Parasitic fauna of spiny eel *Mastacembelus mastacembelus* from Greater Zab river in Iraq

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Summary

A total of 128 spiny eels *Mastacembelus mastacembelus* (Mastacembelidae) were collected from the Greater Zab river at Iski Kalak town, west of Erbil city, Kurdistan region, Iraq, from July 2007 to the end of June 2008. The fish were examined for ecto and endo parasites. The study revealed the existence of 16 species of parasites (with a total prevalence of 89.06%) including: two species of protozoans (*Trypanosoma* sp. and *Trichodina pediculus*), one species of monogenetic trematodes (*Mastacembelocleidus heteranchorus*), six species of digenetic trematodes (*Allocreadium transversale*, *Asymphylodora macracetabulum*, *Pseudochetosoma salmonicola*, *Clinostomum complanatum*, *Diplostomum flexicaudum* and *D. spathaceum*), two species of cestodes (*Polyonchobothrium magnum* and *Ligula intestinalis*), two species of nematodes (*Agamospirura* sp. and *Procamallanus viviparus*), one species of leech, crustacean and arachnoide (*Cystobranchus mammillatus*, *Argulus foliaceus* and *Arrenurus* sp. respectively). Five species of these parasites (*T. pediculus*, *A. transversale*, *D. flexicaudum*, *C. mammillatus* and *Arrenurus* sp.) were recorded for the first time in Iraq. In addition, *M. mastacembelus* was regarded as a new host for *A. macracetabulum*, *C. complanatum* and *A. foliaceus*.

Key words: Parasite fauna, Spiny eel, *Mastacembelus mastacembelus*, Iraq, Greater Zab river

Introduction

Species of spiny eel belong to Class Actinopterygii, Order Synbranchiformes, Family Mastacembelidae (Moyle and Cech, 2004). This family includes five genera and 74 species of freshwater fish of tropical Africa, eastwards to Korea, Malaysia and extends far into central Iran and Iraq (Froese and Pauly, 2007). One of them, *Mastacembelus mastacembelus*, is known in Iraq. The common names for this fish are marmarij or mar masi (Coad, 2007).

Because of the increase in the demand for this fish in the Kurdistan region nowadays, and in addition to its importance in the medical field since it carries the larva of nematodes (*Anisakis* sp.) that can be transmitted to humans in its adult stages (Abdullah, 2002), this research was conducted to investigate the parasites which infect this fish that are collected from the Greater Zab river in the Kurdistan region, adding them to those reported in Iraq.

Materials and Methods

Study area

The Greater Zab river is a large river (392 km), located to the east of the Tigris river in the north of Iraq (Kurdistan region). The sampling area in this study was located near Iski-Kalak city (36°37'0"N, 43°44'0"E) 40 km west of Erbil city.

Sampling

One hundred and twenty eight fresh specimens of *M. mastacembelus* (25-78 cm long) were collected from the Greater Zab river, from July 2007 to the end of June 2008. The fish specimens were collected by gill netting, cast netting and electro fishing by local commercial fishermen. In the laboratory, the fish were measured and examined externally and internally for parasites. Skin, fin and buccal cavity smears

were prepared by slight scraping and examined. The gill arches and filaments were examined under a dissecting microscope. The fish were opened from the abdominal side and each organ was separated and examined carefully under a dissecting microscope (Amlacher, 1970).

Methods used for collecting, fixing, staining and mounting of the parasite specimens were as follows:

Protozoa

For the study of haematozoans, thin blood films were made by taking the blood directly from the heart (from living fishes only). Blood films were air-dried, fixed in absolute methyl alcohol, stained with Giemsa's stain and mounted with Canada balsam (Amlacher, 1970). If trichodinids were present, samples of mucus from the skin, fin and gills were collected. Air dried smears were prepared using klein dry silver impregnation method for staining, the slides were stained with an aqueous solution of 2% AgNO3 for 8 min followed by 20 min exposure to UV light to demonstrate the morphology of the adhesive disc (Albaladejo and Arthur, 1989).

Monogenea

Worms were separated from the gills and fixed under coverlid according to Gussev (1985) in glycerol-gelatin or a mixture of neutral red and glycerin (1:3).

Digenea and Cestoda

Live worms were washed in 0.6% saline solution and fixed in 5% hot formalin, stained with haematoxylin or acetocarmine, then cleared in xylene and mounted in Canada balsam (Scholz, 1989).

Nematoda

Nematoda were fixed in 70% hot alcohol, then cleared in glycerine solution or lactophenol and mounted in jelly glycerine (Chai *et al*., 1986).

Annelida

Annelida were collected from the skin and fins, relaxed by gradual addition of 50% ethanol and fixed in 70% ethanol (Siddall, 2001).

Crustacea and Arachnoidea

Specimens were cleared with 85% lactic acid, and permanent slides were prepared by using jelly glycerin (Lin and Ho, 1998).

Photos were taken with an Olympus camera. Measurements of the parasites were made with an Olympus ocular micrometer, and the detected parasites identified according to their morphology. The following keys were consulted for identification: Bykhovskaya-Pavlovskaya *et al*. (1962) and Hoffman (1998).

Results

Sixteen parasite species were recorded and identified during the present study. The distribution of these parasites and their location on or in the fish host body are summarized in Table 1. The following is an account of the description and measurements of these parasites, especially those which are recorded here for the first time in Iraq.

Trypanosoma sp.

This hemoflagellate was found in the blood. The body of the trypanosome is elongated, slender and uniformly wide except near the extremities. Pointed at both ends, but at the posterior end in a lesser degree. The organisms are markedly monomorphic, slender with an S shape. The length of the body, except the free part of the flagellum, 20-28 µm, width 1.6-2 µm, length of the free part of the flagellum 9-14 µm. The nucleus is elongated, lying approximately in the first third of the body, the length of the nucleus is 3-4 µm. Kinetoplast spherical is present near the posterior end of the body (terminal), with a length of 1.2-1.4 µm. The undulating membrane was clear, with 5-6 undulations (Fig. 1).

Trichodina pediculus (Ehrenberg, 1838)

This ciliated protozoan was found on the skin. The diameter of the body was 55-70 µm, height 11-24 µm, the diameter of the adhesive apparatus 43-66 µm, corona 28.3-44.6 µm, and consists of 25-30 teeth, and the central part of the tooth is conical. External processes are in the form of falcate hooks in

Table 1: The distribution of the parasites recorded in different organs of *Mastacembelus mastacembelus* (No. 128) from Greater Zab river in Iraq

Parasites	No. of fish infected	Prevalence (%)	Mean intensity	Site of infection
Trypanosoma sp. (N)	3	3.3	-	Blood
Trichodina pediculus[*]	2	1.56	3	Skin
Mastacembelocleidus heteranchorus	109	85.15	33.5	Gills
Allocreadium transversale[*]	5	3.9	5.2	Intestine
Asymphylodora macracetabulum[**]	3	2.34	5	Intestine
Pseudochetosoma salmonicola	6	4.68	2.16	Gall bladder
Clinostomum complanatum	1	0.78	1	Branchial cavity
Diplostomum flexicaudum[*]	34	26.56	15.35	Eye lens
Diplostomum spathaceum	105	82.1	31.01	Eye lens
Polyonchobothrium magnum	25	19.53	4.4	Intestine
Ligula intestinalis	1	0.78	1	Intestine
Agamospirura sp.[**]	4	3.12	1	Intestinal wall
Procamallanus viviparus	70	54.68	9 .02	Stomach and Intestine
Cystibranchus mammillatus[*]	12	9.37	2.25	Skin and fins
Argulus foliaceus[**]	5	3.9	2	Skin and fins
Arrenurus sp.[*]	1	0.78	2	Gill cavity

[*]New parasite record in Iraq, [**]New host record in Iraq and (N): Blood taken from 90 living fish

which the thickening margin stains were darker than the finer portion, while the free ends are blunted or more rounded and the length of the external processes 5-6 µm. Internal processes were in the form of long slender needles, usually somewhat curved, with lengths averaging 9-9.6 µm. Macronucleus lies in the center of the body around the vestibulum, and the short rod-like micronucleus lies external to one end of the macronucleus (Fig. 2).

Mastacembelocleidus heteranchorus (Kulkarni, 1969)

Body proper with a long trunk, tapered cephalic region, and short broad peduncle. The body length is 380-520 µm, width 65-95 µm, Haptor length 60-94 µm, width 78-101 µm, and copulatory organ 12-18 µm. The ventral anchor length is 48-54 µm, width 26-34 µm. Dorsal anchor length is 28-36 µm, width 27-33 µm, with ventral bar 45-63 µm, dorsal bar 41-67 µm, and hook length 13-16 µm (Fig. 3).

Allocreadium transversale (Rud, 1802)

Flukes are of medium size with smooth cuticle. The body is long and tapered at both ends, and the prepharynx, small pharynx and esophagus are of varied length. The ventral sucker is larger than the oral sucker, and the intestinal trunks extend to the posterior end of the body or to the middle of the hind testis. Excretory vesicle saccular may reach the level of the posterior margin of the hind testis, with the genital pore anteroacetabular medial or slightly displaced. The genital bursa is small, oval and feebly pronounced, between the pharynx and the ventral sucker. The testes are round, and each are slightly-anterior to the other in the hind part of the body. The ovary is round pretesticular, and the vitellary well developed, partly covering the intestine. The uterus is usually short between the testes and the ventral sucker. Eggs are large and relatively few in number, light yellow. Body length 1.5-2.8 mm, width 0.4-0.7 mm; oral sucker 0.14-0.28 × 0.21-0.27 mm; the ventral sucker is transversely oval, almost twice as large as the oral sucker 0.28-0.32 × 0.38-0.49 mm, pharynx 0.07-0.15 mm, and eggs 0.086-0.115 × 0.045-0.098 mm (Fig. 4).

Asymphylodora macracetabulum (Belous, 1953)

This parasite was recorded from the intestine. This fluke is covered with small spines particularly anteriorly, expanded centrally and narrowed or pointed terminally. The oral sucker is well developed, the pharynx small and muscular, the esophagus possibly extremely long. The intestinal caeca is usually narrow and short. The ventral sucker is markedly larger than the oral sucker. The body length 2-3 mm, width 0.9-1.3 mm; the oral sucker 0.45-0.53 × 0.4-0.46 mm, and ventral sucker 0.75-0.85 mm (Fig. 5).

***Pseudochetosoma salmonicola* (Dollfus, 1951)**

This parasite was found in the gall bladder. The body is small, fusiform, covered with unpinned cuticle, and white or slightly yellowish in color. The mouth leads into a fairly developed pharynx which is highly muscular. The intestine gives off lateral branches in the oral sucker region. Body length 0.9-2.65 mm, width 0.5-1.1 mm, oral sucker 0.21-0.3 × 0.21-0.28 mm, and ventral sucker 0.26-0.4 × 0.28-0.4 mm (Fig. 6).

***Clinostomum complanatum* (Rud, 1819)**

One metacercaria was found in the branchial cavity. It is commonly known as yellow grub, ligulae shaped larva, body length 3.1-5.9 mm, width 0.8-1.4 mm. The ventral sucker is larger than the oral sucker and lies in the first third of the body. The diameter of the oral sucker is 0.25-0.35 mm and the ventral sucker 0.72-0.81 mm (Fig. 7).

***Diplostomum flexicaudum* (Cort et Brooks, 1928)**

This metacercariae was obtained from the eye lens. The body is oval and elongated, 0.35-0.45 mm in length, the forebody foliate. The oral sucker is considerably larger than the ventral, and the ventral sucker is deeply sunk into the parenchyma, and may extend anteriorly in the form of a highly protuberant papilla on both sides of the ventral sucker. Oral sucker 0.040-0.055 mm, ventral sucker 0.040-0.050 mm, hindbody 0.05-0.093 × 0.085-0.14 mm, and Brandes's organ 0.07-0.098 mm (Fig. 8).

***Diplostomum spathaceum* (Rud, 1819)**

This metacercaria was found in the eye lens. The body is broad and leafy shaped. The body length 0.40-0.61 mm, width 0.18-0.21 mm, the length of the lateral sucker is 0.011 mm the diameter of the ventral sucker, 0.019-0.024 mm, oral sucker 0.006-0.01 mm and intestinal caeca not branched 0.29-0.33 × 0.09-0.12 mm (Fig. 9).

***Polyonchobothrium magnum* (Zméev, 1936)**

This cestode was reported from the intestine. The adult worm length is 160-630 mm width 2-4 mm. The scolex trapezoidal, armed along the border by a single row of hooks numbering 42-63, and reaching a length of 0.021-0.058 mm. Emargination is present on the disc and region of the dorsal and ventral bothria. The scolex length is 0.58-1.0 mm, width 0.72-1.15 mm, and mature segment length 0.9-1.1 mm (Fig. 10).

***Ligula intestinalis* (Linnaeus, 1758)**

This plerocercoid was found in the intestine. A slender shape, white creamy in color, non-segmented worm with obvious dorsal and ventral grooves. The anterior end was almost round. Body length 0.5-3 cm (Fig. 11).

***Agamospirura* sp. (Dogel´ et Bykhovskii, 1939)**

Four encapsulated larvae were isolated from the intestinal wall. The larva twisted into a flat spiral, reaching a length of 3-5 mm (Fig. 12).

***Procamallanus viviparus* (Ali, 1956)**

A large number of adult males and females were obtained from the stomach and intestine. The males are smaller than females; they vary in length between 3 and 3.5 mm and in diameter between 0.09 and 0.12 mm. It possesses two spicules, unequal in length. The female measures 5-7 mm in length and 0.1-0.2 mm in diameter (Fig. 13).

***Cystobranchus mammillatus* (Malm, 1863)**

This leech was found on the skin and fins. The body is relatively short, more or less conspicuously differentiated into the neck and body proper. The posterior sucker is almost centrally attached, its dorsal surface slightly longer than the ventral. Eyelike spots are absent on the posterior sucker. Two pairs of eyes on the anterior sucker were slightly developed or absent. The posterior sucker was beset with stellate pigment cells. Radial bonds, lateral vesicle 11 pairs. The general color of the body was gray to dirty pink. Both sides of the body with metamerical transverse dark bands, having diffuse margins, somite consisting of 7 annuli. Body length 7-15 mm, with a

maximum width of 2-3 mm (Fig. 14).

Argulus foliaceus (Linnaeus, 1758)

This parasite was found on the skin and fins. The body is oval, the total body length is 5-6 mm, and the head coalesces with the first thoracic segment. Cephalothorax is covered by broad carapace extending posteriorly to two broad lobes (Fig. 15).

Arrenurus sp. (Dugis, 1834)

This parasite was isolated from the gill cavity. Mites with epimera cover a great part of the ventral surface of the body, the excretory pore is located on well developed chitinous plate, lying at the posterior tip of the body. Large maxillary organ in larval stage, with short strong 5-jointed palps. Palp joints 2 and 3 are relatively broad, the terminal joint has a strong falcate claw. The mandible is short and powerful; all epimera lie separately and cover a large part of the ventral surface. At the posterior epimer the free edge of the body lies an excretory plate bearing 1-2 pairs of cilia on its surface beside the excretory pore. The dorsal surface of the body is covered with oval, generally reticulately engraved dorsal scutellum. The color of the body varied from yellow, green to red. Body length 1-4 mm, width 0.5-2 mm, length of palps 0.03-0.12 mm, the dorsal surface length 0.15-0.25 mm, width 0.09-0.2 mm. Length of cephalothorax 0.04-0.11 mm, width 0.03-0.09 mm (Fig. 16).

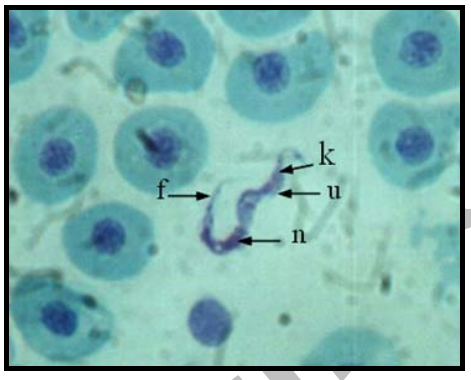

Fig. 1: *Trypanosoma* sp. (×1000). f = flagellum, k = kintoplast, n = nucleus and u = undulating membrane

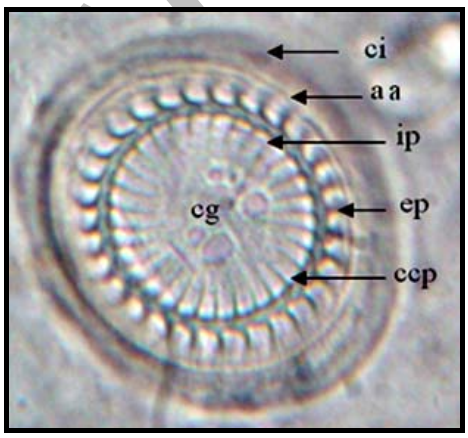

Fig. 2: *Trichodina pediculus* (×1000). aa = adhesive apparatus, ccp = central conical part, cg = central granule, ci = cilia, ep = external processes and ip = internal processes

Fig. 3: *Mastacembelocleidus heteranchorus* (×150). h = hapter

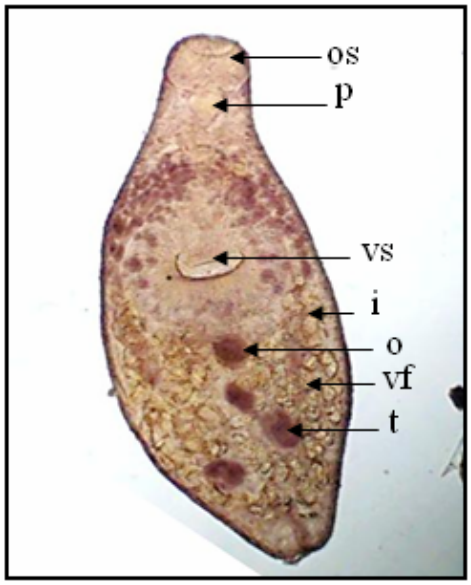

Fig. 4: *Allocreadium transversale* (×40). i = intestine, o = ovary, or = oral sucker, p = pharynx, t = testes, vf = vitellary follicle and vs = ventral sucker

Discussion

The present investigation is on the spiny eel *M. mastacembelus*. It revealed the presence of 16 parasitic species including both ecto and endopatasites. The spiny eel fish showed the highest infection rates with *M. heteranchorus* (85.15%) and *D. spathaceum* (82.1%), while on the other hand, the lowest infection rates were with *C. complanatum*, *L. intestinalis* and *Arrenurus* sp. which was 0.78% for each of them.

In Iraq, there are some reports on non-specified species of the genus *Trypanosoma* from some species of freshwater fish in different parts, including those from *Silurus glanis*, *M. mastacembelus* and *Barbus kersin* (Al-Niaeemi, 1997; Abdullah, 2002; Bilal, 2006). What is certain is that the *Trypanosoma* recorded in the present study is the same species recorded by Abdullah

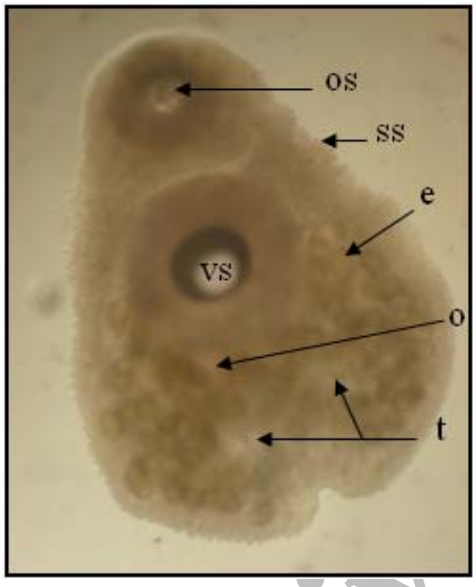

Fig. 5: *Asymphylodora acracetabulum* (×24). e = egg, o = ovary, or = oral sucker, ss = small spine, t = testes and vs = ventral sucker

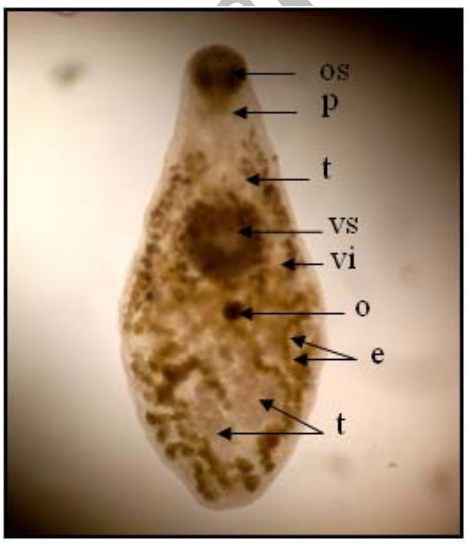

Fig. 6: *Pseudochetosoma salmonicola* (×40). e = egg, i = intestine, o = ovary, os = oral sucker, p = pharynx, t = testes, vi = vitellaria and vs = ventral sucker

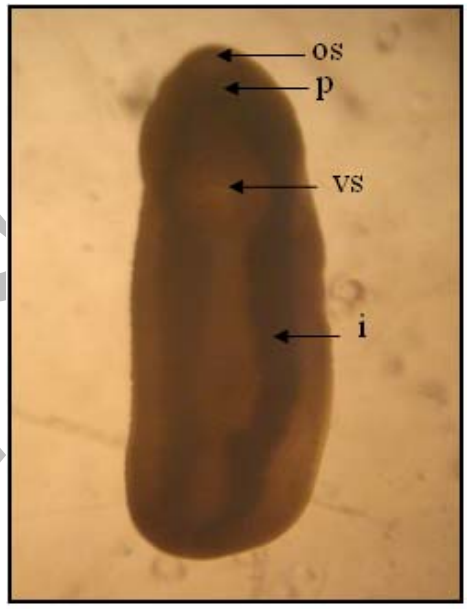

Fig. 7: Metacercaria of *Clinostomum complanatum* (×24). i = intestine, p = pharynx, os = oral sucker and vs = ventral sucker

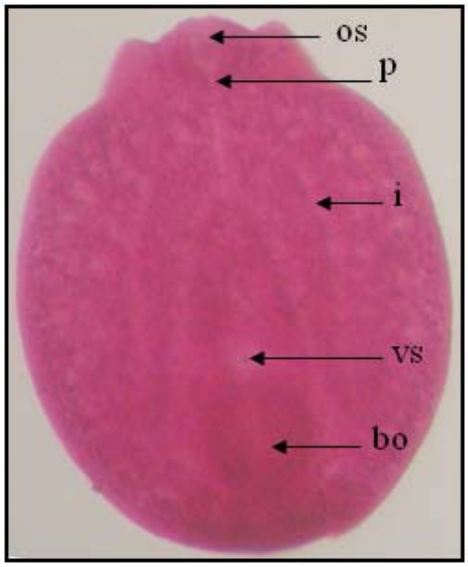

Fig. 8: Metacercaria of *Diplostomum flexicaudum* (×120). bo = Brandes's organ, i = intestine, os = oral sucker, p = pharynx and vs = ventral sucker

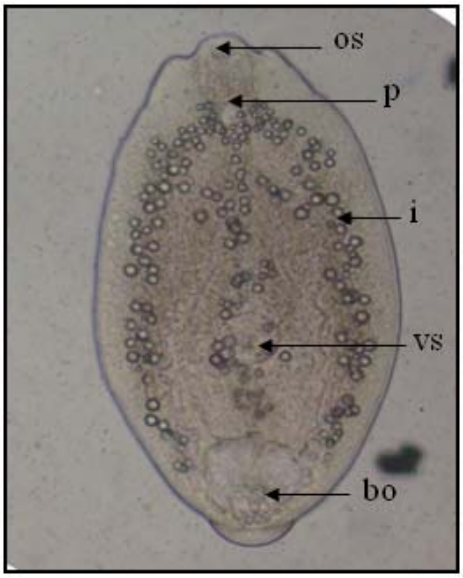

Fig. 9: Metacercaria of *Diplostomum spathaceum* (×120). bo = Brandes's organ, i = intestine, os = oral sucker, p = pharynx and vs = ventral sucker

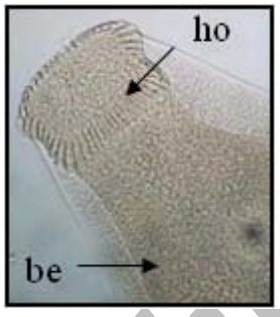

Fig. 10: Scolex of *Polyonchobothrium magnum* (×40). be = bothrial edge and ho= hook

Fig. 11: *Ligula intestinalis* (×100)

(2002) from *M. mastacembelus* collected from Iski Kalak town. Moreover, there are some classified species of *Trypanosoma* recorded in Iraq from various fish species

Fig. 12: *Agamospirura* sp. (×40)

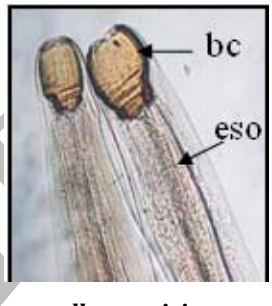

Fig. 13: *Procamallanus viviparous* (×40). bc = buccal cavity and eso = esophagus

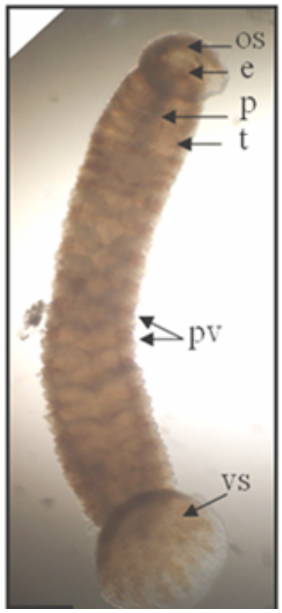

Fig. 14: *Cystobranchus mammillatu* (×10). es = eye spot, os = oral sucker, p = pharynx, pu = pulsatile vesicles, t = testes and vs = ventral sucker

such as *T. acanthobramae*, *T. neinavana*, *T. mystuii*, *T. garrae*, *T. arabica*, *T. basrensis* and *T. cyprinioni* (Mhaisen, 2008).

Concerning ciliated protozoans, in Iraq eight species of *Trichodina* were previously recorded namely: *T. acuta*, *T. cottidarum*, *T. domerguei*, *T. gracilis*, *T. heterodentata*, *T. mutabilis*, *T. nigra* and *T. nobilis* from different species of fish (Al-Marjan and

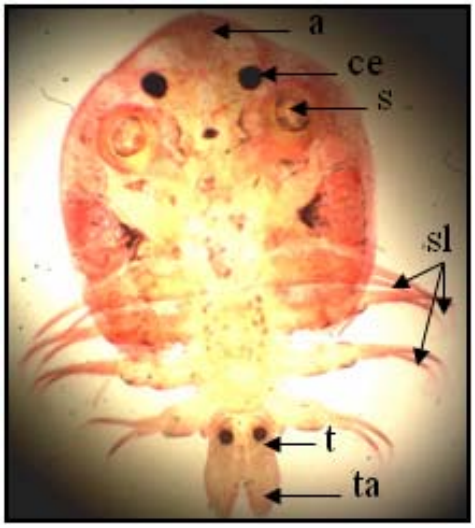

Fig. 15: *Argulus foliaceus* (×20). a = anterior, ce = compound eye, s = sucker, sl = swimming legs, t = testis and ta = tail

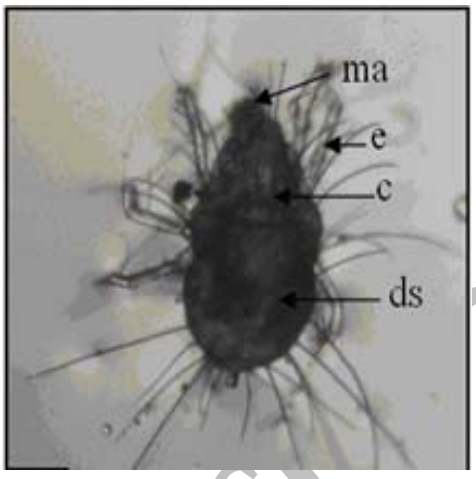

Fig. 16: *Arrenurus* sp. (×100). c = cephalothorax, ds = dorsal surface, e = epimera and ma = maxillary organ

Abdullah, 2007). These results indicate that this is the first record of *T. pediculus* in Iraq.

The present species, *M. heteranchorus*, has been described for the first time in Iraq from the same host (*M. mastacembelus*) by Kritsky *et al*. (2004). It seems that *M. heteranchorus* is a specific parasite of *M. mastacembelus*. This result is also confirmed by Jalali *et al*. (2008).

In this survey, six species of digenetic trematodes were recorded, most of which have been reported in other researches and other fish hosts previously, except for *A. transversale* and *D. flexicaudum*. The present record represents the first record of trematodes in Iraq (Mhaisen, 2008). Their description and measurements are similar to those reported by Bykhovskaya-Pavlovskaya *et al*. (1962), where *A. transversale* was detected in the stomach and intestine of thunder fish, spine loach, and crucian carp in freshwater of Moscow, and *D. flexicaudum* detected in the eye lens of freshwater fish, *Catastomus commersonii* in Lake Douglas, U.S.S.R. Furthermore, *M. mastacembelus* of the present study represents a new host for *A. macracetabulum* in Iraq, which was described for the first time in Iraq from the intestine of *B. luteus*, *B. grypus*, *Cyprinon kais* and *C. carpio* from Al-Musaib city at the Euphrates river (Al-Sáadi, 2007).

The description and measurements of *P. magnum* are similar to those reported by Ali *et al*. (1988) and Al-Sáadi (2007), who recorded it from *C. macrostomum* from two manmade lakes in Baghdad city and from *M. mastacembelus* collected from the Euphrates river, respectively. The other species of cestode which was found in this study was *L. intestinalis*. This parasite has been recorded previously from six different species of Iraqi freshwater fish, not including *M. mastacembelus* (Abdullah and Rasheed, 2004). Therefore, this fish represents a new host for this parasite in Iraq.

Two species of nematodes were recorded in this study, *Agamospirura* sp. and *P. viviparous*. The present parasites show a great similarity to specimens which were recorded previously in Iraq by Ali *et al*. (1987), from the surface of the liver of *Heteropneustes fossilis*, and in the stomach of *Mystus halpensis*, respectively from the Tigris river in Baghdad. According to Mhaisen (2008), *M. mastacembelus* is considered a new host for both nematodes in Iraq and the present study represents the first record in the Kurdistan region of Iraq.

According to Mhaisen (2008) five species of leeches are recorded in different freshwater fish in Iraq namely: *Cystibranchus mastacembeli*, *Hemiclepsis marginata*, *Piscicola geometra*, *Cystibranchus* sp. and *Piscicola* sp. Since there is no previous report on *C. mamillatus* in Iraq, the present record represents the first one in this country.

In this study, only one species of crustaceans was found, *A. foliaceus*. This

louse was recorded in Iraq for the first time on the skin, dorsal fin and gills of *Carasbarbus luteus* and *C. carpio* by Herzog (1969). After that, it was reported on ten fish species, excluding *M. mastacembelus* (Mhaisen, 2008). Therefore, *M. mastacembelus* is a new host for this parasite.

Several genera of mites have been reported from the skin, gills and esophagus of fish in Europe and North America. The larvae are usually encapsulated. Most authors consider them accidental parasites, but if they are present in large numbers in the water, the fish can become heavily infected and injured (Hoffman, 1998). Since there is no previous report on the recording of this parasite in Iraq, the present study represents the first record of *Arrenurus* in this country.

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