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Infestation of External Ciliated Protozoan in Red Swamp Crayfish (*Procambarus clarkii*)

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Abstract

The current study was aimed to investigate ciliated protozoa that can infest *Procambarus clarkii* in Assiut governorate, Egypt. Hymenostomatida (*Ichthyophthirius multifiliis*), *Peritrich* ciliates (*Epistylis* sp., *Vorticella* sp. and *Trichodina* sp.), Suctorians ciliates (*Tokophrya cyclopum*, *Tokophrya infusionum*, *Tokophrya quadripartite* and *Podophrya* sp.), Ichthyophonida (*Psorospermium haeckeli*) and Cyrtophorids (*Chilodonella* sp.) were all identified from gills, branchiostegite and pleopods of infested individuals. Gills melanization was recorded in 11.5% of infested individuals associated with granulomas or encapsulation reaction filled with hemocytes aggregations (3.84%). Abdomen, pleopods and tail (telson and uropod) were also melanized and covered with brown slime like material in 38.46% of examined samples. Histopathological examination showed lamellar disorganization and distortion of the tip of gills lamellae with vacuoles formation. Hyperplasia and hypertrophied, strongly basophilic nuclei of lamellar epithelial cells and damaged gill lamellae with cuticle lysis were recorded. Lesions of epithelial cells varied from degeneration and necrosis to complete epithelial cell lysis. Melanization and granuloma formation were also recorded. It is obvious from the results that infestation of external ciliated protozoa induced pathogenic lesions in tissue of *Procambarus clarkii*.

KEYWORDS
Epistylis, *Ichthyophthirius multifiliis*, *Procambarus clarkii*, *Psorospermium haeckeli*, *Trichodina*.

INTRODUCTION

Food deficiency is one of the biggest problems worldwide. In Egypt, the human population increases rapidly with a need of more animal protein sources. Fish and shellfish are one of the most important sources of animal protein obtained either by capture of fish from wild fishery stocks or aquacultures (Subasinghe *et al*., 2009). Red swamp crayfish, *Procambarus clarkii* is a cheap source of animal protein (El-Sherif and Abd El-Ghafour, 2015).The crayfish farming are popular at southern united states, Australia and Europe with production rate varies between 40 000 to 60 000 tons per year. Nearly the same amounts are captured from wild especially in North America, China, Australia, Kenya, Turkey and Europe (Holdich, 1993).

Procambarus clarkii is an invasive species. It belongs to phylum Arthropoda; class *Crustacea*; order *decapoda* and family *Cambaridae* (Huner and Barr, 1991). It has a rapid growth and maturity rates and a great adaptation to seasonal water changes. That make it the most dominant freshwater crayfish species in the world (Henttonen and Huner, 1999). It was introduced to Egypt in 1980 via crayfish farm in Giza (Manial-Sheiha) and fast spread across the River Nile (Fishar, 2006).

In 1995, *P. clarkii* annual yield was about 4.6 tons in Egypt (Emam and Khalil, 1995). El-Sherif and Abd El-Ghafour (2015) analyzed the crayfish, *P. clarkii* meat and reported that it has a high protein content (15.22%). They mentioned that consumption of about 150 gram of canned crayfish meat can cover the mineral recommended daily allowances for an adult man (80-95% of phosphorus, 72-92% of calcium, 47-75% of magnesium, 31-36% of sodium and 19-24% potassium). It also contains amino acids; isoleucine, leucine, lysine, threonine, valine, histidine, methionine and cystine, and phenylalanine and tyrosine (El-Sherif and Abd El-Ghafour, 2015).

Epibiosis is a facultative and interspecific relation between two organisms, the epibiont and basibiont organisms. The basibiont organism act as a substrate for the epibiont organism attachment (Wahl, 2008). The relation between the two organisms is dynamic and changeable as the costs and the gains from this relation can change according to the environmental conditions (Fernandez-Leborans, 2010)

Epibionts ciliates are normally found on crayfish and causing no harm to their host. They are commonly present on the external surface of crustacean e.g., pleopods, periopods, telsons, gills and carapace (Longshaw, 2011). The crustacean exoskeleton is a calcified surface supports a perfect habitat for epibiosis (Fernandez-Leborans, 2010). Some epibionts ciliates preferred specific area for attachment, while others can attach to any part of the host surface. The host metabolism plays an important role in this interaction to be permanent or temporary (Fernandez-Leborans and Gabilondo, 2006). When the parasitic load increases, it af-

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fects the host movement, causes lethargy, stress and facilitates secondary infections (Görtz, 1996; Puckett and Carman, 2002). Mortalities in aquacultures due to parasitic infestation are associated with poor water quality, raised temperature and high stocking densities (Morado and Small, 1995).

The crustacean gills are the main respiratory organ and play an important role in osmo-regulation, gas exchange and acid base equilibrium. Changes in gills tissues affect respiration and ions uptake in crayfish (Cerqueira and Fernandes, 2002). Gills were reported to be a major target of waterborne contaminants and the first organ to display histological changes (Desouky *et al*., 2013). Gills were also considered to be a good indicator of water quality (Wendelaar Bonga and Lock, 1992).

The most reported ciliates associated with freshwater crayfish were *Epistylis*, *Vorticella*, Zoothamnuim, *Opercularia, Lagenophrys, aralagenophrys, Podophyra, Tokophyra, Acineta, Discophyra, Cothurnia, Hyalophysa, Hyalophysa, Psorospermium haeckeli* and *Chilodonella* (Morado and Small, 1995; Vogt and Rug, 1995, 1999; Edgerton *et al*., 2002; Romero and Jiménez, 2002; Nekuie *et al*., 2011).

In Egypt, there are few data about the ciliated protozoa affecting freshwater crayfish, *Procambarus clarkii*. The aim of the current study was to provide more data about the ciliated protozoan parasites affecting *P. clarkii* in Assiut governorate, Egypt and record any induced pathological effect.

MATERIALS AND METHODS

Biometrics

Total of 52 Red swamp crayfish *Procambarus clarkii* (22 males and 30 females) were collected from River Nile canals and drains at Assiut governorate, Egypt. Samples were collected from October 2018 to September 2019, 17 of them were collected from October and November 2018. The other 35 were collected from August to September 2019. The specimens were transported live in a plastic tank (25 width X 40 length) containing about 1 cm height of water to the laboratory of Animal Health Research Institute, Assiut. Immediately upon arrival of the samples, the weight, length, and sex were recorded then samples were directly sent for parasitological examination. The animal study was approved by "Institutional Review Board" of the Faculty of Medicine in Assiut University, Assiut, Egypt (IRB Local Approval number: 17300868).

Parasitological examination

For each specimen, the branchiostegite was removed to expose the branchial cavity. Gills was examined by naked eye and magnified lens to record any gross lesions found. Wet mount was prepared as follow, piece of gills was clipped, put on a slide and a drop of water was added to examine the samples under microscope. For the carapace samples, wet mount was prepared by scrapping the internal side of the branchiostegite including subepidermal connective tissue and blood vessels using a cover slide. Pleopods (swimmerets) samples were rinsed with water to remove any attached waste. Pleopods was removed by a scissors and examined. All wet mounts were examined directly after preparation using light microscope with 10x and 40x magnification. Parasites images were captured using Sony Cyber shots Exmor R CMOS sensor AVCHD 16.2 megapixels camera. To determine the health state of affected individuals, tissue samples were excised in 1 cm pieces and immersed immediately into Davidson fixative solution. After 24 hours, samples were transferred to 70% alcohol until processed. Statical analysis were done using Microsoft excel (Microsoft 365 enterprise V 16.0.15601.20088).

Identification of parasites

Tokophrya cyclopum was identified according to Ramírez-Ballesteros and Mayén-Estrada (2019),*Tokophrya infusionum* according to Rudzinska and Chambers (1951), *Tokophrya quadripartite* according to Ramírez-Ballesteros and Mayén-Estrada (2019), *Capriniana* sp. according to Al-Musawi (2018); National Institute for Environmental Studies (2018c) and Theodore *et al*. (2019), *Podophrya* sp. according to National Institute for Environmental Studies (2018b) and Ramírez-Ballesteros and Mayén-Estrada (2019). *Psorospermium haeckeli* identification was done according to Henttonen *et al*. (1997), *Chilodonella* according to Khaj (2014) and National Institute for Environmental Studies (2018a).

Histopathological examination

The present study was carried out on the gills of *P. Clarkii*. Gills were fixed in Davidson's fixative for standard processing for light microscope examination (115 ml glacial acetic acid, 330 ml 95% ethanol, 335 ml distilled water, 220 ml 100% formalin). Fixative was added 10x volume to one volume of tissues. Samples were fixed for 24 hours at room temperature. After fixation, the tissues were transferred to store in 70% ethanol. After dehydration in graded ethanol series to absolute ethanol, samples were embedded in paraffin. Slices of 5 micrometer thickness were obtained using microtome, then routinely stained with hematoxylin and eosin and examined under the light microscope (Lightner, 1996).

RESULTS

Biometrics

Biometrical characters of the samples are recorded in Table 1.

Clinical, postmortem and microscopical examination

No apparent clinical signs were observed in the examined crayfish specimens. During gross examination, melanization was recorded in 11.5% of examined gills (n=6/52) (Fig.1A). During microscopical examination of the gills wet mount, granulomas or encapsulation reaction filled with hemocytes aggregations were observed in 3.84% (n=2) of gills samples. One sample was infested with *Epistylis* sp., *Trichodina* sp. and *Chilodonella* sp. and the other was infested with *Epistylis* sp. and *Ichthyophthirius multifiliis* sp.

Melanization was also recorded in abdomen, pleopods and tail (telson and uropod) in 38.46% (n=10/26) of examined samples (Fig.1B). Brown slime like material was covering the infected parts. Melanization in the gills were associated with *Epistylis* sp.(n=1), mixed infection of *Epistylis* sp., *Trichodina* sp. and *Ichthyophthirius multifiliis* (n=1), mixed infection of *Epistylis* sp. and *Tokophrya cyclopum* (n=1), mixed infection of *Epistylis* sp., *Tokophrya quadripartite*, *Podophrya* sp., and *Psorospermium haeckeli* (n=1). While in the pleopods melanization was associated with *Epistylis* sp. (n=7), *Chilodonella* sp. (n=1), mixed infection of *Epistylis* sp. and *Chilodonella* sp. (n=1).

Parasitological examination of the wet mount

Eleven species of protozoa were identified affected different parts of *P. Clarkii*. This includes *peritrich* ciliates (*Epistylis* sp., *Vor-*

ticella sp. and *Trichodina* sp.), *Suctorians* (*Tokophrya cyclopum*, *Tokophrya infusionum*, *Tokophrya quadripartite*, *Capriniana* sp. and *Podophrya* sp.), *Ichthyophonida* (*Psorospermium haeckeli*), Cyrtophorids (*Chilodonella* sp.) and *Hymenostomatida* (*Ichthyophthirius multifiliis*). The presence of protozoan parasites during different seasons was summarized in Table 2.

Peritrich ciliates

Epistylis sp.

Epistylis sp. infestation was highest in pleopods 50% (n=13/26) followed by gills 40.38% (n=21/52) and lowest in branchiostegite 7.69% (n=2/26). Colonies of *Epistylis* sp. were found attached to gills filaments and pleopods. Zooids were elongate ovoid in shape with no lorica. The peristome was disc shaped. The oral cavity was present in the apical free end. The stalk was symmetrically branched. The zooid has one contractile vacuole located in the apical part under the peristomial disc (Fig. 2A). When *Epistylis* startled, the zooid contracted like an accordion and the stalk does not contract (Fig. 2B). Zooids were found detached from their stalks (Fig. 2C). Binary fission of zooid and teletroch (larval stage) were also observed (Fig. 2 D & E).

Vorticella sp.

Solitary *peritrich*s ciliate, *Vorticella* sp. infestation percent was 1.92% (n=1/52) in gills and (3.84%) in pleopods (n=1/26). The zooids shape looked like inverted bell and tended to be somewhat ovoid. One contractile vacuole located ventrally. Its characteristic coiling contraction pattern was observed in infested tissues (Fig. 2F).

Trichodina sp.

Trichodina sp. was round, or saucer shaped. Its margin carried a ring of cilia. The adhesive disk with denticular ring was located at the convex side of the parasite. It was found gliding over tissue of gills and between gills lamellae (44.23%, n=23/52), branchiostegite (57.69%, n=15/26) and Pleopods 3.84% (n=1/26) (Fig.3, C).

Suctorians ciliates

Tokophrya sp.

Tokophrya cyclopum, *Tokophrya infusionum* and *Tokophrya quadripartite* were restricted to the gills. The percent of infesta

Table 1. Biometrical characters of the collected samples.

Time of collection	Number	Mean weight (g)	Mean length (cm)	Sex	Male to female ratio
October and November (2018)	17	Not recorded	10.6 ± 2.15 cm	Males (n=9) Females (n=8)	1:0.9
August to September (2019)	35	Males (25.6±12.05) g Females (20.95±8.13)	8.91 ± 1.21 cm	Males (n=13) Females (n=22)	1:1.7

Table 2. Prevalence of parasites in sample collected during different seasons.

Protozoan ciliates	October to November (2018) (n=17)	August to September (2019) (n=35)
Trichodina sp.	+ (n=12/17) 70.59%	+(n=12/35) 34.29%
Epistylis sp.	+ (n=11/17) 64.71%	+(n=17/35) 48.57%
Psorospermium haeckeli	+ (n=9/17) 52.94%	+(n=3/35) 8.57%
Tokophrya quadripartita	+ (n=3/17) 17.65%	-
Chilodonella sp.	-	+(n=3/35) 8.57%
Podophrya sp.	+ (n=3/17) 17.65%	-
Capriniana sp.	-	+(n=2/35) 5.71%
Tokophrya infusionum	+ (n=1/17) 5.88%	-
Tokophrya cyclopum	-	+(n=1/35) 2.86%
Vorticella sp.	+ (n=1/17) 5.88%	+(n=1/35) 2.86%
Ichthyophthirius multifiliis	-	+(n=1/35) 2.86%

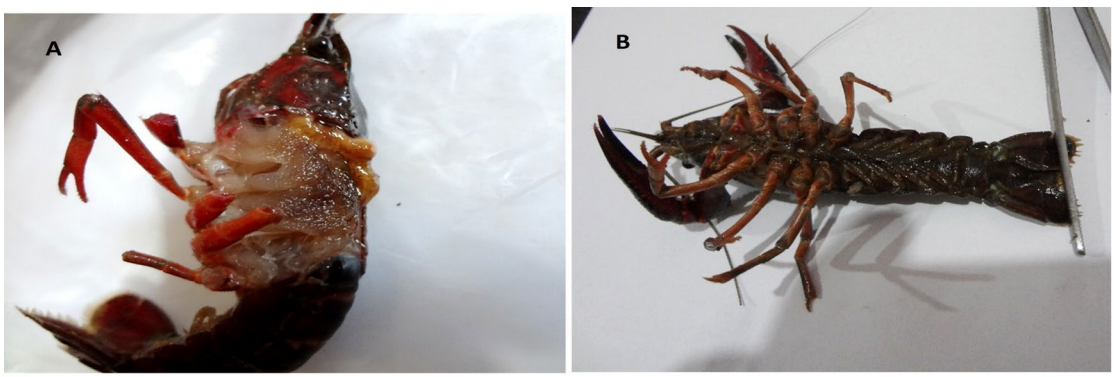

Fig 1. Melanization in the gills (A), abdomen, pleopods and tail (telson and uropod) (B)

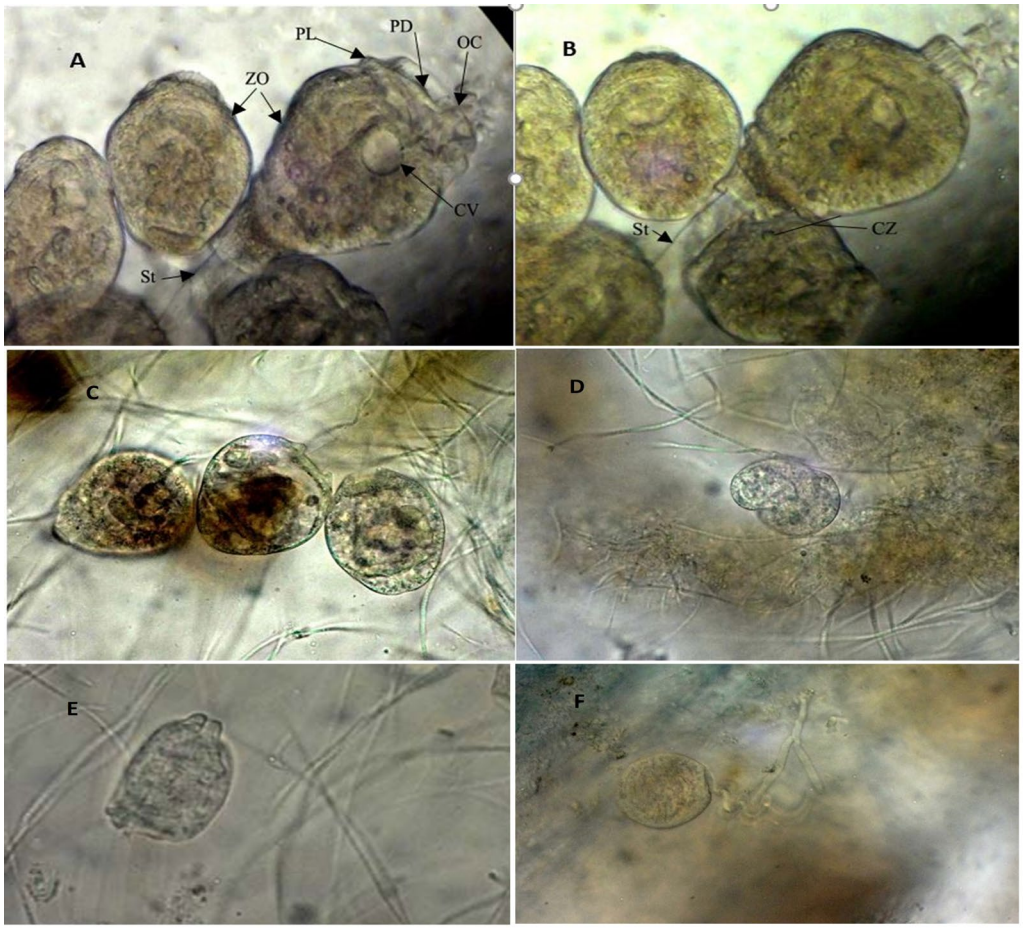

Fig. 2. *Epistylis* colony of zooids attached to gills filaments (A & B) and in swimmerets (C& D & E) in wet mount. (A) showing; ZO: zooids, PL: peristomial lip, PD: peristomial disc, OC: oral ciliature, CV: contractile vacuole and St: stalk. (B) showing the contraction of zooid without contraction of the stalk. (C) Zooids start to detach from their stalks. (D) Showing binary fission of the zooid. (E) teletroch. (F) *Vorticella* attached to pleopods with contracted stalk (400X).

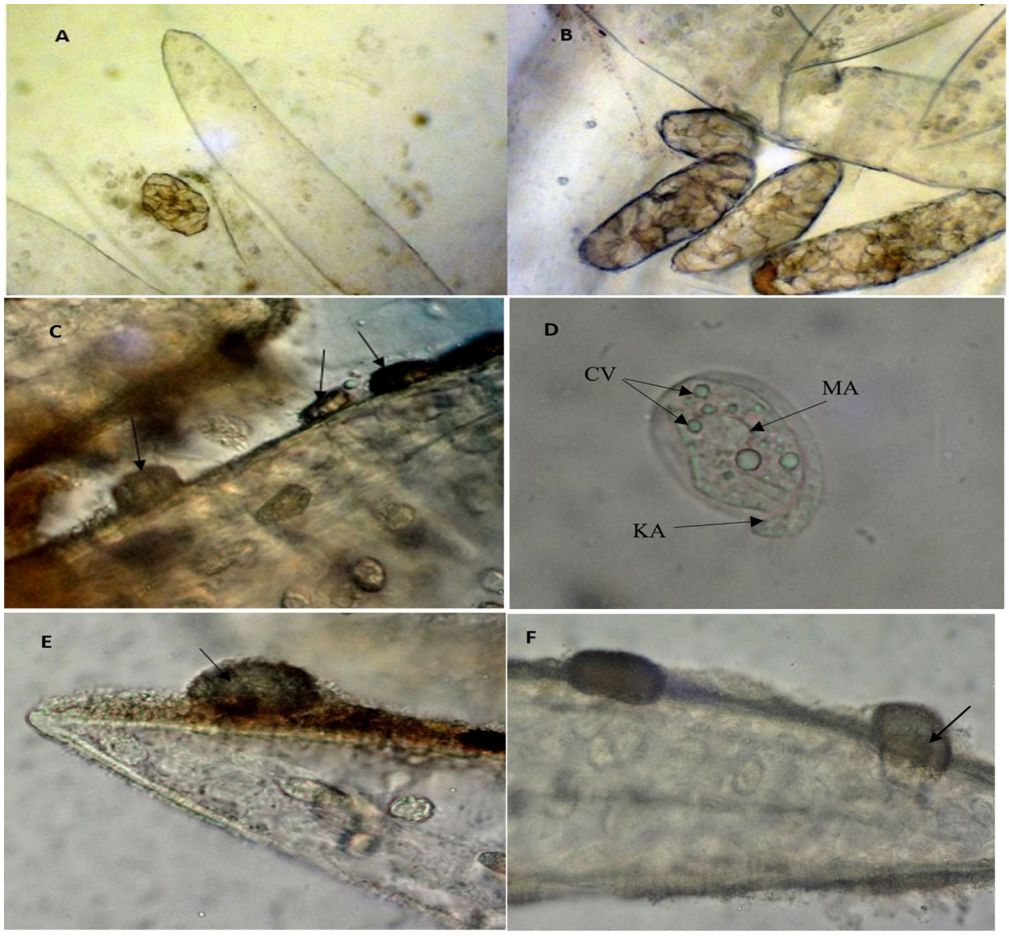

Fig 3. *Psorospermium haeckeli* between gills filaments of *P. Clarkii*. Naked form (A) and Large naked form (B). *Trichodina* infesting gills lamellae (C). *Chilodonella* infesting pleopods (D); CV: contractile vacuoles, MA: macronucleus and KA: Kinetal arc. *Ichthyophthirius multifiliis* trophont on gills lamellae showing C-shaped "horseshoe" nucleus (E&F). Wet mount (400X).

tion were 1.92% (n=1/52), 1.92% (n=1/52) and 5.77% (n=3/52) respectively.

Tokophrya cyclopum body was pyramidal in shape with central round nucleus. Tentacles arranged in two fascicles at the top of the body (Fig. 4B).

Tokophrya infusionum had a pyriform body shape with macronucleus. The body had no lorica. Body was filled with dark food vacuoles. Two tentacles fascicles were projecting from the apical part of the body. There was one contractile vacuole in the apical region (Fig.4A).

Tokophrya quadripartite body was ovoid or rounded in shape. The corners of the apical regions carry 4 prominent actinophores. Each actinophores carried a fascicle of capitate tentacles. The body had a central round macronucleus. The body was attached to long thin stalk (Fig. 4C).

Capriniana sp.

Capriniana sp. (previously called *Trichophrya* sp.) was attached to pleopods (3.84%, n=1/26). It was oval shaped with elongated macronucleus and long tentacles arranged regularly in fascicles (Fig. 4E). In gills (1.92%, n=1/52), tentacles were absent. The body dark orange colored pigment was obvious (Fig. 4F).

Podophrya sp.

Podophrya sp. was only found in the gills (5.77%, n=3/52). It had an ovoid body with no lorica. Capitate tentacles distributed

Table 3. Showing the prevalence of protozoan parasite infested RED swamp crayfish, and the percent of infestation in gills, branchiostegite and pleopods.

Parasites detected	Gills (n=52)	Branchiostegite (n=26)	Pleopods (n=26)
Trichodina sp.	44.23% (n=23)	57.69% (n=15)	3.84% (n=1)
Epistylis sp.	40.38% (n=21)	7.69% (n=2)	50% (n=13)
Psorospermium haeckeli	23.08% (n=12)	7.69% (n=2)	0
Tokophrya quadripartita	5.77% (n=3)	0	0
Chilodonella sp.	5.77% (n=3)	0	7.69% (n=2)
Podophrya spp.	5.77% (n=3)	0	0
Capriniana sp.	1.92% (n=1)	0	3.84% (n=1)
Tokophrya infusionum	1.92% (n=1)	0	0
Tokophrya cyclopum	1.92% (n=1)	0	0
Vorticella sp.	1.92% (n=1)	0	3.84% (n=1)
Ichthyophthirius multifiliis	1.92% (n=1)	0	0

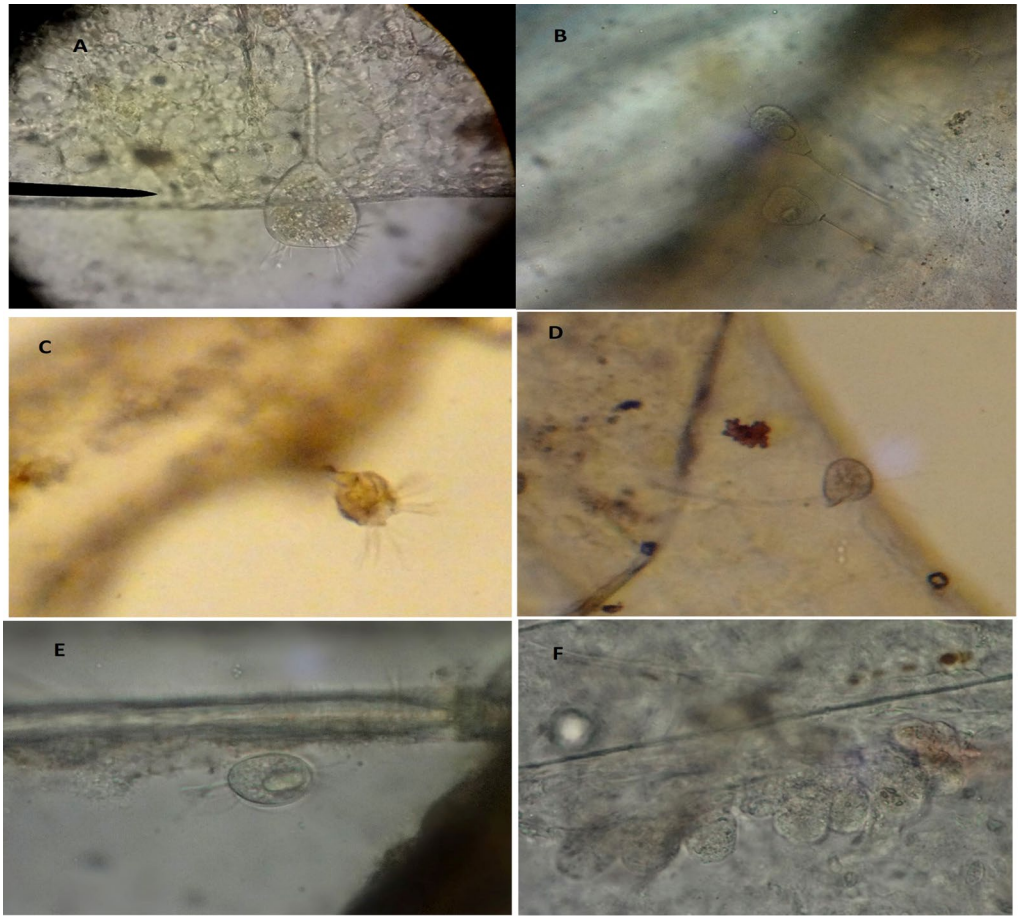

Fig. 4. *Tokophrya infusionum* (A), *Tokophrya cyclopum* (B), *Tokophrya quadripartite* (C) and *Podophrya* spp.(D) in the gills. *Capriniana* spp. in pleopods (E) and gills (F) (400X).

over the whole-body surface. Round macronucleus located in the body center (Fig.4D). It was found in gills only.

Ichthyophonida

Psorospermium haeckeli was found in the gills (23.08%, n=12/52) and branchiostegite (7.69%, n=2/26). Two *Psorospermium haeckeli* life forms were observed between gills filaments. The Naked form, the cell was oval, filled with large globules and surrounded by thin membranous layer. The large naked form, the cell was elongated in shape, filled with large globules, and surrounded by thin membranous layer (Fig. 3A & B).

Cyrtophorids

Chilodonella was identified in the gills (5.77%, n=3/52) than pleopods (7.69%, n=2/26). It was oval, flattened dorsoventrally. Somatic cilia were attached to the ventral surface. Macronucleus, contractile vacuoles and kinetal arc were all observed (Fig.3, D).

Hymenostomatida

Ichthyophthirius multifiliis trophont was found on gills lamellae of one sample (1.92%, n=1/52). The large parasite was oval. The C-shaped "horseshoe" nucleus was observed (Fig. 3E & F).

Prevalence and percent of infestations in gills, branchiostegite and pleopods of each protozoan parasite were summarized in Table 3.

Histopathological examination

The histopathological examination of gills revealed variable changes. Gills showed lamellar disorganization and distortion of the tip of lamellae with vacuole formation (Fig. 5). Abnormal gill lamellae lead to damage with complete destruction of some gill lamellae and vacuolar degeneration in the intralamellar space (Fig. 6). Hyperplasia and hypertrophied, strongly basophilic nuclei of lamellar epithelial cells and damaged gill lamellae with cuticle lysis were recorded. lesions of epithelial cells varied from degeneration and necrosis to complete epithelial cell lysis (Fig. 7). Congestion with hemocytic and eosinophilic cells infiltration, swollen and necrotic gill lamellae with necrotic epithelium were detected (Fig. 8). Necrotic changes of gill lamellae with melanization were seen (Fig. 9). The histopathological results also revealed granuloma formation with inflammatory cells infiltration and presence of *Epistylis* parasite (Fig.10). Appearance of other protozoan parasites in different stages infesting gills of *Procambarus clarkii* such as *Epistylis*, *Chilodonella*, Myxobolus, *Trichodina* and *Ichthyophthirius multifiliis* (Figs.10,11,12 & 13).

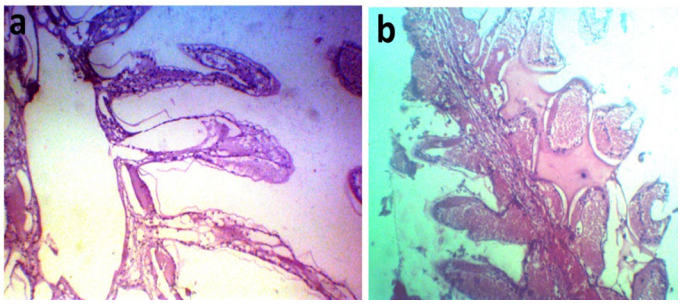

Fig. 5. Histopathological sections of gills lamellae of RED swamp crayfish, *P. Clarkii*. (a) Longitudinal section of gills lamellae showing distortion of the tip of lamellae (X100). (b) Longitudinal section of gill lamellae showing disorganization of gill lamellae (X100).

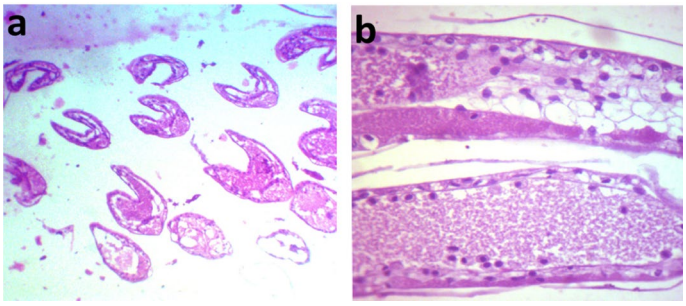

Fig. 6. Histopathological sections showing (a): cross section of damaged gill lamellae (X100). (b) Vacuolar degeneration in intralamellar space (X400).

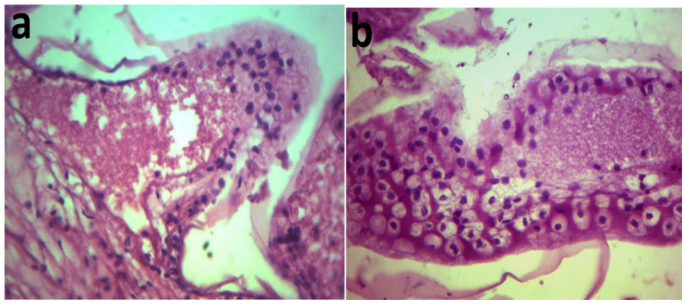

Fig. 7. Histopathological section showing (a): hyperplasia of lamellar epithelial cells (X400). (b) Lysis of cuticle (X400).

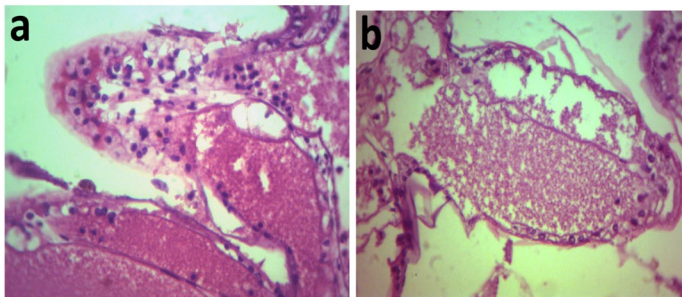

Fig. 8. Histopathological sections showing: (a) congestion with haemocytic and eosinophilic cells infiltration (X400). (b) swollen gill lamellae showing necrotic epithelium (X400).

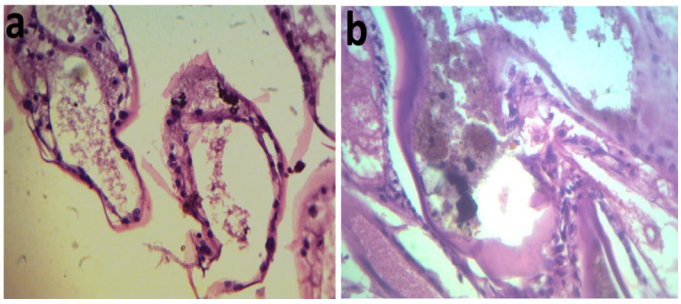

Fig. 9. Histopathological section of gill lamellae showing (a): melanization (X200). (b) Focal area of melanized necrosis in gill lamellae (X400).

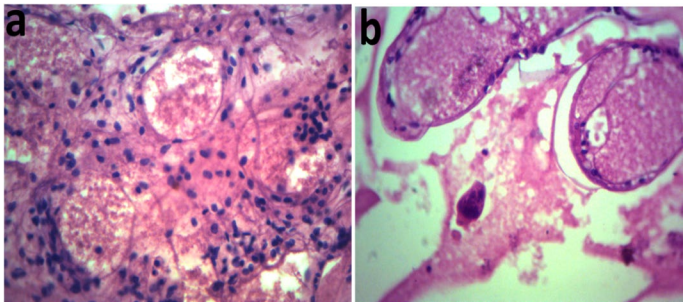

Fig. 10. Histopathological sections showing: (a) granuloma formation (X400). (b) presence of *Epistylis* parasite (X400).

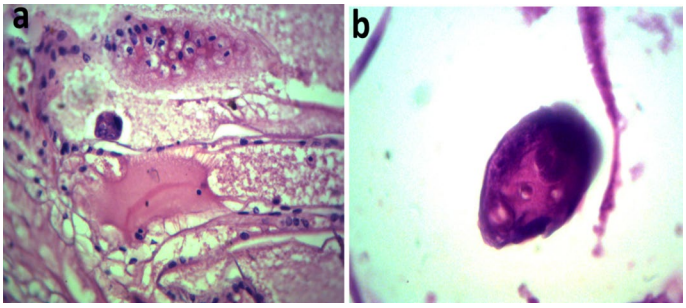

Fig. 11. Histopathological sections showing: (a) *Chilodonella* parasite (X400). (b) *Chilodonella* Parasite (X1000).

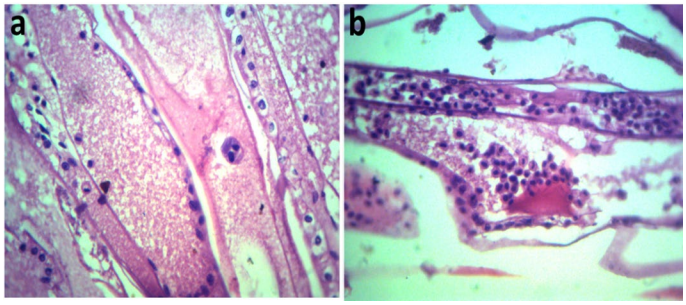

Fig. 12. Histopathological sections showing (a) Myxobolus parasite (X400). (b) Hemorrhage with mixed parasitic infestation (X200).

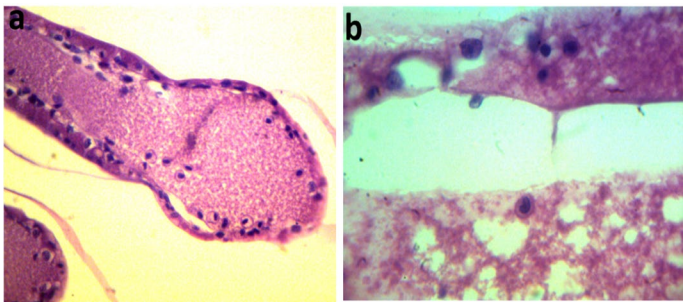

Fig. 13. Histopathological sections showing: (a) *Trichodina* parasite (X400). (B) *Ichthyophthirius multifiliis* (X1000)

DISCUSSION

Epistylis sp. infestation was found in gills, branchiostegite and pleopods. *Epistylis* spp. were found in the gills (Scott and Thune, 1986; Vogelbein and Thune, 1988; Quaglio *et al*., 2004; Zhou *et al*., 2019), exoskeleton cuticle (Quaglio *et al*., 2004) and pereopods (Zhou *et al*., 2019) of red swamp crayfish, *Procambarus clarkii*. were also found in gills and branchial cover of white clawed crayfish, *Austropotamobius pallipes* (Quaglio *et al*., 2006) and in cephalothorax of The red claw crayfish, *Cherax quadricarinatus* and marron (Herbert, 1987). Different species of *Epistylis* were identified e.g., *Epistylis semiciculus* n. sp. from pereopods and gills of *Procambarus clarkii* (Zhou *et al*., 2019). *E. astaci* from the gills of european crayfish; *Astacus astacus*, danube crayfish; *Astacus leptodactylus* and stone crayfish; *Austropotamobius torrentium*, *E. cambarid* from gills of *Cambarus* species, *E. crassicollis* from gills of *Astacus astacus* and *Astacus leptodactylus* and *Epistylis bimarginata* from *Astacus astacus* appendages (Fernandez-Leborans and Tato-Porto, 2000a). *E. branchiophila, Epistylis bimarginata, E. carinogammari; E. gammari, E. lacustris, E. stammeri, E. niagarae and E. variabilis* on the exoskeleton of the mexican dwarf crayfish, *Cambarellus patzcuarensis* while *E. gammari* was attached to its gills (Mayén-Estrada and Ma. Antonieta Aladro-Lubel, 2001). It was identified in the branchial chambers, the gill filaments and the exoskeleton of *P. Clarkii* at Sharkia, Dakahlia and Kafr El-Sheikh provinces in Egypt (El-Moaty *et al*., 2016).

Epistylis infestation rate was high. This may be due to the collection of *Procambarus clarkii* from water canals and drains with high organic pollution and slow water current. The better the water quality, the lower the incidence of *Epistylis* sp. (Scott and Thune, 1986; Brown *et al*., 1993; Quaglio *et al*., 2004). No host tissue reactions were observed in the gills except in 6 samples. Vogelbein and Thune (1988) observed *Epistylis* sp. cause no harm to the gills cuticle nor stimulate significant host immune reaction. They explained, the stalk of *Epistylis* is formed from an outer membrane surround equally spaced tubules. Those tubules are located within a fine microfibrillar matrix. This microfibrillar matrix become more condensed at the junction between basal disk and epicuticle. The stalk's tubules branched in the basal disk above the epicuticle and attach the stalk to this condensed microfibrillar layer at the gills surface. Melanization in 30.76% of infested pleopods with *Epistylis*. Brown slime like material was covering those areas. Brown *et al*. (1993) observed, the cephalothorax, abdomen, periopods, claws, head and maxillipeds of infested rusty crayfish, *Orconectes rusticus* were all covered by black filamentous material. While Herbert (1987) recorded no harmful effect in the cephalothorax of *C. quadricarinatus* or marron infested by *Epistylis* spp.

The percent of infestation by *Psorospermium haeckeli* was higher in gills than branchiostegite. *Psorospermium* was detected in the gills of *Astacus astacus* (Henttonen *et al*., 1994, 1997; Vogt and Rug, 1995), Calico crayfish; *Orconectes immunis*, Rusty crayfish; *Orconectes rusticus*, Virile crayfish; *Orconectes virilis* and signal crayfish; *Pacifastacus leniusculus* (Henttonen *et al*., 1994), marron; *Cherax tenuimanus* (Evans and Jussila, 1996) and stone crayfish; *Austropotamobius torrentium* (Vogt *et al*., 1996).

No marked immune response was recorded in the host tissue due to such infection. That may be due to the low number of parasitic spores' present. Longshaw *et al*. (2012) mentioned that *Psorospermium* spp are not a life threaten parasite to crayfish *Austropotamobius pallipes*. They noticed no marked host response in hepatopancreas connective tissues. They referred the results to the low number of parasite spores found in the infected tissues. Rogers *et al*. (2003) explain the lack of the host response to a protein coat formed by the parasite and hiding it from the host immune reaction. While Thörnqvist and Söderhäll (1993) experiment showed *P. haeckeli* lead to degranulation of the semigranular cells which lowering the total haemocytic count. This will not affect healthy crayfish individuals, but the carrier infected individuals with other infections will not be able to resist both infections at the same time leading to more mortalities as a result. Also, Vey (1978) reported *Psorospermium haeckeli* to be pathogenic and causing death to infected individuals. He noticed the increase in the mortality rate especially during molting. Evans and Jussila (1996) reported the lack of host tissue immune response in most examined specimens but if the infection is heavy, the parasite was encapsulated by fibrocytes and hemocytes especially semigranular hemocytes. But in the immature form of the parasite, hemocytes aggregation was rarely observed. No melanization were recorded in any sample during histological examination.

Capriniana spp. was identified in gills and pleopods of *P. Clarkii*. No apparent lesions were recorded. Different *Trichophrya* spp. were previously identified from different crayfish species. *T. astaci* was found in pereiopods and gills of *A. leptodactylus*. *T. cambari* was detected on cambarus species telson (Fernandez-Leborans and Tato-Porto, 2000b) and *T. epistylidis* from chelae of *Procambarus* (*Austrocambarus*) spp. (Ramírez-Ballesteros and Mayén-Estrada, 2019). Little data were found on the pathological effect of *Trichophrya* on tissues of crustacean. *Capriniana piscium* was detected in the gills of *Coho salmon, Oncorhynchus kisutch*. No inflammation or necrosis were recorded (Ferguson *et al*., 2011). Noga (2010) mentioned that it can slightly damage the epithelium of the gills in light infestation. In heavy infestation, large number of parasites can mechanically clog the gills tissue causing respiratory distress.

Tokophrya quadripartita, *T. infusionum* and *T. cyclopum* were found in gills only. *Tokophrya* was found attached to giant freshwater prawn; *Macrobrachium rosenbergii* uropods and gills (Granados and Chinchilla, 1990). *T. cyclopum* was attached

to pleopods only of yabby; *Cherax destructor* causing no disease (O'Donoghue *et al*., 1990). Tahir and Wang (2017) identified *T. huangmeiensis* in the carapace of redclaw crayfish; *Cherax quadricarinatus*. Ramírez-Ballesteros and Mayén-Estrada (2019) reported *T. cyclopum* on antennae and carapace, and *T. quadripartite* on mandible and chelae of *Procambarus* (*Austrocambarus*) crayfish. *T. quadripartite* was also found on antennae and pereiopods of Crayfish, *Cambarellus patzcuarensis* (Estrada and Lubel, 1998).

Podophrya was found only in the gills. Ramírez-Ballesteros & Mayén-Estrada (2019) identified *Podophrya maupasi* on antennae and *P. sandi* on carapace of freshwater crab, Raddaus bocourti. P. sandi was found on carapace and uropods of *Cambarellus patzcuarensis* (Estrada and Lubel, 1998) and P. fixa on cephalothorax of *Astacus leptodactylus* (Nekuie *et al*., 2011). *P. astaci* was recorded from *Astacus fluviatilis* (Sprague and Couch, 1971). *Tokophrya* and *Podophrya* cause no harm to freshwater crayfish but they can cause hypoxia in case of heavy infestation of the gills (Edgerton *et al*., 2002)

Vorticella was only found in 2 specimens. one in gills and the other in the pleopods. The parasitic infestation was mild and not associated with any lesions. *Vorticella* was detected in pleopods, uropods and gills of giant freshwater prawn; Macrobrachium rosenbergii (Granados and Chinchilla, 1990). *V. similis* was identified on gills and legs of *A. leptodactylus* (Nekuie *et al*., 2011). *Vorticella* campanula, *V. communis*, *V. infusionum, V. latifunda, V. microstoma, V. natans, V. striata* and *V. fromenteli* were found on Mexican dwarf crayfish; *Cambarellus patzcuarensis* exoskeleton and gills (Mayén-Estrada and Ma Antonieta Aladro-Lubel, 2001). *V. jaerae, V. convallaria, V. calciformis, V. flexulosa,* and two other unidentified species were identified in yabby; *Cherax destructor* and marron; *Cherax tenuimanus* pleopods and gills (O'Donoghue *et al*., 1990). The infestation percent was highest on pereiopods, pleopods and uropods and lowest in the gills (O'Donoghue *et al*., 1990; Mayén-Estrada and Ma Antonieta Aladro-Lubel, 2001). This ciliate considered ectocommensal or pollution parasite and causing no harm to its host (O'Donoghue *et al*., 1990).

Chilodonella species was found in gills and pleopods. Nekuie *et al*.(2011) recorded the presence of *Chilodonella* in low number in gills of *A. leptodactylus*. scarce data were found on its pathological effect on crayfish species.

Ichthyophthirius multifiliis was found in gills of *P. Clarkii* in mixed infection with *Epistylis* and *Trichodina*. Melanization and granulomatous reaction were associated with such infestation. No or scarce data were found about *Ichthyophthirius multifiliis* infestation and its pathological effect on crayfish. Further investigation of this point is needed.

The histopathological examination of gills of red Swamp Crayfish; *Procambarus clarkii,* showed variable changes. As the gills are the primary site of infection for protozoan parasites (Noga, 2010). The obtained results revealed hyperplasia and hypertrophied, strongly basophilic nuclei of the lamellar epithelial cells. Parasites cause a reactive hyperplasia of the epithelium and increased mucous production and these histopathological changes if occur on gills resulted in hypoxia and respiratory failure (Noga, 2010; Yasser and Naser, 2011). Changes like hyperplasia and hypertrophy of the epithelial cells, are examples of defense mechanisms (Fernandes and Mazon, 2003).

Also Ding *et al*. (2013) explained the hypertrophied and intensely basophilic nuclei as an indication of more advanced infection. In gills, congestion, hemocytic and eosinophilic cells infiltration with macroscopic and microscopic melanization appeared. Same results were found by Johansson *et al*. (2000) who reported that in crustaceans, hemocytes play important roles against foreign agents including phagocytosis, melanization, cytotoxicity and cell- cell communication.

Abnormal gill lamellae showed vacuolar degeneration in the intralamellar space. Desouky *et al*. (2013) explained vacuolation of epithelial cells can increase the diffusion distance for respiratory gases and ions, so it affects the physiological processes of gills.

Gills showed swollen gill lamellae with necrotic epithelium, lamellar disorganization, and distortion of the tip of lamellae. The same results found by Abd El-Atti *et al*. (2019) who studied effects of titanium dioxide nanoparticles on gills of red swamp crayfish *Procambarus clarkii*. Necrosis of gills consider one of the lethal lesions causing higher crayfish mortality (Bianchini and Monserrat, 2007).

As different parasites were seen in gills including *Chilodonella*, *Trichodina*, Ichthyphthirius and *Myxobolus* sp. Bruno *et al* (2006) reported that gill lesions in *Chilodonellosis* include hyperplasia, damaged gills function and necrosis. Respiratory failure because of gill hyperplasia is the primary cause of fish mortality. Also, Bruno *et al*. (2006) found that infection by *Trichodina* and *Ichthyophthirius* associated with epithelial hyperplasia especially in gills. Maftuch *et al*. (2018) recognized damaged gill with congestion which occurred due to *Myxobolus* sp.-infected Koi carp. In addition, Hadi and Alwan (2012) stated that congestion, as a passive process, was caused by increased blood volume in a vessel placed in gill lamellae.

CONCLUSION

Most of protozoan parasites appear to cause no harm to *P. Clarkii*. Melanization are mostly associated with heavy infestation only. Maintaining good water quality is the key to prevent any future parasitic infestation.

CONFLICT OF INTEREST

The authors declare no conflicts of interest associated with this study.

REFERENCES

Abd El-Atti, M., Desouky, M.M.A., Mohamadien, A., Said, R.M., 2019. Effects of titanium dioxide nanoparticles on red swamp crayfish, *Procambarus clarkii*: Bioaccumulation, oxidative stress and histopathological biomarkers, The Egyptian Journal of Aquatic Research 45, 11–18.

Al-Musawi, A.M.K., 2018. First record of *Trichophrya intermedia* Prost, 1952 (*Ciliophora, Suctoria*. in Iraq from the Mugilid fish Planiliza abu. Journal of University of Babylon for Pure and Applied Sciences 26, 174–178.

Bianchini, A., Monserrat, J.M., 2007. Effects of methyl parathion on *Chasmagnathus granulatus* hepatopancreas: protective role of sesamol, Ecotoxicology and Environmental Safety 67, 100–108.

Brown, P.B., White, M.R., Swann, D.L., Fuller, M.S., 1993. A Severe Outbreak of Ectoparasitism Due to *Epistylis* sp. in Pond-Reared Orconectid Crayfish. Journal of the World Aquaculture Society 24, 116–120.

Bruno, D.W., Nowak, B., Elliott, D.G., 2006. Guide to the identification of fish protozoan and metazoan parasites in stained tissue sections, Diseases of Aquatic Organisms 70, 1–36.

Cerqueira, C.C.C., Fernandes, M.N., 2002. Gill tissue recovery after copper exposure and blood parameter responses in the tropical fish *Prochilodus scrofa*. Ecotoxicology and environmental Safety 52, 83–91.

Desouky, M.M.A., Abdel-Gawad, H., Hegazi, B., 2013. Distribution, fate and histopathological effects of ethion insecticide on selected organs of the crayfish, *Procambarus clarkii*. Food and Chemical Toxicology 52, 42–52.

Ding, Z., Yao, W., Du, J., Ren, Q., Li, W., Wu, T., Xiu, Y., Meng, Q., Gu, W., Xue, H., 2013. Histopathological characterization and in situ hybridization of a novel spiroplasma pathogen in the freshwater crayfish *Procambarus clarkii*. Aquaculture 380–383, 106–113.

Edgerton, B.F., Evans, L.H., Stephens, F.J., Overstreet, R.M., 2002. Synopsis of freshwater crayfish diseases and commensal organisms. Aquaculture 206, 57–135.

El-Moaty, A., Shimaa, M., Sharaf, H.M., Khalil, A.E.-M.M., Ahmed, S.S., 2016. Survey on the parasites infested crayfish *Procambarus clarkii*, Girard, 1852 (*Crustacea, Cambaridae*. in Egypt. Journal of Bioscience and Applied Research 2, 395–400.

El-Sherif, S.A.E.H., Abd El-Ghafour, S., 2015. Nutritive value of canned River Nile Crayfish (*Procambarus clarkii*. products. The Egyptian Journal of Aquatic Research 41, 265–272.

Emam, W.M., Khalil, M.T., 1995. Population dynamics and stock assessment of the newly introduced crayfish *Procambarus clarkii* in the river Nile, Egypt. Proc. Zool. Soc. AR Egypt 26, 131–143.

Estrada, R.M., Lubel, M.A.A., 1998. Tres especies de suctores (Protozoa: *Ciliophora*. ectosimbiontes del acocil *Cambarellus patzcuarensis*, Anales del Instituto de Biología. Serie Zoología 69, 1–12.

Evans, L.H., Jussila, J., 1996. Morphology and prevalence of *Psorospermium* sp. in farmed and wildstock freshwater crayfish populations in Western Australia. Freshwater Crayfish 11, 481–493.

Ferguson, J.A., St-Hilaire, S., Peterson, T.S., Rodnick, K.J., Kent, M.L., 2011. Survey of parasites in threatened stocks of coho salmon (*Oncorhynchus kisutch*. in Oregon by examination of wet tissues and histology. Journal of Parasitology 97, 1085–1098.

Fernandes, M.N., Mazon, A.F., 2003. Environmental pollution and fish gill morphology, In: Val, A. L. & B. G. Kapoor (Eds). Fish adaptations. Enfield, Science Publishers, pp. 203–231.

Fernandez-Leborans, G., 2010. Epibiosis in Crustacea: an overview. Crustaceana 5, 549–640.

Fernandez-Leborans, G., Tato-Porto, M.L., 2000a. A review of the species of protozoan epibionts on crustaceans. I. *Peritrich* ciliates, Crustaceana 73, 643–683.

Fernandez-Leborans, G., Tato-Porto, M.L., 2000b. A review of the species of protozoan epibionts on crustaceans. II. *Suctorian* ciliates. Crustaceana 73, 1205–1237.

Fernandez-Leborans, G., Gabilondo, R., 2006. Taxonomy and distribution of the hydrozoan and protozoan epibionts on *Pagurus bernhardus* (Linnaeus, 1758. (*Crustacea, Decapoda*. from Scotland). Acta Zoologica 87, 33–48.

Fishar, M.R., 2006. Red swamp crayfish (*Procambarus clarkii*. in River Nile, Egypt, Biodiversity Monitoring and Assessment Project (BioMap.. Nature Conservation Sector. Egyptian Environmental Affairs Agency, Ministry of State for Environmental Affairs. Available at: https://www.cbd.int/cms/ui/forums/attachment.aspx?id=238.

Görtz, H.D., 1996. Symbiosis in ciliates, In: Hausmann, K., Bradbury, P. C., Eds... Ciliates: cells as organisms. Stuttgart: Gustav Fischer, pp. 441–462.

Granados, L.C., Chinchilla, M., 1990. Distribución de ciliados epibiontes en *Macrobrachium rosenbergii* (*Decapoda: Natantia*. Revista de Biología Tropical 38, 1–5.

Hadi, A.A., Alwan, S.F., 2012. Histopathological changes in gills, liver and kidney of fresh water fish, *Tilapia zillii*, exposed to aluminum. International Journal of Pharmacy and Life Sciences 3.

Henttonen, P., Huner, J.V., 1999. The introduction of alien species of crayfish in Europe: a historical introduction, In: Gherardi, F and Holdich, D.M, Editors, 1999. Crayfish in Europe as Alien Species. Crustacean Issues 11, A.A. Balkema, Rotterdam, pp.13–22.

Henttonen, P., Huner, J.V., Lindqvist, O.V., 1994. Occurrence of Psorospermium sp. in several North American crayfish species, with comparative notes on *Psorospermium haeckeli* in the European crayfish, *Astacus astacus*. Aquaculture 120, 209–218.

Henttonen, P., Huner, J.V., Rata, P., Lindqvist, O.V., 1997. A comparison of the known life forms of Psorospermium spp. in freshwater crayfish (Arthropoda, decapoda. with emphasis on *Astacus astacus* L., Astacidae., *Procambarus clarkii* (Girard) (Cambaridae). Aquaculture 149, 15–30.

Herbert, B., 1987. Notes on diseases and epibionts of *Cherax quadricarinatus* and *C. tenuimanus* (*Decapoda: Parastacidae*., Aquaculture 64, 165–173.

Holdich, D.M., 1993. A review of astaciculture: freshwater crayfish farming, Aquatic Living Resources 6, 307–317.

Huner, J. V and Barr, J.E., 1991. Red Swamp Crayfish: Biology and Exploitation, in Red Swamp Crayfish: Biology and Exploitation. 3rd edn. Baton Rouge, the Louisiana Sea Grant College Program, Center for Wetland Resources, Louisiana State University, p. 35. Available at: https://ntrl.ntis.gov/NTRL/dashboard/searchResults/titleDetail/PB91163451.xhtml.

Johansson, M.W., Keyser, P., Sritunyalucksana, K., Söderhäll, K., 2000. Crustacean haemocytes and haematopoiesis. Aquaculture 191, 45–52.

Khaj, H., 2014. Studying the prevalence of *Chilodonella* in fish ponds of Sistan region in Iran. Advances in Environmental Biology, 8, 469–472.

Lightner, D.V., 1996. A handbook of shrimp pathology and diagnostic procedures for diseases of cultured penaeid shrimp, Baton Rouge (La.. : World Aquaculture Society, c1996. ISBN: 09-624-52998

Longshaw, M., 2011. Diseases of crayfish: a review. Journal of invertebrate pathology 106, 54–70.

Longshaw, M., Stebbing, P.D., Bateman, K.S., Hockley, F.A., 2012. Histopathological survey of pathogens and commensals of white-clawed crayfish (*Austropotamobius pallipes*. in England and Wales. Journal of Invertebrate Pathology 110, 54–59.

Maftuch, M., Sanoesi, E., Farichin, I., Saputra, B.A., Ramdhani, L., Hidayati, S., Fitriyah, N., Prihanto, A.A., 2018. Histopathology of gill, muscle, intestine, kidney, and liver on *Myxobolus* sp.-infected Koi carp (*Cyprinus carpio*. Journal of Parasitic Diseases 42, 137–143.

Mayén-Estrada, R., Aladro-Lubel, Ma Antonieta, 2001. Distribution and prevalence of 15 species of epibiont *peritrich* ciliates on the crayfish *Cambarellus patzcuarensis* Villalobos, 1943 in Lake Pátzcuaro, Michoacán, Mexico. Crustaceana 74, 1213–1224.

Mayén-Estrada, R., Aladro-Lubel, Ma. Antonieta, 2001. Epibiont *Peritrich*ids (Ciliophora: *Peritrich*ida: *Epistylididae*. on the Crayfish *Cambarellus patzcuarensis* in Lake Pátzcuaro, Michoacán, Mexico. Journal of Crustacean Biology 21, 426–434.

Morado, J.F., Small, E.B., 1995. Ciliate parasites and related diseases of *Crustacea*: a review. Reviews in Fisheries Science 3, 275–354.

National Institute for Environmental Studies, 2018a. *Chilodonella*, *Chilodonella* Strand, 1928 (ref. ID; 2013, 3331. [Preprint]. Available at: https://www.nies.go.jp/chiiki1/protoz/morpho/ciliopho/chilodon.htm.

National Institute for Environmental Studies, 2018b. *Podophrya* Morphology, *Podophrya Ehrenberg*, 1838 (ref. ID; 2013. [Preprint]. Available at: https://www.nies.go.jp/chiiki1/protoz/morpho/ciliopho/podophry.htm (Accessed: 25 August 2020..

National Institute for Environmental Studies, 2018c. *Trichophrya*, *Trichophrya* (*Trichophrya Claparede* & Lachmann, 1859 (ref. ID; 2013. [Preprint]. Available at: https://www.nies.go.jp/chiiki1/protoz/morpho/ciliopho/trichoph.htm (Accessed: 19 August 2020..

Nekuie, F.A., Motalebi, A.A., Jalali, J.B., Aghaadeh, M.M., Azadikhah, D., Afsharnasab, M., 2011. Survey on fungal, parasites and epibionts infestation on the *Astacus leptodactylus* (Eschscholtz, 1823., in Aras Reservoir West Azarbaijan, Iran.

Noga, E.J., 2010. Fish disease: diagnosis and treatment. John Wiley & Sons.

O'Donoghue, P., Beveridge, I., Phillips, P., 1990. Parasites and ectocommensals of yabbies and marron in South Australia, Vetlab report. central veterinary laboratory. South Australian Department of Fisheries. S.A. Department Agriculture, Adelaide.

Puckett, G.L., Carman, K.R., 2002. Ciliate epibiont effects on feeding, energy reserves, and sensitivity to hydrocarbon contaminants in an estuarine harpacticoid copepod. Estuaries 25, 372–381.

Quaglio, F., Morolli, C., Galuppi, R., Bonoli, C., Marcer, F., Nobile, L., De Luise, G., Tampieri, M.P., 2006. Preliminary investigations of disease-causing organisms in the white-clawed crayfish Austropotamobius pallipes complex from streams of northern Italy. Bulletin Francais de la Peche et de la Pisciculture 1271–1290.

Quaglio, F., Morolli, C., Galuppi, R., Tampier, M.P., Marcer, F., Rotundo, G., 2004. Pathological investigation on crayfish (*Procambarus clarkii*, Girard 1852. from canals in Padana Plain, in XV Symposium of the International Association of Astacology. London, p. 45.

Ramírez-Ballesteros, M., Mayén-Estrada, R., 2019. Suctorians (Ciliophora: Suctoria. as epibionts of decapods of families Cambaridae and Pseudothelphusidae, Zootaxa, 4648.

Rogers, D., Hoffmann, R., Oidtmann, B., 2003. Diseases in selected *Austropotamobius pallipes* populations in England, in Holdich, D.M., Sibley, P.J., eds. Management & Conservation of Crayfish. Proceedings of a conference held on 7th November, 2002. Nottingham, UK.: Environment Agency. Bristol., pp. 169–174.

Romero, X., Jiménez, R., 2002. Histopathological survey of diseases and pathogens present in redclaw crayfish, *Cherax quadricarinatus* (Von Martens., cultured in Ecuador. Journal of Fish Diseases 25, 653–667.

Rudzinska, M.A., Chambers, R., 1951. The Activity of the Contractile Vacuole in a *Suctorian* (*Tokophrya infusionum*., Biological Bulletin 100, 49–58.

Scott, J.R., Thune, R.L., 1986. Ectocommensal protozoan infestations of gills of red swamp crawfish, *Procambarus clarkii* (Girard., from commercial ponds. Aquaculture 55, 161–164.

Sprague, V., Couch, J., 1971. An annotated list of protozoan parasites, hyperparasites, and commensals of decapod Crustacea. The Journal of Protozoology 18, 526–537.

Subasinghe, R., Soto, D., Jia, J., 2009. Global aquaculture and its role in sustainable development. Reviews in Aquaculture 1, 2–9.

Tahir, U., Wang, Z., 2017. First record of a new epibionts suctorian ciliate *Tokophrya huangmeiensis* sp.n., Ciliophora, Phyllopharyngea. from redclaw crayfish *Cherax quadricarinatus* von Martens 1868, Zootaxa 4269, 287–295.

Theodore, M., Tamara, B., Collette, B., Jayde, F., Davis, S., Norman, S., 2019. Diseases of wild and cultured fishes in Alaska. Alaska Department of Fish and Game. Fish Pathology Laboratories. Available at: https://www.adfg.alaska.gov/static/species/disease/pdfs/fish-diseases/*Trichophrya*.pdf.

Thörnqvist, P.O., Söderhäll, K., 1993. *Psorospermium haeckeli* and its interaction with the crayfish defence system. Aquaculture 117, 205–213.

Vey, A., 1978. Recherches sur une maladie des écrevisses due au parasite *Psorospermium haeckeli* Hilgendorf. Freshwater crayfish 4, 1.

Vogelbein, W.K., Thune, R.L., 1988. Ultrastructural Features of Three Ectocommensal Protozoa Attached to the Gills of the Red Swamp Crawfish, *Procambarus clarkii* (*Crustacea: Decapoda*. 1. The Journal of Protozoology 35, 341–348.

Vogt, G., Keller, M., Brandis, D., 1996. Occurrence of *Psorospermium haeckeli* in the stone crayfish *Austropotamobius torrentium* from a population naturally mixed with the noble crayfish *Astacus astacus*. Diseases of Aquatic Organisms 25, 233–238.

Vogt, G., Rug, M., 1995. Microscopic anatomy and histochemistry of the crayfish parasite *Psorospermium haeckeli*. Diseases of Aquatic Organisms 21, 79–90.

Vogt, G., Rug, M., 1999. Life stages and tentative life cycle of *Psorospermium haeckeli*, a species of the novel DRIPs clade from the animal-fungal dichotomy. Journal of Experimental Zoology 283, 31–42.

Wahl, M., 2008. Ecological lever and interface ecology: epibiosis modulates the interactions between host and environment. Biofouling 24, 427–438.

Wendelaar Bonga, S.E., Lock, R.A.C., 1992. Toxicants and osmoregulation in fish. Netherlands Journal of Zoology. E.J. Brill, 42, 478–493.

Yasser, A.G., Naser, M.D., 2011. Impact of pollutants on fish collected from different parts of Shatt Al-Arab River: a histopathological study. Environmental Monitoring and Assessment 181, 175–182.

Zhou, T., Wang, Z., Yang, H., Gu, Z., 2019. Morphological and molecular identification of epibiontic sessilid *Epistylis semiciculus* n. sp. (*Ciliophora*, *Peritrich*ia. from *Procambarus clarkia* (*Crustacea, Decapoda*. in China. International Journal for Parasitology: Parasites and Wildlife 10, 289–298.