



First Record of *Calydiscoides difficilis* (Yamaguti, 1953) Young, 1969 (Monogenoidea, Polyonchoinea, Diplectaniade) on Gills of Three Fish species off Marine Waters of Iraq

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Abstract: A parasitological examination of two species of lethrinid fishes; Pink ear emperor *Lethrinus lentjan*, Spangled emperor *L. nebulosus* and Areolate grouper *Epinephelus areolatus* caught in Iraqi marine territorial waters from January to December 2020, revealed that the gills of the fishes were infected with deiplectanid monogenoid, which is identified as *Calydiscoides difficilis* (Yamaguti, 1953) Young, 1969. This study redescribed the parasite and for the first time described the egg and confirmed that the species has a wide range of dimension resulting from intraspecific variations. The measurements of hard and soft parts of the parasite collected from *L. lentjan* are smaller than those from *L. nebulosus*. In addition, the measurements from *E. areolatus* are close to that from *L. lentjan* or intermediate between *L. nebulosus* and *L. lentjan*. The past record of *C. difficilis* from *L. harak* is discussed and hence *L. nebulosus* and *L. lentjan* are considered as new records to the Arabian Gulf. Dimensions of the eggs of the parasite in the current study is given here for the first time. The occurrence of *C. difficilis* from Epinephelidae is considered as a new host family record.

Keywords: Ectoparasite, *Epinephelus areolatus*, Fish, Gills, *Lethrinus lentjan*, *Lethrinus nebulosus*.

Introduction

The family Lethrinidae has five genera and 44 valid species in the world (Fricke *et al.*, 2022). They are distributed in the tropical waters of the Indo-Pacific. Lethrinids are bottom-feeding, carnivorous, near reefs; they typically feed primarily at night on benthic invertebrates or fishes (Froese & Pauly, 2022). Pink ear emperor *Lethrinus lentjan* (Lacepède, 1802) is a marine, brackish water, reef-associated; non-migratory fish. It is distributed in the Indo-West Pacific, widespread from the Red Sea, Arabian (Persian) Gulf and East Africa. It is a

sandy bottoms dweller in coastal areas, deep lagoons and near coral reefs; feeds primarily on crustaceans and molluscs but echinoderms, polychaetes and fishes are also consumed in considerable quantities (Carpenter *et al.*, 1997).

Spangled emperor *Lethrinus nebulosus* (Forsskal, 1775) is a marine, brackish, reef-associated and non-migratory species. It is distributed in the Indo-West Pacific, the Red Sea, Arabian Gulf, East Africa to southern Japan and Samoa. It feeds on echinoderms,

mollusks and crustaceans, and to some extent on polychaetes and fishes (Froese & Pauly, 2022). It has been shown that this species can survive for long periods in salinities as low as 10 parts per thousand and therefore, it is a potential estuarine aquaculture species (Carpenter *et al.*, 1997) and very important commercial and sport fish in some countries (Froese & Pauly, 2022). Areolate grouper *Epinephelus areolatus* (Forsskål, 1775) usually occurs in seagrass beds or on fine sediment substratum close rocky reefs and dead coral (Heemstra, & Randall, 1993), and it feeds on fish and benthic invertebrates, mainly crabs and prawns (Randall & Heemstra, 1991).

There have been few works on monogenoids in the Arabian Gulf; *Tareenia acanthopagri*, (= *Benedenia acanthopagri* [Hussey, 1986]), *Megalocotyloides epinopheli* (= *Allobenedenia epinopheli* (Bychowsky and Nagibina, 1967) and *Polylabris angifer* have been isolated from marine perciform fish cultured in culture tanks at Al-Raas, Kuwait, by Hussey (1986). El-Naffar *et al.* (1992) recorded six genera belonging to five families of monogenoids from United Arab of Emirates coasts. Pur Ebrahim *et al.* (1999) isolated some gill monogenoids from *Eleutheronema tetradactylum*, *Otolithes ruber* and *Pampus argenteus* in Arabian Gulf. Kritsky *et al.* (2000) collected 17 species of diplectanids from gills of 17 species of marine fishes off Kuwait. Kardousha (2002) detected three species of *Capsala* from the mackerel tuna *Euthynnus affinis* off Qatar. In addition, two *Encotyllabe* species were recorded from fishes in Qatar (Kardousha *et al.*, 2002). Kritsky (2012) described *Euryhaliotrema seyi* Kritsky, 2012, from gills of *Lutjanus russellii* off the Arabian Gulf. Hassan *et al.* (2015) recorded *Benedenia acanthopagri* Hussey, 1986, from *Acanthopagrus bifasciatus*, *Diplodus noct* and *Sparidentex hasta* in Eastern Saudi Arabia.

In Iraq, the studies on monogenoids of marine fishes are few; Forty-one monogenoids taxa were reported from 84 fish species; from them 22 taxa belonged to the subclass Polyonchoinea and 19 taxa to subclass Oligonchoinea. Diplectanidae includes only five taxa: *Lamellodiscus iraqensis* Jassim & Al-Salim, 2020, *Lamellodiscus* sp. 1 from Arabian yellowfin seabream *Acanthopagrus arabicus*, *Lamellodiscus* sp. 2 from Twobar seabream *A. bifasciatus*, *Diplectanum* sp. 1 from Sin croaker *Johnius sina* (= *J. dussumieri*) and *Diplectanum* sp. 2 from Short-nosed tripodfish *Triacanthus biaculatus* (Mhaisen *et al.*, 2018; Jassim & Al-Salim, 2020).

Due to little attention on monogenoids of marine fishes of Iraq, and as no previous works on ectoparasites of Lethrinidae were done, apart from that a record of two ascaridoid nematodes (Ali, 2008; Al-Salim & Ali, 2010), one cucullanid nematode (Al-Salim & Ali, 2011), all from intestine of *L. nebulosus*, in addition to one copepod species from gills of *L. nebulosus* (Uyeno & Ali, 2013). Only four studies on endoparasites of Epinephelidae in Iraq were done; Al-Daraji *et al.* (2002) and Bannai (2017) isolated four and one species of trematodes respectively from alimentary canal of orange-spotted grouper *Epinephelus coioides* (Both studies erroneously reported it as *E. tauvina*); and from ovary of the same fish host, Moravec & Ali (2014) described a philometrid nematode. Al-Hajjaj (2021) isolated one species of each of trematode, from *E. areolatus* and Dusky-tail grouper *E. bleekeri*, nematode from *E. areolatus* and Yellowfin hind *Cephalopholis hemistiktos* and acanthocephalan from *E. areolatus*.

The current study is prepared to record and describe one diplectanid monogenoid species which is new to the parasitic fauna of fishes of Iraq.

Materials & Methods

A total of 266 specimens including 158 fish specimens of Lethrinidae (121 *Lethrinus lentjan* and 37 *L. nebulosus*) and 108 *Epinephelus areolatus* (Epinephelidae) were caught by fishermen by using trawl net, from Iraqi marine waters at 29°53'-29°85'N, 48°13-48°40'E during 2020 for parasitological examination. The fishes were transported freshly to the laboratory. The gill lamellae were immediately removed and placed in vials containing a hot 60°C 5% formalin solution for relaxation and fixation of attached helminths. The vials were labelled and then vigorously shaken for 15-30 seconds. Sclerotised structures of several helminths were studied by mounting some specimens on microscope slides unstained in Gray and Weiss medium. Other samples were stained with Gomori's trichrome or Mayer-Schuberg's Aceto carmine and mounted in Canada balsam on a slide to examine delicate anatomical details (Kritsky *et al.*, 1978; Humason, 1979; Palm, 2004). The term minimum prevalence is followed Kritsky *et al.* (2013). The illustrations were made by using a camera Lucida mounted on a Leica compound microscope. All measurements are in micrometres, with a mean between parentheses. The host taxonomy was followed by Carpenter *et al.* (1997) and verified by Fricke *et al.* (2022).

Results & Discussion

Calydiscoides difficilis (Yamaguti, 1953) Young, 1969 of the present study belongs to the family Diplectanidae, order Dactylogyridea, subclass Polyonchoinea of the class Monogenoidea.

Description

The body is fusiform to slender, with a maximum width at the testis level, in some specimens in the male copulatory organ level

(in very slender specimens). The body tapers markedly toward both ends of body. Three bilateral cephalic organs. Eye spots four, posterior pair somewhat larger than anterior pair. Mouth subterminal, pharynx spherical. Esophagus relatively short, bifurcated into two blind intestinal caeca blind. Peduncle broad (Fig. 1A) or narrow (Fig. 2). Haptor bilaterally lobed, wider than body width. Lamellodisc 10, the first three elements complete circle and the latter seven similar crescent chitinous lamellae (semicircle). Dorsal anchor somewhat curve blade point recurved and long, developed ventral root (Fig. 1C). Ventral anchor bifurcate with unequal roots and curved blade recurved point (Fig. 1B). Ventral bar transverse bow-shape very long tapered at both ends with median ventral groove (Fig. 1D). Dorsal bars, pair elongate, with outer hook and bifurcate inner end (Fig. 1E). Male copulatory organ (MCO) huge with two well sclerotized parts (Fig. 1F). Marginal hooks similar with delicate point and shank (Fig. 1G). Genital pore opens between two parts of MCO. Testis ovate and postovarian. Two prostatic reservoirs, one irregular elongated shape around anterior of MCO, the second spherical anterior the first one. Vas deference not observed, and seminal vesicle globular. Ovary variable in shape and the oviduct turned around right caecum. Vitellaria numerous distributed between pharynx and the posterior level of caeca. Egg, single elliptical in shape (Fig. 1H).

Taxonomic summary

Type host: *L. lentjan*, *L. nebulosus* and *E. areolatus*.

Date of collection: *L. lentjan* during January-March, May and August-December 2020; *L. nebulosus* during January-February and August- December 2020.

Infection site: Gill lamellae.

Voucher specimens: Iraq Natural History Museum: INHM-TRC 35-40 from *L. lentjan*, INHM-TRC 41-43 from *L. nebulosus* and INHM-TRC 44-47 from *E. areolatus*).

Minimum prevalence: 74.4% (90 of 121 *L. lentjan*, 35.1% (13 of 37 *L. nebulosus*) and 4.6% (5 of 108 *E. areolatus*).

Table (1): Comparison of the measurements of different soft and hard parts of *C. defficilis* from *L. nebulosus* and *L. lentjan* and *E. areolatus*.

Characters	Mean (Min-Max±SD; n)	Mean (Min-Max±SD;n)	Mean (Min-Max±SD;n)
Host	<i>L. nebulosus</i>	<i>L. lentjans</i>	<i>E. areolatus</i>
Length of body	748 (606-915±103; n=18)	610 (485-727±81; n=20)	641(603-6695±36; n=6)
Width of body	129 (92-229±43;n=15)	97 (75-135±18;n=18)	97 (89-103±5; n=6)
Width of haptor	193 (157-232±27;n=9)	157 (117-182±17;n=15)	156 (130-189±22; n=5)
Lamellodisc inner ring diameter	29 (16-37±4; n=18)	27 (17-32±4; n=11)	21 (16-24±3;n=8)
Length of ventral anchor inner	68 (65-70±1; n=20)	58 (47-65±5; n=20)	57 (54-59±2; n=10)
Length of ventral anchor outer	69 (65-73±3; n=21)	57 (42-62±5; n=22)	61 (59-62±1; n=10)
Length of dorsal anchor inner	47 (43-49±2; n=20)	38 (36-44±2; n=21)	40 (38-41±1; n=10)
Length of dorsal anchor outer	64 (59-67±2; n=20)	53 (47-57±3; n=22)	54 (51-57±2; n=10)
Length of ventral bar	148 (130-173±15; n=13)	112 (86-151±19; n=14)	122 (116-127±5; n=6)
Width of ventral bar	13 (11-14±2; n=13)	14 (14-14±0; n=6)	13 (11-14±2; n=5)
Length of dorsal bar	94 (81-104±7; n=21)	66 (57-84±7; n=21)	73 (70-76±3; n=12)
Width of dorsal bar	13 (10-16±2; n=14)	12 (10-14±2; n=10)	14 (11-14±1; n=11)
Length of MCO	80 (73-87±5; n=12)	74 (62-96±10; n=19)	79 (76-83±2; n=8)
Length of lamellodisc	76 (68-86±6; n=16)	70 (57-81±7; n=21)	64 (51-70±8; n=12)
Width of lamellodisc	70 (60-80±5; n=18)	54 (42-68±6; n=20)	53 (51-57±2; n=12)
Length of hook	12 (11-14±1;n=12)	11 (10-12±1;n=14)	11 (11-12±1; n=12)
Width of pharynx	38 (33-49±5;n=10)	31 (26-36±3;n=14)	28 (27-32±2; n=6)
Length of testis	102 (76-119±13;n=13)	70 (55-83±9;n=13)	81 (78-85±3; n=4)
Width of testis	75 (49-97±13;n=12)	51 (42-65±6;n=13)	60 (54-68±7, n=4)
Width of ovary	39 (30-43±4;n=9)	44 (30-60±9;n=13)	30 (27-33±2; n=5)
Length of egg	85 (73-97±8; n=13)	82 (62-94±9; n=10)	-
Width of egg	40 (35-49±4; n=12)	37 (31-46±6; n=9)	-
Length of egg filament	60 (49-73±8; n=8)	36 (26-42±7; n=8)	-

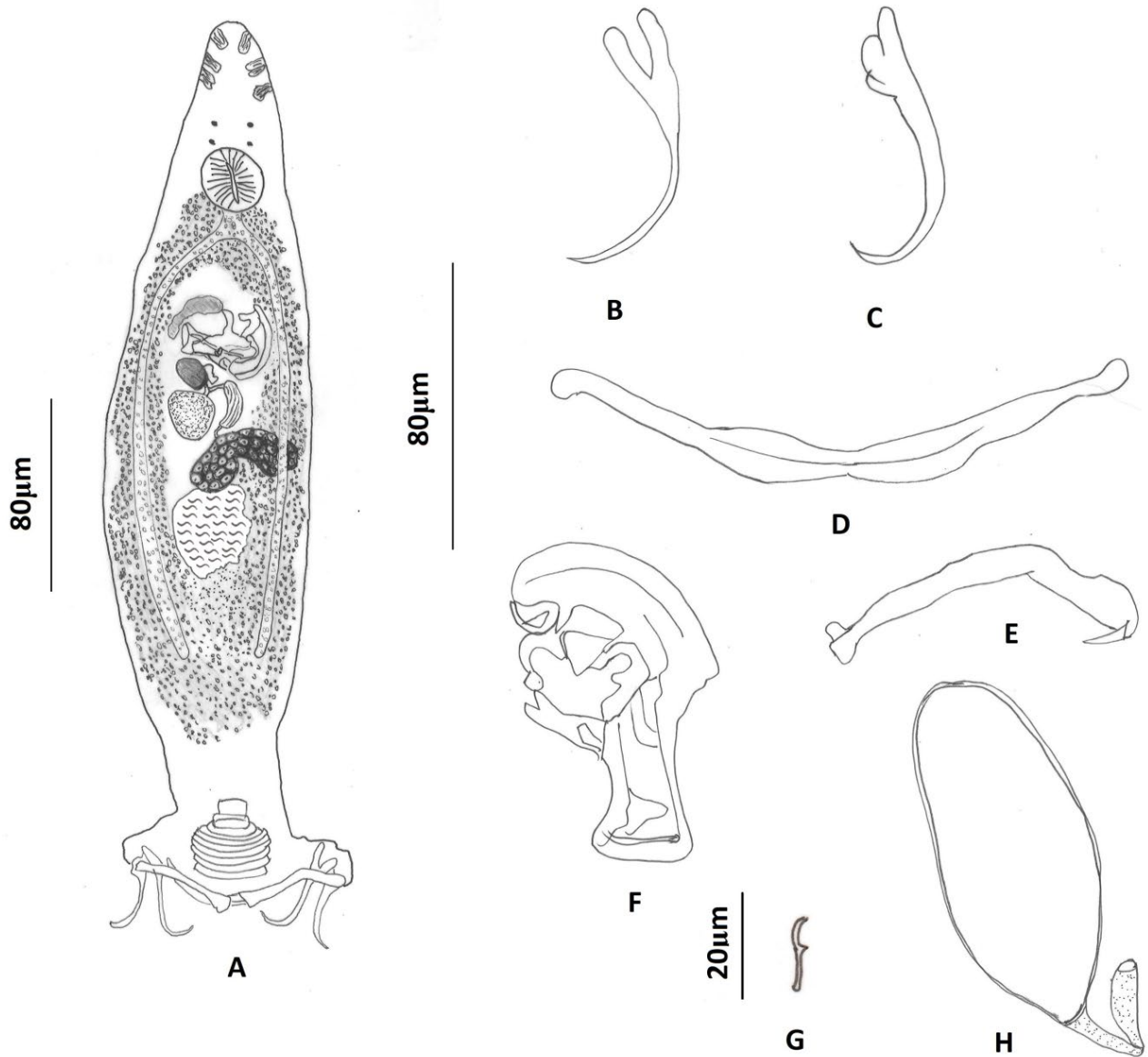


Fig. (1): *C. difficilis* from *L. nebulosus*. A- whole body, B- ventral anchor, C- dorsal anchor, D- ventral bar, E- dorsal bar, F- male copulatory organ, G- hook, H- egg.

Yamaguti (1953) described *Lamellodiscus difficilis* from gill of *Lethrinus* sp. from Indonesia. Young (1969) moved this parasite to *Calydiscoides* Young, 1969 depending on presence of telescoping lamellae from *Lethrinus fletus* (= *L. laticaudis*) off eastern Australia.

Young (1970) reported it again from *L. reticulatus*. Oliver (1984) recorded this worm from *Lethrinus chrysostomus* (= *L. miniatus*) and *L. nebulosus* from eastern Australia. Rohde *et al.* (1994, 1995) recorded this worm

from *L. miniatus* from eastern Australia. Rascalou & Justine (2007) reported it from *Lethrinus harak*, *L. lentjan*, *L. nebulosus*, *L. obsoletus* and *L. ravus* off New Caledonia, South Pacific.

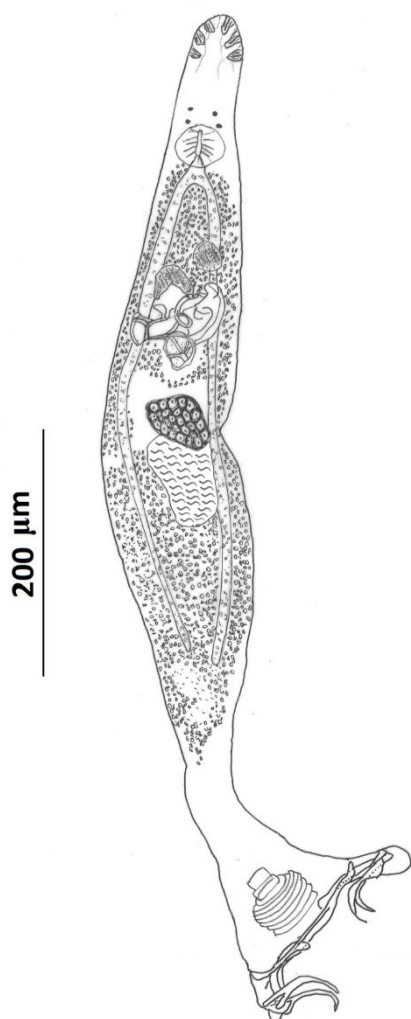


Fig. (2): A slender shape of *C. difficilis* from *L. nebulosus*.

Calydiscoides Young, 1969 has 18 valid species, eight species parasitic in Lethrinidae and 10 species from Nemipteridae (Justine, 2007; Domingues & Boeger, 2008; Justine & Brena, 2009; WoRMS, 2022). The members of *Cyclodiscoides* parasites of Nemipteridae are more specific in comparison with those from Lethrinidae. Seven from nine species of *Calydiscoides* parasites have single nemipterid host, while just three out of seven species of *Calydiscoides* are parasitic on a single host species of Lethrinidae. Furthermore, *C. difficilis* had nine hosts from *Lethrinus* spp., hence it is considered as a very wide range of hosts in Australia, Indonesia and Arabian Gulf (Justine, 2007; Rascalou & Justine, 2007;

Domingues & Boeger, 2008). Justine (2007) studied *Calydiscoides* species from lethrinids and found that they could be divided into two groups: the “difficilis” group with massive MCO, which includes *C. difficilis* and *C. rohdei* Oliver, 1984 and the “australis” group with branched MCO which includes *C. australis* Young, 1969, *C. duplicostatus* (Yamaguti, 1953) Young, 1969 and *C. gussevi* Oliver, 1984.

Rascalou & Justine (2007) compared the measurements and the descriptions the type specimens of *C. difficilis*, from Indonesia and museum specimens from Australia and the Arabian Gulf, and they found that the hard parts of specimens from the same host and from different hosts exhibited wide morphometric variations and they concluded that *C. difficilis* has a high degree of variability. This finding agrees with the present result of *C. difficilis* from *L. nebulosus* and *L. lentjan* from Iraqi waters. The measurements of soft organs, hard parts and MCO of parasites from *L. lentjan* are smaller than those from *L. nebulosus* (see table 1). In addition, different degrees of developed peduncle and the place of widest area in the body (MCO level in very slender specimens vis testis level in fusiform specimens) can be noticed in specimens from the two host species. These wide differences in the diagnosis dimensions within intraspecific variations might come from effect of some environmental criteria, season, intensity of infection and size of the host which was reported in *C. flexuosus* (Yamaguti, 1953) Young, 1969 from *Nemipterus* spp. from different geographical area (Kritsky *et al.*, 2000). According to Rascalou & Justine (2007), the museum specimens of monogenoides deposited by I. Al Mathal were identified as *C. difficilis* from *L. harak*. Indeed, this lethrinid host have not distribution in the

Arabian Gulf, but in the Gulf of Oman and Arabian Sea (Froese & Pauly, 2022). Many similar species, bought in the market, came from the Gulf of Oman (Randall *et al.*, 1994; Ali *et al.*, 2018). Accordingly, the record of *C. difficilis* from *L. nebulosus* and *L. lentjan* is considered as a new record to the Arabian Gulf, and dimensions of the eggs of the parasite in the current study is given here for the first time. Furthermore, the record of *C. difficilis* from *E. areolatus* is considered as for the first time in the world (New host family record). Consequently, *C. difficilis* became not just has wide range of hosts from Lethrinidae, but also as a parasite of one species of Epinephelidae.

Conclusion

The parasitological investigation of monogenoid parasites of the families Epinephelidae and Lethrinidae in Iraqi marine waters in the current study was carried out for the first time. The Lethrinidae represents here by two species: *Lethrinus lentjan* and *L. nebulosus* and both host species were found infected with *C. difficilis*, which has a broad range in measurements of both soft and hard parts of the parasite even within the same host species. In addition, two different shapes (fusiform and slender) of parasite were existed. The occurrence of monogenoideans of the genus *Calydiscoides* on gills of Epinephelidae (*E. areolatus*) is documented for the first time and the Epinephelidae is added as the third family within hosts of *Calydiscoides* in addition to Lethrinidae and Nemipteridae.

Contributions of Authors

A.A.R.A: Collection of specimens, Laboratory techniques, wrote and revised the manuscript.

A.H.A: Suggestion the proposal of the article, wrote and revised the manuscript, identification of the parasite.

H.A.S: Suggestion the proposal of the article, revised the manuscript.

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Ethical approval

All ethical guidelines related to Fish and care issued by national and international organizations were implemented in this report.

Conflicts of interest

The authors declare that they have no conflict of interests.

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أول تسجيل للدودة أحادية المنشأ (*Calydiscoides difficilis* (Yamaguti, 1953) (الديدان أحادية المنشأ: تحت صنف بوليونكوييني: عائلة دبليكتانيدي) على ثلاثة أنواع من الأسماك من المياه البحرية العراقية

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المستخلص: نتيجة الفحص الطفيلي لغلاصم نوعين من أسماك الشعري والتي شملت الشعري وردى الأذن *Lethrinus lentjan* والشعري اللماع *L. nebulosus* والهامور اريولاتاس *Epinephelus areolatus* في المياه البحرية العراقية خلال الفترة من كانون الثاني-كانون الأول 2020 ظهر أن غلاصم الأسماك مصابة بنوع واحد من الديدان أحادية المنشأ العائدة للعائلة Diplectanidae وهو النوع *Calydiscoides difficilis* (Yamaguti, 1953) Young, 1969. تناولت الدراسة الحالية إعادة وصف النوع ووصفت البيوض لأول مرة وأكدت مانتشر في الدراسات الأخيرة بأن الطفيلي يمتلك مدى واسع من الصفات المظهرية التي تقع ضمن إختلافات النوع نفسه. وكانت قياسات للأجزاء الصلبة والرخوة للنماذج المعزولة من سمك الشعري وردى الأذن أصغر من تلك المعزولة من الشعري اللماع. كما أن القياسات من الهامور اريولاتاس أقرب الى تلك المسجلة من الشعري وردى الأذن أو تكون وسطية بين القياسات المعزولة من الشعري والشعري وردى الأذن. التسجيل السابق للنوع *C. difficilis* من الشعري *L. harak* نوقشت وعليه فأن تسجيل الطفيلي يعدّ الاول في الخليج العربي من كلا نوعي الأسماك. وصف بيض الطفيلي في الدراسة الحالية يعدّ للمرة الاولى. ان تواجد *C. difficilis* على غلاصم عائلة الهامور يعدّ بمثابة تسجيل عائلة مضيئة جديدة لهذا الطفيلي.

الكلمات المفتاحية: طفيليات خارجية، *Epinephelus areolatus*، أسماك، غلاصم، *Lethrinus lentjan*، *Lethrinus nebulosus*.