | 5 | 1 | 0 | 7.69 | 0 | 5.56 | |
| Su. | | 34 | 17 | 17 | 1 | 0 | 5.88 | 0 | 2.94 | | |
| Total | | 52 | 30 | 22 | 2 | 0 | 6.67 | 0 | 3.85 | | |
| Gulf of Suez | Sp. | 45 | 33 | 12 | 0 | 0 | 0 | 0 | 0 | | |
| | Su | 257 | 137 | 120 | 12 | 12 | 8.76 | 10.0 | 9.34 | | |
| | Au | 83 | 51 | 32 | 5 | 2 | 9.80 | 6.25 | 8.43 | | |
| | Win. | 7 | 3 | 4 | 0 | 0 | 0 | 0 | 0 | | |
| | Total | 392 | 224 | 168 | 17 | 14 | 7.59 | 8.33 | 7.91 | | |
| Total all sites | | | 691 | 400 | 291 | 23 | 15 | 5.75 | 5.15 | 5.50 | |
| | | | | | | 38 | | | | | |

Note that: Au= autumn, Win.= winter, Sp.= spring, and Su.= Summer.

C - Position and Occurrence of Externae:

The occurrence of externae on the abdominal segments of the host crab, *L. exaratus*, was varied according to the number (single or double) of extruded externae (Table, 3). It was found that, out of the recorded 38 infected individuals, 35 were carrying only single externa and represented 92.11 %, compared with three individuals have double externe (7.89 %) on separate two abdominal segments of each. Single externae were occurred and distributed either on the 2nd, 3rd, 4th, 5th and 6th segments in the sequence of 3, 3, 1, 11 and 12 respectively, or occurred in-between the segments of 2nd -3rd, 5th - 6th and 6th -7th (telson) and represented by 1, 3 and 1 externae, respectively. The double externae were also varied and occurred on the 2nd, 3rd, 5th, and 6th segments as 2, 1, 1 and 2, respectively (Table, 3).

It was also noticed that, no specific sequence for the occurrence of externae based on their size. In two specimens with double externae, the largest ones were found on the 5th and 6th segments, but in the third, the largest externa was found on the 2nd segment, while the smallest externa was lying on the 6th abdominal segment

These results indicated that, the highest number of extrenae (13) has occurred in the 6th segment, represented 31.70 % of all, followed by 11 on the 5th segment, represented 26.82 %, then 5 on the 2nd segment, represented 12.2 % and 4 on the 3rd (9.76 %), but only one on the 4th segment (2.44 %); while those in-between segments recorded 1, 5 and 1, represented 2.4 %, 12.2% and 2.4%, respectively (Table, 3).

Table 3: Numbers and positions of *S. leptodiae* externae on the abdominal segments of the host crab, *L. exaratus* during this study.

| Externae | Abdominal segments | | | | | | | | |
|-----------------------|--------------------|-----------------|----------|-----------------|-----------------|-----------------|----------|-----------------|----------|
| | No | 2 nd | 2-3 | 3 rd | 4 th | 5 th | 5-6 | 6 th | 6-Telson |
| Single (n=35) | 35 | 3 | 1 | 3 | 1 | 10 | 5 | 11 | 1 |
| Double (n=3) | 6 | 2 | 0 | 1 | 0 | 1 | 0 | 2 | 0 |
| Total externae | 41 | 5 | 1 | 4 | 1 | 11 | 5 | 13 | 1 |
| Percentage (%) | - | 12.2 | 2.44 | 9.76 | 2.44 | 26.82 | 12.2 | 31.70 | 2.44 |

D- Size of Externae:

The present results showed that *S. leptodiae* externae were varied according to developmental stages of maturity, the incidence of double infections and even between sexes. The externae varied in size from 1.0 – 10.4 mm in breadth (B), 1.5- 7.1 mm in height (H) and 1.0 - 4.0 mm in thickness (Th).

The overall breadth of externae averaged 6.06 ± 2.89 mm, and was 5.77 ± 2.65 mm in males and 7.19 ± 2.81 mm in females, without significant difference between sexes ($P > 0.05$, $DF = 34$). Externae were varied in height and thickness averaged 4.49 ± 1.43 and 5.90 ± 1.39 mm in height, 1.87 ± 0.79 and 2.76 ± 1.14 mm in thickness in males and females, respectively, with the only significant difference in thickness between males and females ($P < 0.05$).

Externae breadth showed sharp seasonal variations between sexes. It averaged 7.58 ± 2.11 and 3.77 ± 3.30 mm during summer and autumn between males, respectively, with a statistically significant difference ($P < 0.05$). In females, externae breadth averaged 5.06 ± 2.56 mm in autumn and 5.31 ± 3.10 mm in summer at Ain Sokhna without significant differences between seasons ($P > 0.05$).

II- External Changes in The Host:

Results in Table (1) show values of changes in the external secondary sexual characters in both normal and infected individuals of *L. exaratus* infected with *S. leptodiae* particularly those who carry maturing exterane. The most prominent changes were noticed in remarkable increasing abdominal width with obvious sternum invagination and broadness in infected males (Plate III c&d) compared with normal uninfected ones. In infected males, the ratio of abdominal width in carapace width (CW/ADW) averages 6.81 ± 2.07 compared with that in normal males which average 9.70 ± 1.26 , and the difference was statistically significant ($P < 0.05$). In females, the increasing ratio was slightly low averaged 3.62 ± 0.47 in infected females and 4.28 ± 0.53 in normal females without significant difference ($P > 0.05$). In addition, there are obscuring segmentation and appearance aberration at lateral abdominal borders.

The second prominent morphological changes were detected in the increasing densities and lengths of abdominal setae in infected males (Table 1 and Plate II). The fringed abdominal setae were varied from 0.20 to 1.0 mm and averaged 0.58 ± 0.23 mm, but cannot precisely measure in normal males. In females, a remarkable increase was noticed and increased from an average value of 59 ± 1.72 mm in normal females to an average of 1.98 ± 0.43 mm in infected females, with maximum setae length up to 2.9 mm (Table, 1).

Other changes in the ratios of right chelae length and height in carapace width had increased slightly in infected females and averaged 1.72 ± 0.04 and 3.35 ± 0.45 , respectively, compared with 1.66 ± 0.17 and 3.67 ± 0.37 in normal females (Table, 1).

On the other hand, in infected males, the ratio of pleopodal length in carapace length did not greatly vary, but the first pleopods appeared weak and slender in general morphology. Also, both the carapace length and carapace width showed a wide range in size and have higher averages in infected males and females (Table, 1).

III- Internal Changes in The Host Crab:

A- Gonad Morphology of The Normal Crabs:

The present investigation indicated that the gonads (testes and ovaries) in normal examined males and females of *L. exaratus* crab are paired structure, located under the carapace directly alongside the stomach. Ovaries are thin, very small, slender and translucent in early immature stages, become lobulated during the maturing stage. Their colours varied from whitish to faint yellowish at the beginning transferred to deep yellow and orange at end of this stage. The ovaries extend anteriorly as lobulated lobes along the laterodorsal sides of the stomach and posteriorly under the pericardium and connect together by a transverse bridge of the gonadal tissues forming an H-shaped structure. The anterior lobes of ovaries extend between the hepatopancreas and gill chamber and posteriorly until the beginning of abdomen. At H-shaped, paired of short oviducts emerge vertically and open in a posterior sac-like structure, called “spermathecae”, in which sperms are stored. The spermathecae open with two short ducts on the sterinte of the 6th thoracic segments by a pair of obscure gonopores (Plate I).

In males, testes are also paired structure and have H- shaped, laying also along-side the laterodorsal side of the stomach. Testes are conspicuous in mature individuals, and each testis connects to a vas deferens which extends as deferential connected together forming an H- shape alongside the stomach and appear as a pair of thin whitish tubules. The vasa different extend behind the stomach and become swollen with stored sperms and run beneath the pericardium. The vasa differentia lead into ejaculatory ducts opens on the ventral side of the coxal segments of the last walking legs (5th legs). They have tiny papillae that extend into the bases of the first pleopods (Plate I).

B- Changes in Gonads:

In infected males, particularly those carrying large and maturing externae (≥ 7.6 mm), the testes were either destroyed or enlarged have swollen empty vasa deferentia, with dark brown color without any traces of sperms. In other infected males carrying maturing externa of ≥ 8.0 mm breadth, and those infected with an immature one of 3.3 mm, testes were found but invaded with heavy rootlets of internal parts of the parasitic *Sacculina*. In the infected female, ovaries varied from completely destroyed and undeveloped in most specimens, and when present they were vacuolated and occupied by irregular and resorbed eggs. Rootlets of the parasite was detected and present in high density.

C- Changes in Hepatopancreas:

These results showed also that, internal rootlets of *S. leptodiae* were observed around and within hepatopancreas, and around the intestine, as well as in all spaces of the crab's body cavities.

IV-Parasitic Castration:

The present investigations showed that internal changes were well represented by the complete disappearance of testes in 10 infected males. In females, ovaries were either destroyed or undeveloped and if present no ovulation was detected among infected females. Moreover, no evidence for the presence of ovigerous females infected with *Sacculina* during the spawning season in spite of obtained 50 ovigerous females from all studied sites. Therefore, the infected females were characterized by increasing abdominal setae length and dense, and remarkable segmentation of abdomens a phenomenon called “ hyperfeminization” (Plate, II E&F).

The disappearance of testes and androgenic glands in infected males, lead to modifications and changes in the secondary sexual characters, represented mainly by broadness and segmentation of male abdomens, fringed with marginal abdominal setae, and obvious invagination of the male sternum in some males is known as “Parasitic castration” with external remarkable changes (Plate, III, C-D).

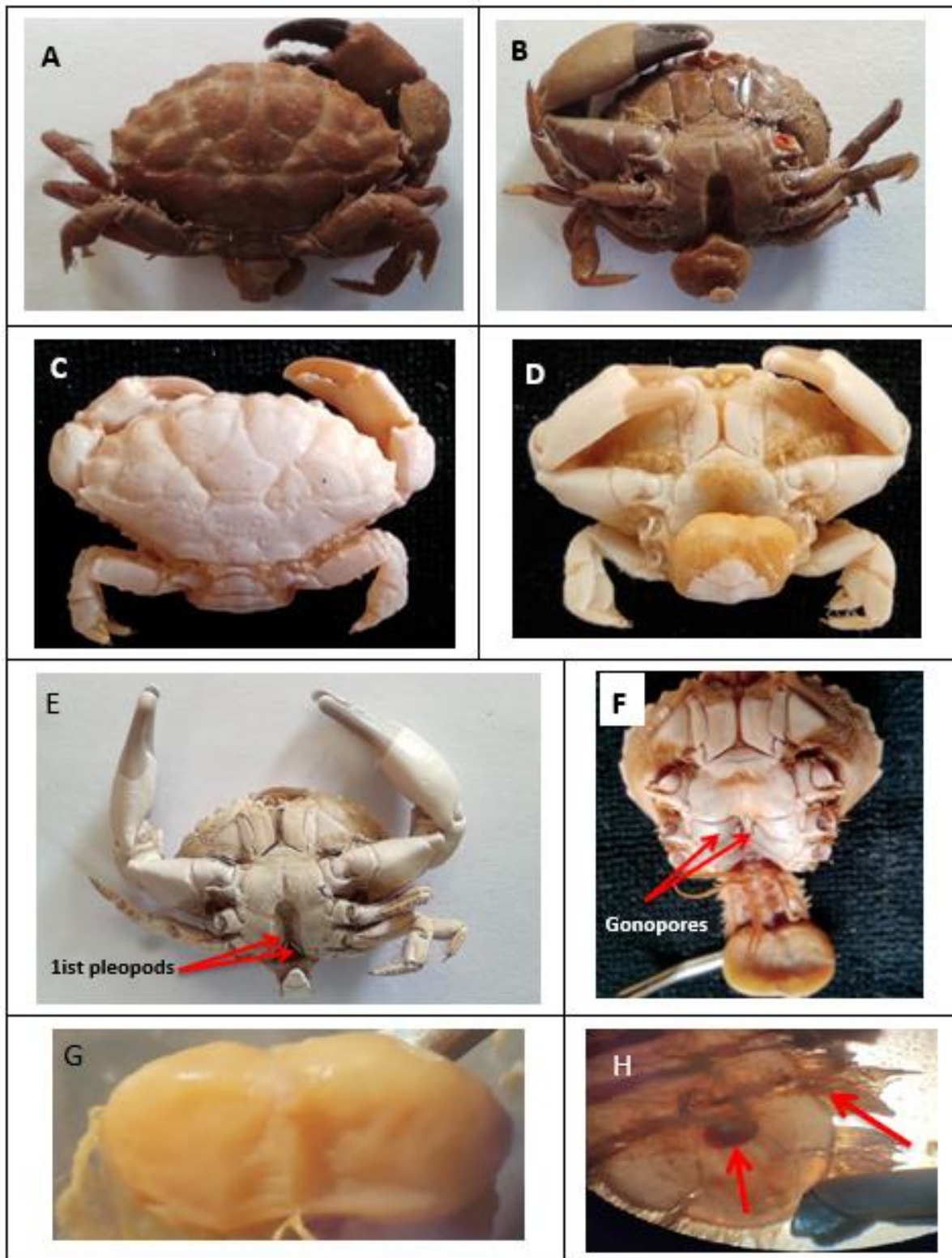


Plate I: Shows dorsal and ventral views of infected male (A &B), infected female (C&D); ventral view of normal male showing pair of pleopds (E), ventral view of female showing gonopores and pleopods (F), Isolated full mature externa (G), and scar of discarded *Sacculina* on female's abdomen with dense fringed setae (H).

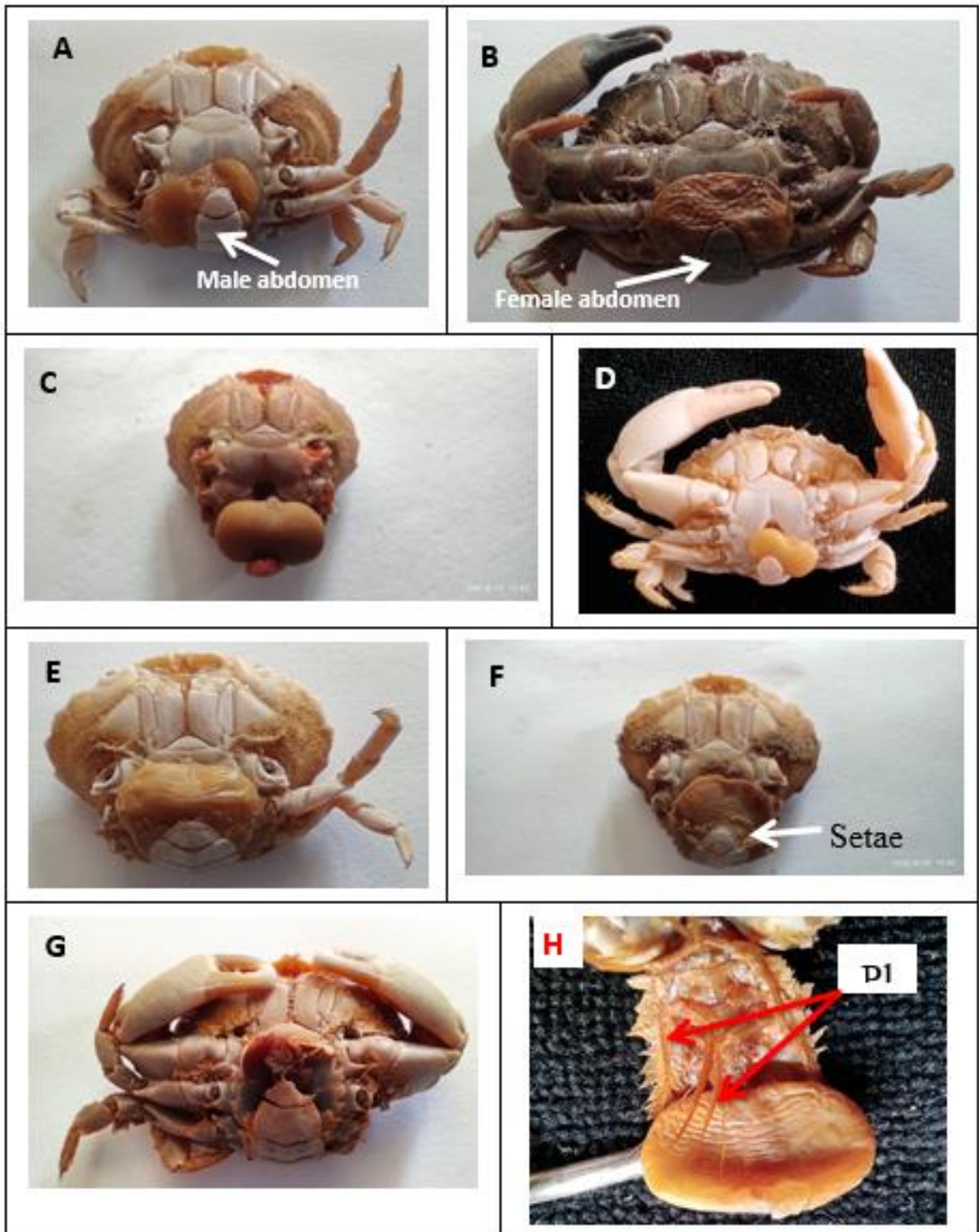


Plate II: Shows infected males of *L. exarartus* carrying externae of *S. leptodiae* at variable sizes (A-F), arrows refer to broad abdomens (A& B); infected females with externae (E-H), notice abdominal setae "S"(F-H) and female pleopods "PI" (H).

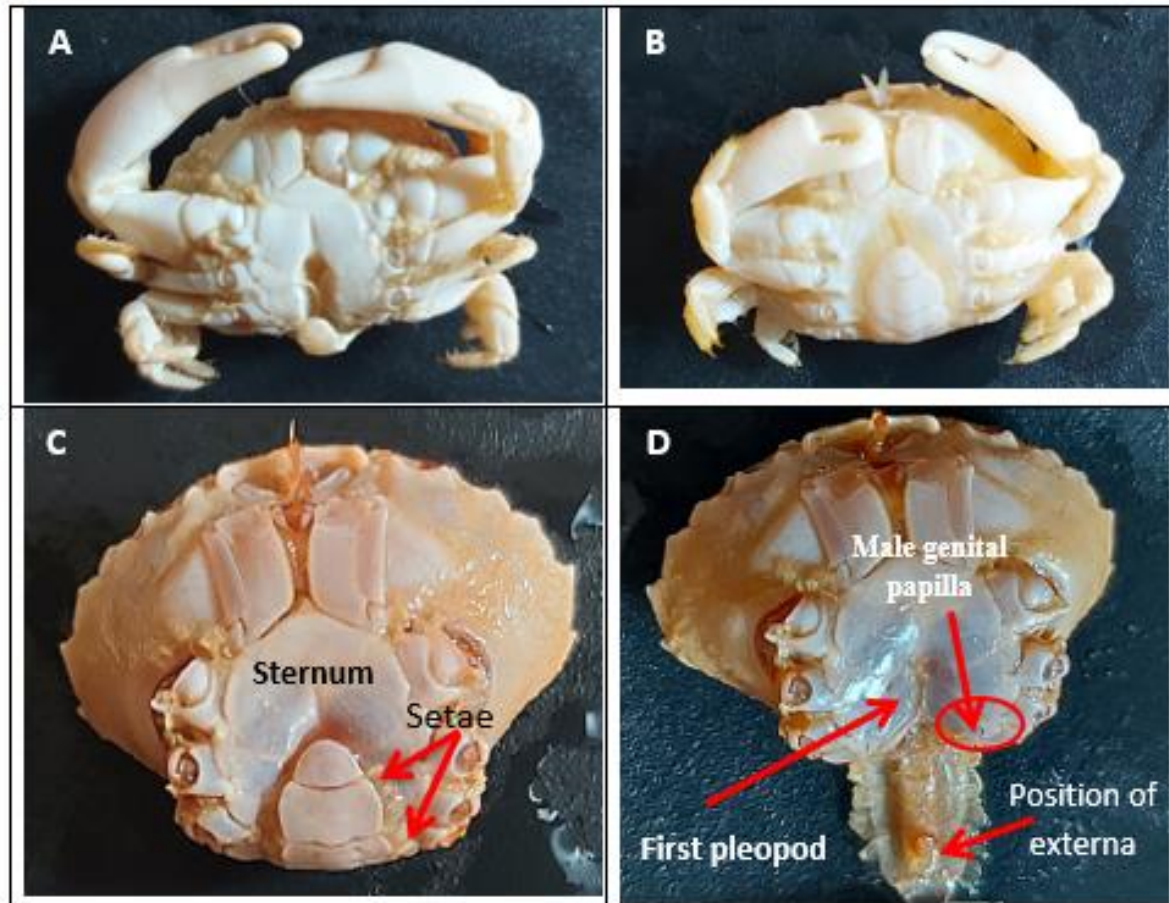


Plate III: Shows ventral views of *L. exaratus* normal male (A), normal female (B), broad abdomen fringed with setae in male infected with *S. leptodiae* (C), and infected male (D) has scar of externa, fringed setae, weak and fragile pleopod, invagination of sternum.

DISCUSSION

During this study, a total of 41 externae (35 single and 3 double) of the parasitic rhizocephalan crustacean, *Sacculina leptodiae* Guerin- Ganivet (1911), family Sacculinidae, were detected on the abdomens of 38 individuals (23 males and 15 females) of *L. exaratus* collected from the Red Sea, Gulf of Suez and Gulf of Aqaba. The infection of *L. exaratus* with this parasite was recorded for the first time and described as a new species by Guerin- Ganivet (1911) on the type specimens of *Chlorodius exatarus* (= *Xantho hydrophilus* a synonym of *Leptodius exaratus* from Djibouti, the Gulf of Aden and east coast of Africa. The occurrence of this parasite was reported by Boschma (1936, 1948, 1955a, b) on the individuals of *Xantho exaratus* (= *Leptodius exaratus*) from the Gulf of Suez and the Red Sea as well as from Zanzibar and Grand Comoro Island (Indian Ocean). The parasitic *S. leptodiae* not specific for *L. exaratus*, but it infects other brachyuran crabs including *Leptodius gracilis*, *Pseudozium caystrus*, *Lachnopodus* sp. (Xanthidae), *Thalamita stimpsoni* (Portunidae), and *Trapezia* sp. family Trapezoidea (Boschma, 1936, 1947, 1948, 1955a,b), in addition to *Camposcia retusa* (Majidae) and the portunid, *Carupa laeviuscula* which need further confirmation as pointed out by Boschma (1948). This parasite has a wide distribution and infects *L. exaratus* in all Indo-Pacific regions as reported by Boschma (1936, 1947, 1948, 1954a,b, 1955a,b; Chan, *et al.*, 2005). It was reported infects *L. exaratus* at coasts of Pakistan (Arabian Sea) by Siddiqui and Ahmed (1993) and Moazzam and Moazzam (2004) and from

the Red Sea by El-Sayed *et al.* (1997, 1988) and Alazaly (2017).

However, the xanthid carb, *Leptodius exaratus*, is infected with other rhizocephalan parasites, which comprise *Loxothylacus amoenus*, *L. brachybris* and *Sacculina sinensis* (Boschma, 1933 a, 1954; Chan *et al.*, 2005). The infection of *L. extaratus* with *S. sinensis* is restricted in China only due to the short larval development of this parasite (Boschma, 1933 b; Chan, *et al.*, 2005). It is worth mentioning that, *S. sinensis* beings very similar to *Sacculina leptodiae* in size of their externae but they are morphologically different in several characters and general outline as described by Boschma (1933 b) and Chan *et al.* (2005).

The obtained results showed that the overall rate of infection was 5.50 % for all populations at the studied sites. It was relatively higher in females than in males and showed spatial and temporal variations. The highest rate was 7.91 % recorded at Suez Gulf populations, followed by 3.85 % at Gulf of Aqaba, but the lowest rate (2.43 %) recorded at all Red Sea populations comprised Ras Mohamed, Hurghada and southern coasts, reached the minimum rate of infection (0.57 %) at the southern Red Sea populations. The overall rates of infection at the present results are higher than that reported by El-Sayed *et al.* (1997, 1998), which recorded 3.52 % at the same sites, which was higher in males (4.17) than in females (2.73 %). However, the obtained values of overall infection rates are lower than 7.49 % reported by Alazaly (2017) among the population of *L. extaratus* at Hurghada infected with this parasite and was higher in females (11.49) than in males (4.91 %). It was also higher than that reported by Siddiqui and Ahmed (1993) along Karachi coast which recorded 3.44% at Buleji and varied from 3.21% in males to 3.72% in females at Manora (Karatchi, Pakistan). However, these results are generally very low compared with that reported by Chan, *et al.* (2005) on the same species infected with *S. sinensis* in China reached 71.7 % and in other species of true crabs studied by Lutzen and Takashi (1997) and Raffi (*et al.*, 2012).

The rate of infection was also seasonally varied, recorded 9.34 %, 10.34 % and 5.56 % during summer, autumn and spring at Suez Gulf, Hurghdad and Gulf of Aqaba populations, respectively. However, it declined sharply to 2.04 % during autumn at Gulf of Suez and reached 0.49 % during summer at the southern Red Sea coasts and showed variations between sexes within each site. These results are in agreement with that reported by El-Sayed, *et al.* (1997), but are in contrast with Alazaly (2017), recorded the highest rate (21.74 %) during autumn and the lowest rate (5.78 %) in winter, and was moderate in summer and spring, with high rate within females than males during all seasons.

The fluctuations in the infection rate during this study indicate that, *S. leptodiae* may be breed and spawn all the year-round. But it depends upon the prevailing environmental conditions as strong waves and currents, in addition to activities and molting stages of the host during different seasons which enable cypris larvae to infect their hosts. These results are in accordance with that recorded by Siddiqui and Ahmed (1993) indicated that, *Sacculina* breed throughout the year whereas April to August is the main recruitment season.

The present results study showed that, the position and occurrence of externae were variables, but they were restricted either on or in-between the 2nd and 6th abdominal segments. The highest numbers of externae were 13 and 11 recorded on the 6th and 5th segment respectively, while other segments were carrying low numbers varied from one to five. These results are in agreement with that reported on the same species by El-Sayed *et al* (1997, 1998) from the Red Sea and its associated gulfs (Suez and Aqaba) or at Hurghada by Alazaly (2017). The occurrence of the highest numbers of externae on the 6th and 5th segments may be attributed to the flexibility of articular membrane between those abdominal segments, particularly in males due to the fused nature of the 3rd to 5th in one unit without segmentation. The occurrence of externae on the last abdominal segments may provide wide space for maturation and help in fanning movements on the externae as a physiological behavior for infected hosts in both males and females as described by Hartnoll (1962, 1967),

Warner (1977), Schmedit and Hinam (1977); Goddard *et al.* (2005), and Waiho, *et al.* (2017).

Boschma (1948) stated that, the externae may be extruded from any segments, but those on the last abdominal segments have large space for increasing size due to the effects of depressing between the host's abdomen and sternum. Lutzen and Takashi (1997) mentioned that externae of *Sacculina polygenea* can be extruded on the abdominal segments from 1-4 in *Hemigrapsus sanguineus*.

For double extrane, no specific sequence for their occurrence based on size was detected. In the two male specimens carrying double extrane, the largest ones are arising on the 2nd and the 5th segments; while the smallest ones occurring on the 3rd and 5th segments, respectively. In the female, the largest externa was on the 6th and the small one on the 4th segments, respectively. El-Sayed *et al.* (1997, 1998) recorded only one female with double extrane, carrying small externae on the 4th segment and large externa on the 6th segment. At Hurghada (Red Sea), Alazaly (2017) recorded two females carrying double extrane. In one female the two extrane were similar in size (6.5 mm for each) and arising on the 3rd and 4th segments, while the second female has the largest externa on the 6th and small one on the 4th segment. Multiple extrane were recorded by Boschma (1933 a) on *L. exaratus* infected with *S. sinensis*. They reached five and two externae, in addition to single one, while Chan *et al.* (2005) reported three extrane of this parasite on *L. exaratus* from Hong Kong (China).

Hartnoll (1967) stated that the occurrence of double or multiple externae on the same host is due to either re-infection with the new larvae as demonstrated by Rees and Glenner (2014) or to polyembryony for the parasite species. Lutzen and Takashi (1997) attributed multiple externae of *Sacculina polygenea* parasitizing the intertidal crab *Hemigrapsus sanguineus* at Japan to numerous internal tumors (primordial parasites) which arise by asexual reproduction through budding from the root system of the parasite. They stated that the old externae die and eaten by the host, and then are replaced 1-3 times during the life of a crab. Consequently, the average number and size of the externae increase at each instar. Therefore, *Sacculina polygenea* offers the first proven case of asexual reproduction in the family Sacculinidae according to Lutzen and Takashi (1997), Takashi *et al.* (1997), and Takashi and Lutzen (1998).

The size of *S. leptodiae* externae was varied from 1.0 – 10.4 mm in breadth, 1.5- 7.1 mm in height and from 1.0 to 4.0 mm in thickness. They have an overall breadth averaged 5.9 ± 2.97 mm, and varied from 5.19 ± 2.76 mm in males to 5.63 ± 3.17 mm in females. These results are very close to sizes of externae of the same species reported by Guerin-Ganivet (1911), Boschma (1936, 1948, 1955a), Siddiqui and Ahmed (1993), El-Sayed *et al.* (1997, 1998), Moazzam and Moazzam (2004), and Alazaly (2017) on *L. exaratus* from the Red Sea and other Indo-Pacific localities.

The effects of infection with *S. leptodiae* were accompanied by pronounced alterations in some external characters and internal organs in both infected males and females of *L. exaratus* during this study. A significant increase in the breadth of abdominal segments of infected males was obvious in comparison with those in the normal individuals. Also, a remarkable invagination in the thoracic sternites, increasing density and lengths of associated fringed setae in addition to the loss of locking mechanism, as well as disappearance of testes were detected. However, the changes in porpodal chelae length and height and pleopodal length were relative and may be attributed to parasitism. These results are in agreement with those recorded by Siddiqui & Ahmed (1993) and El-Sayed *et al.* (1997, 1998) but are relatively lower than those reported by Alazaly (2017). El-Sayed *et al.* (1998) attributed these changes to disturbance or increasing testosterone levels in infected crabs. It is worth mentioning that, all reduction in male secondary sexual characters are affected by the hypertrophy of the androgenic gland and ceasing of androgenic hormones which acquiring the female-type, due to the effecting of female sex hormones released from the ovaries of the

hermaphrodite parasite over dominated by ovarian size (Barington, 1967; Highnam & Hill, 1977; Schmidt & Roberts, 1977; Warner, 1977; Sumpton *et al.*, 1994; El-Sayed *et al.*, 1997, 1998; Takashi and Lutzen, 1998; Rees *et al.*, 2014). Therefore, the infected males behave as ovigerous females due to the occurrence of externae on their abdomens, where fanning the externae by the rapid movement of the abdomen (Jeffrey *et al.*, 2005; Waiho, *et al.*, 2017). This behavior is common in all infected brachyuran males and due to secreted peptide pheromones from externae similar to that secreted from eggs of ovigerous females (Hartnoll, 1967 and De Vries, *et al.*, 1989; Waiho, *et al.*, 2017).

Highnam and Hill (1977) indicated that the changes in the external characters are not due to the destruction of testes as a result of parasitism, but they reflect a reduction in the number of androgenic hormones in the blood necessary to maintain the male secondary sexual characters. The presence of parasite leads to hypertrophy of androgenic glands, consequently, ceases releasing of androgenic hormone into the blood of parasitized males which regulate the appearance and development of male secondary sexual characters at the subsequent molts, a phenomenon known as “parasitic castration”. This phenomenon was discussed and recorded in several brachyuran species (Boschma, 1948; Hartnoll, 1962, 1967; Schmidt & Roberts, 1977; Warner, 1977; Sloan, 1984; Siddiqui and Ahmed 1993; Galil & Luetzen, 1995, El-Sayed, *et al.*, 1997, 1998; Lutzen and Takashi, 1997; Yamoguchi and Aratake, 1997; Moazzam and Moazzam, 2004; Raffi *et al.*, 2012; Alazaly, 2017).

During this study, the careful examination showed that, out of the 25 infected males, 10 have broad abdomens with obvious scars varied from 1-3 for previous old *Sacculina*. These scars indicate previous infections which lead to the subsequent molts for these changes after the last molting which are in agreement with that denoted by Lutzen and Takashi (1997) on the asexual reproduction in *Sacculina polygonea*.

On the other hand, the external changes in females were limited, but remarkable degrees of hyperfeminization in most infected females were observed with a tendency towards larger sizes. The present study showed a slight broadness in the female's abdomen, fringed with dense and long marginal setae, while chelae not sharply affect. Examined ovaries in the infected females refer to underdeveloped and disintegrated or almost absent in few specimens and when present no ovulation was detected, therefore, a hypofeminization phenomenon was noticed. These results are in good agreement with that reported on the same species by Siddiqui & Ahmed (1993) and El-Sayed *et al.* (1997, 1998), Alazaly (2017) and that reported by Hartnoll (1967), Weng (1987) and Sumpton *et al.* (1994) on the other brachyuran species. El-Sayed *et al.* (1998) exhibited that, hyperfeminization beings as a result of the high level of ovarian hormones released from the ovaries of the parasite in addition to that released from the host's ovaries if present. They attributed increasing levels of testosterone in infected females to that released from parasite's testes of maturing externae causing hyperfeminization,

The parasitizing of true crabs with *Sacculina* spp. maybe has direct or indirect economic importance for humans. It can be used as a biological control against invasive species particularly those non-infected with the introduced parasite (Høeg, *et al.*, 2000). It was found that, *Carcinus maenas* infected with parasitic *S. carcini* cease molting and change their sex ratios and so do not grow to a suitable size for eating. This species considers of gastronomic importance and is frequently eaten in many European countries (Høeg, *et al.*, 2000; Jones, 2007).

Generally, in spite of the obtained results, there is still a lack of information about *Sacculina* species parasitizes *L. exaratus* due to their difficulty in identification, therefore, further biological and molecular studies are necessary along the Red Sea coasts and both Gulfs of Suez and Aqaba.

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ARABIC SUMMARY

التغيرات المورفولوجية الخارجية والداخلية المحدثة باصابة طفيل ساكولينا ليبثودي (ريزوسيفلا: ساكولينيدي) لسرطان الزانثيدي "الليبتوديس إكساراتس" (زانثويديا: زانثيدي) في البحر الأحمر وخليجي السويس والعقبة، مصر

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- 3- قسم علم الحيوان، كلية العلوم، جامعة الأزهر فرع أسبوط، مصر.
- 4- قسم علوم الحياة، كلية العلوم، جامعة الجوف، ساكا، المملكة العربية السعودية.

تم في هذه الدراسة تجميع 691 عينة شملت 400 ذكرا و 291 أنثى من سرطان المنطقة بين المدية " ليبثوديس إكساراتس" (عائلة الزانثيدي- السرطانات الأصلية)، من شواطئ البحر الأحمر وخليجي السويس وخليج العقبة، ولقد تم فحصها حيث تبين إصابة 38 عينة (23 ذكرا و 15 أنثى) تحمل 41 كيسا لطفيل ساكولينا ليبثودي بما يمثل 5.5 % من مجموع كافة العشائر في مناطق الدراسة قاطبة، وتبين ارتفاع نسبة الإصابة في الذكور عنها في الإناث لهذا النوع حيث سجلت النتائج إصابة 5.75% في الذكور مقابل 5.15 % في الإناث. ويظهر طفيل الساكولينا على هيئة أكياس تعرف بـ " إكسترننا" علي بطن العائل المصاب من أفراد هذا النوع من السرطانات يتصل بواسطة جذور داخلية تعرف بـ " إنترنا" تمتد وتتفرع داخل جسم العائل، ويتباين شكل وحجم ولون تلك الأكياس (الإكسترننا) تباينا واضحا. كما يتباين معدل الإصابة بهذا الطفيل حسب المواسم والمناطق وبين الشقين (الذكور والإناث)، فلقد سجل أعلى معدل للإصابة (7.91%) بين عشائر خليج السويس مع وجود ارتفاع نسبي في الإناث (8.33 %) عنها في الذكور (7.59%)، إلا أنه انخفض إلى 3.85% في عشائر خليج العقبة و 2.43% في عشائر البحر الأحمر حتى وصل إلى أدنى معدل (0.57%) بين عشائر شواطئ البحر الأحمر الجنوبية.

ولقد أوضحت الدراسة أيضا تسجيل أعلى معدل للإصابة في الخريف حيث وصل إلى 10.34% بين عشائر منطقة الغردقة (شمال البحر الأحمر)، مقابل انخفاض ملحوظ في الصيف إلى 9.34% و 7.69% في خليج السويس ورأس محمد (شمال البحر الأحمر) على الترتيب، كما تباين معدل الإصابة بين المواسم داخل المناطق المختلفة حيث سجل 2.94% بين عشائر خليج العقبة في الصيف و 5.65% خلال الربيع. كما أوضحت نتائج الدراسة أيضا تباين عدد أكياس الطفيل بين الأفراد المصابة، حيث تبين إصابة 35 عينة (92.11% من إجمالي المصاب) بكيس واحد فقط، مقابل ثلاث عينات أخرى تحمل أكياسا مزوجة بنسبة 7.89%. كما تباين تواجد إكسترننا الطفيل على بطن العائل حيث وجد أن العدد الأكبر (13 كيسا) ويمثل 31.70% تواجد علي العقلة البطنية السادسة، تلتها العقلة البطنية الخامسة بتواجد 11 كيسا بنسبة 26.82%، أما العقل الأخرى فحملت أعداد محدودة تراوحت من 1-5 أكاس. وأوضحت الدراسة اختلاف أحجام السكولينا الذي تراوح ما بين 1 – 10.4 مم في الطول بمتوسط 5.19 ± 2.76 مم في حجم إكسترننا الذكور و 5.63 ± 3.17 مم في إكسترننا الإناث.

كما أظهرت النتائج امتداد الجذور الداخلية للطفيل داخل الخصي والمبايض والكبد البنكرياسي وجميع الفراغات الداخلية لجسم السرطان، وتنسب هذه الجذور في تدمير خصي الذكور المصابة وملحقاتها من الغدد التناسلية المسؤولة عن افراز هرمونات الذكورة مما يتسبب في ظهور تغيرات مورفولوجية تمثلت بشكل واضح في استعراض وتمفصل العقل البطنية الملتحمية من 3-5 واضمحلال نسبي وملحوظ في الأرجل الذكرية والكلابية وظهور الشعيرات المسحفة بكثافة واستطانتها على حافة البطن في الذكور المصابة وكذلك تفلطح منقطة القص بشكل واضح في بعض الذكور المصابة، مما يشير إلى حدوث ظاهرة الخصي أو العقم بها، أما الإناث المصابة فقد أدت الإصابة بالطفيل إلى تدمير المبايض أو توقف مراحل تطورها مع ظهور زيادة ملحوظة في استعراض البطن وزيادة في طول وكثافة الشعيرات البطنية، مما يعطي مظهرا أنثويا مبالغاً فيه بسبب تواجد الطفيل الذي يغلب عليه الجانب الإنثوي.