Prevalence and intensity of internal parasitic helminthes infected Black sole fish, *Brachirus orientalis* (Bloch and Schneider, 1801) in the Persian Gulf

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Abstract

This study was conducted to identify intestinal helminthes parasites. The helminthes fauna is collected from body cavity and intestines of 108 specimens of *Brachirus orientalis* which were obtained from Abadan port (waterfront) and investigated during the years 2009-2010. Most of the 4 species of helminthes were found in the intestine and body cavity of the examined fishes. The helminthes found was composed of 2 digenetic Trematodes (*Lepocreadioides zebrine, Allocreadium brachirusii*) all adults, in the intestine. The general prevalence of Lepocreadioides zebrini and *Allocreadium sp.* was in the intestine, 58% and 73% respectively, nematode *Hysterothylacium aduncum* with a prevalence of 23% was the only cestod Protocephalus sp. with 16%, except *Pterobothrium sp*., which was reported previously. Most parasite species found in this study have been reported for the first time from Iran and Allocreadium sp. is introduced as a new species and described here.

Keywords: Helminthes fauna, Intestine, *Brachirus orientalis*, Persian Gulf, Iran

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Introduction

Lack of investigation about this species as one of the most familiar edible sources of marine fish in the Persian Gulf is tangible. Almost 60% of flat fish are caught from Khuzestan. These fish have been captured in Abadan, which is the most important port of Khuzestan, and this study has been done in this harbor.

The Flounder fish contained 6 families, 117 genus and 538 species. Flat fish order (Pleuronectiformes) is one of the most diversified fishes (Yasemi et al., 2008). This order is one of the most significant species among near coast commercial fish resources. Bottom trawls on the inner continental shelf is the major catching method of this species. They are categorized as high valued species in the world (Nelson, 2006).This order is widely distributed in the Persian Gulf and Oman Sea. 1700 Mt out of 38000 Mt consists of flat fishes which are considered as 4.5% of the total catch amount harvested in Khuzestan province and to which the highest catch amount among 4 southern provinces belongs. The catches of Boushehr, Sistano-Baluchestan and Hormozgan provinces were 140, 300 and 760 Mt, respectively (Iran Fisheries Organization, 2009).

The pattern of food and feeding habits of *Brachirus orientalis* were studied during April 1987 to June 1988 by Khan et al. (1993). The food content consisted of annelids (2.82%), crustaceans (14.23%), molluscs (17.85%), platy-helminthes (4.16%), nematohelminthes (4.70%), fish (3.09%), sand grains (30.07%) and miscellaneous food items such as semi-digested parts of fish, crustaceans, and shells etc. (56.51%). The food and feeding habits varied with reference to season (Khan et al., 1993). Geffen et al. (2007) reported that it feeds predominantly on bottom-living invertebrates, especially small crustaceans. Ambicoloration is an abnormality described in flatfishes (Carnikián et al., 2006).

The maximum total length of the species is about 30 cm, commonly 10 to 12 cm **(**Khan and Shamsulhoda., 1998). Flatfish metamorphosis includes a unique physical character with morphological and physiological changes associated with eye migration, a 90° rotation in posture and asymmetrical pigmentation **(**Geffen et al, 2007). Distribution of these fish are common throughout most tropical coastal waters of the Indo-West Pacific area from Gulf of Thailand northward to Taiwan and southern Japan, southward through the Philippines, New Guinea to north-central Australia (both coasts); westward to India and the Persian Gulf (Menon, 1978). Marine fish parasites have received little attention in Iran until recently studies of helminthes Parasitology have been developed in the recent years (Peighan and Hoghoghi, 2004; Peighan et al., 2006; Abdi, 2010; Bazari moghadam, 2010; Raissy, 2010). In recent years, several known and some unknown Helminthes species have been reported and described by Iranian researchers (Raissy et al., 2010**)**. Many species of helminthes were observed in the Persian Gulf by Abdi (2010)., but in the neighboring countries many of fish parasites in this region were reported by Khalil and Polling (1997) and by some researchers from Kuwait and Emirates (Sey

et al., 2003). The objective of this study was to identify intestinal helminthes parasites. Mandani, (1994) reported the Trypanorhynchus larva in flesh of flat fishes from the Persian Gulf. Amin et al. (1984) have found Acanthocephal *Neoechinorhynchida* and *serrasentis sagittifer* from flatfish intestines in Kuwait shores. Ahmad et al. (1987) have also reported *Lasiotocus guptai* from intestine of flat fish in the Arabian Sea. Nahhas et al. (2002) have reported *Erilepturus Hamati* in flat fish intestines originated from Kuwait shores in the Persian Gulf. Gonzales et al. (2001) have reported 12 parasite species in big eye flat fishes from northern Chile which 7 species were internal parasites; Digens from Hemiuridae family and Cestodes , *Floridosentis sp Corynosoma australe* and *Neobothriocephalus adspinosus*. Oliva et al. (1996) have reported nematodes; Capillaria sp, Anisakis sp, Philometra sp and *Nybelinia surmenicola* in *Paralichthys adspersus* which is a type of flat fish. Alkuwari (2000) from Qatari waters was found to be infected with 2 species of digenetic trematodes which have been identified as *Treptodemus latus* and *chauhantrema spiniacetabulum.* According to Saoud et al. (1986) 18 identified genera of digenetic trematodes were recorded for the first time in the Persian Gulf. Shimazu et al. (2000) have reported *Allocreadium patogonicum* sp.n. (Alloocreadiidae) which has been identified from the intestine of *Percichthys colhuapiensis* and *percichthys trucha* in Argentina. Richard arthurand and Abu tweb (2002) have reported 8 species from Order Plagiorchiida, Allocreadioidea Superfamily and Allocreadiidae Family whose names were *Allocreadium bengalensis, A.glossogobium, A.handiai, A.mahaseri, A.mehrai, A.minutum, A.mymensinghi and A.ovatum*, all of which were found in the intestine. Sheng-fa et al. (2010) reported 5 species of genus Lepocradidae (Trematoda) from Chinese marine fish. *L. hunghuaensis* from *Cynoglossus semilaevis*, *L. orientalis* from *Cynoglossus robustus*, *L.epocreadioides discum* and *L. pagrosomi* from *Pagrus major* and *L. zebrini* from *Zebrias zebra*. Bray and Gibson (1989) reported a new species, *Neolepidapedon smithi* of the Lepocreadiidae (Digenea) family, it is described from the fish *Mora moro* in the north-eastern Atlantic Ocean and is distinguished from other members of the genus. Lile (1998) has reported eighteen species of helminths which were analysed in relation to host–parasite specificity and the effect of host ecological preferences on the establishment of parasite fauna in the alimentary tract of four flatfish were also analysed. The Persian Gulf in the Southwest Asian region is an extension of the Indian Ocean located between Iran and the Arabian Peninsula, historically and commonly known as the Persian Gulf. The length and width of the Persian Gulf basin region is about 1000 Km and 200-300 Km respectively. The Persian Gulf is situated between 26/30 31' latitude and 56 20 longitude. The average temperature within the sampling region is 35 degrees in August and 13 degrees in February. The salinity of the Persian Gulf is 40-41 ppt and this figure reaches 50 ppt in shallow regions. The helminthes fauna found in the intestine of 108 specimens of Brachirus orientalis (Osteichthes: Soleidae) was

obtained from the Persian Gulf. Fresh catch specimens of Black sole were acquired from Abadan port during spring 2008 to winter 2009 in the Persian Gulf, Iran.

Materials and methods

A total of 108 *B. orientalis*, Black sole fishwere obtained from Abadan ports, during February 2009 to January 2010. Both commercial and non commercial sized fish (18-40cm) were collected, these collected fish were kept on ice and brought to the laboratory of Aquatic Department, Faculty of Veterinary Science, Chamran University. An Iranian ichthyologist in accordance with Etemad and Mokhayer (1979) carried out identification of fish host. The methods and techniques used for collection, relaxation, fixation, staining and mounting of helminthes are basically those described by Hanek and Fernando (1972) and Roberts (2001).

Figure1: Brachirus orientalis-Black sole (Soleidae)

Each fish was measured, the total length to nearest 0.1cm, the total weight to nearest 10 g and the sex was determined internally. Fishes were examined only for internal parasites, each fish was opened and the intestines were fully examined for parasites. The abdominal cavity of each fish was cut open and the intestine was separated from the other visceral organs and placed in a Petri-dish containing physiological saline and the helminthes were found with loop. The digeneans were washed in a 0.6% saline solution and fixed in 70% ethanol alcohol. They were stained with alum carmine, dehydrated and then cleaned in xylene and mounted in Canada balsam for nematodes. Fish were dissected and the intestine and body cavity were observed carefully and nematodes were collected from the fish intestine and washed in saline (0.6%-0.8%). With the nematode kept extended, they were fixed in 76% alcohol and cleaned in lacto phenol for seasonal days. Permanent mount was made by using azocarmin stains and the identification of parasites was carried out according to keys given by Gussev (1985), Moravec (1994) and Yamaguti (1971). Prevalence and mean intensity were calculated according to the definitions given by Bush et al. (1997). Measurements expressed in micrometers and in parentheses are calculated for those species with measurements done on three

specimens; numbers are rounded to the nearest decimal. Drawings were prepared by Camera Lucida and photographs were taken by a Canon digital camera (A1000).

Results

The material collected during the present investigation was used for identification of the helminthes fauna currently occurring on *Brachirus orientalis* in Abadan, and also for estimating the prevalence and intensity of infestation of fish. All 4 helminthes were found in the body cavity and intestine of dissected fish. The following helminthes found are as follows.

Table 1: Helminthes species in flounder fishes in the Persian Gulf

parasites	Locality(ies)	helminthes	Catching site(s)	Reference(s)
Erilepturus hamati	Intestine	Trematode	Kuwait waters	Nahhas et al 2002
Lasitocus guptai	Intestine	Trematode	Arabian sea	Ahmad et al 1987
Trypanorhynca larva	Muscles	Cestoda	Persian Gulf	Mandani et al 1994
Serrasentis sagittifer	Intestine	Acanthocephal	Kuwait waters	Amin et al 1984
Lepocreadioides zebrini	Intestine	Trematode	Persian Gulf	Present survey
Allocreadium Brachirusi	Intestine	Trematode	Persian Gulf	Present survey
Hysterothylacium aduncum	Intestine	Nematode	Persian Gulf	Present survey
Protocephalus sp	Intestine	Cestoda	Persian Gulf	Present survey

Trematodes

Intestine prevalence of the Lepocreadioides zebrini Yamaguti, 1936 was assessed as 58% (table2) but according to table 6 the mean intensity was calculated as 4.65.

Yamaguti, 1936, found and described the two new species of the Lepocreadidae family observed in marine fish. *L. zebrini* from Zebrias zebrinus, which is distinguished from the closely related Lepocreadium based on the position of the genital pore. This helminthes has a cirrus sac with a small valve and all measurements are given in table 4 with a micrometer that seems to be the first report in the region (Figs. 2, 3).

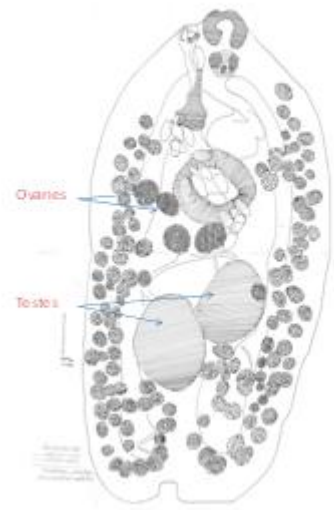


Figure 2: Lepocreadioides zebrini

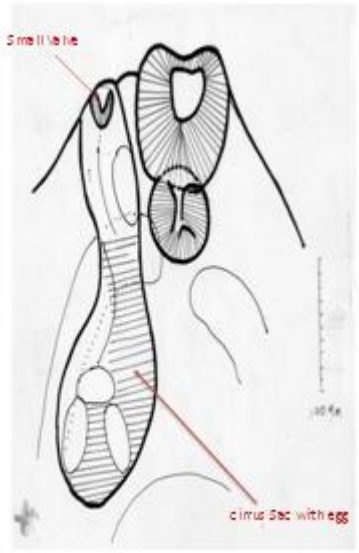


Figure 3: cirrus sac L.zebrini

Intestine *prevalence of Allocreadium* sp Looss, 1894 (New species) was 73% (table 2) but according to table 6 the mean intensity was calculated as 2.03. This helminthes has a cirrus sac in double folding, all measurements are given in table 4 and this species has been reported from the Persian Gulf for the first time (Figs. 4, 5).

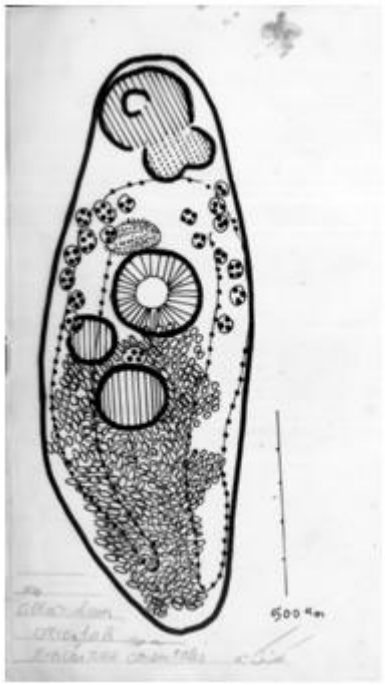


Figure 4 Allocreadium (New species)

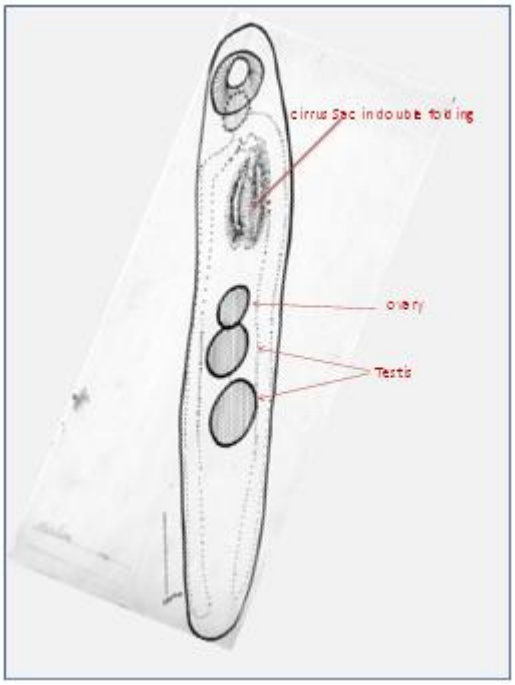


Figure 5: cirrus sac Allocreadium sp.

Table 2: Prevalence of Internal parasites in examined fish in north of the Persian Golf

Parasites	Fish infected	Fish uninfected	Prevalence (%)
Hysterothylacium Aduncum	25	83	23
Allocreadium Sp	79	21	73
Lepocreadioides zebrini	63	37	58
Proteocephalus Sp	18	90	16

Table 3: Prevalence of different helminthes in Black sole in the Persian Gulf 2009-10

Parasites	Spring (%)	Summer (%)	Autumn (%)	Winter (%)
Hysterothylacium Aduncum	56	4	16	16
Allocreadium Sp	92	84	48	68
Lepocreadioides zebrini	76	74	28	52
Proteocephalus Sp	20	0	4	40

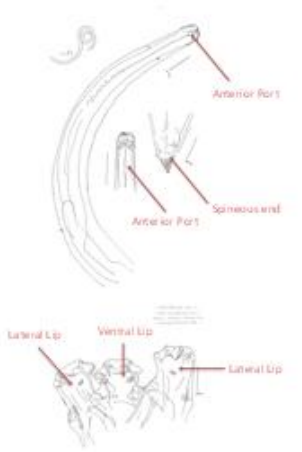
Table 4: Measurements of *Allocreadium* sp and *Lepocreadioides zebrini*

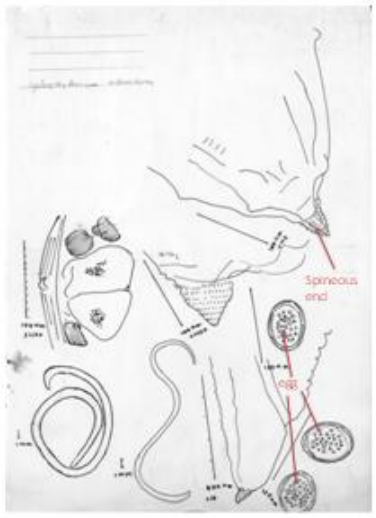
Species Measurement	Measurement
Allocreadium sp	
Eggs	10×15-65×50(33×25)
Pharynx	40×40-162×125(93×76)
Ventral sucker	70×72-212×200(135×124)
Oral sucker	80×70-223×182(143×129)
Ovary	110×100-50×45(95×90)
Testes	170×150-80×70(130-120)
Body width	150-550(297)
Body length	454-1625(915.5)
Lepocreadioides zebrini	
Eggs	25×30-40×40(35.8×32)
Pharynx	15.2×18.3-65×80(44×53)
Ventral sucker	39.8×37.6-185×225(118.2×152.5)
Oral sucker	43×45-77×90(45.9×51)
Ovary	90×70-65×60(70×65)
Testes	230×190-150×140(200×165)
Body width	400-645.8(541.9)
Body length	790-1187.5(969.1)

Nematode

H. auctum (syn. *H. aduncum)* is a common nematode occurring in many regions of the world in the fish of sea and brackish waters (Hartwich, 1975). *Hysterothylacium aduncum* is known as a parasitic nematode of marine fish, which act either as a final or

paratenic host (Rudolphi, 1802). Adult worms and larvae are found in the alimentary canal and in the body cavity, respectively. No case of human infection with the nematode has been reported (Yoshinaga, 1987). *H aduncum* has two telogonic ovaries. Larvae encysted third-stage larvae and adults were found in intestines of examined fish. Mentioned parasites, have typically occurred in several flatfish in the European sea (Szostakowska and Sulgostowska, 2001). *Hysterothylacium aduncum* was a common species reported in flounder from the Baltic Sea in various seasons by Sulgostowska et al. (1987). In the present study the general prevalence was 23% (table 2) and according to table 6 the mean intensity was 1.16 parasites in a fish. All measurements are given in table 5 with a micrometer (Figs. 6, 7, 8).





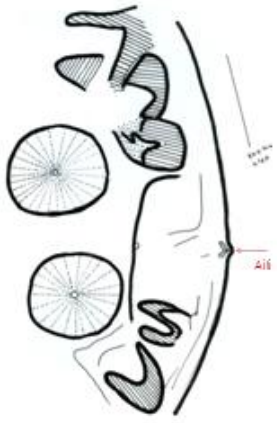


Figure 6: ***Hysterothylacium aduncum*** **Figure 7:** ***H aduncum*** **(eggs)** **Figure 8:** ***Lateral section***

Table5: Measurements of *Hysterothylacium Aduncum*

Spineous end	30-41(35)
Ventral lip	160×90-170×115(163×103)
Dorsal lip	180×64-190×80(185×73)
Caudal pappies	8-14(12)
Anterior cecum	790×263-861×290(850×285)
Posterior cecum	890×503-931×566(920×550)
Spicule	2400-3209(2700)
Egg	53×41-60×50(58×44)
Ventricle	140×110-160×130(150×124)
Body width	500-700(600)
Body length	16200-45800(32060)

Cestoda

Protocephalus sp reported by Weinland, 1858 is the only cestod which was found in examined fish. Sculex has five suckers. The general prevalence is 16%, the mean intensity is 1.5 parasites in a fish and measurements of scolex in this Cestoda were 165×170-185×200(170×175) (fig. 9).

Azadikhah (2009) showed that the parasite *silususglanis* species from Aras reservoir caused pathological changes by the scolex in the attaching site of intestines of infected fish.

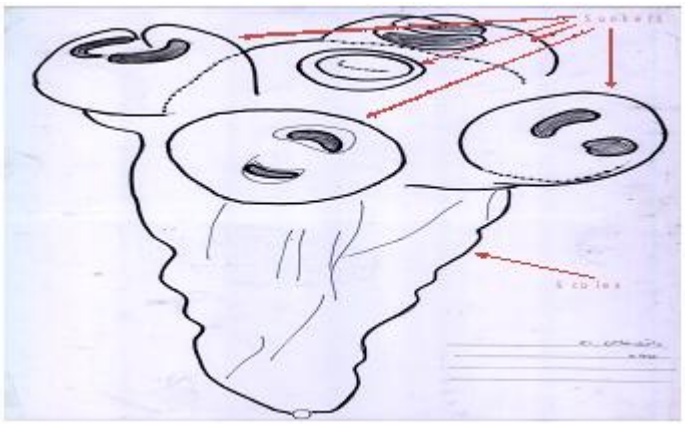


Figure 9: *Protocephalus sp* (sculex)

Table 6: Intensity of parasites in Black soles in the Persian Gulf

Parasites name	The lowest No. of parasites in infected fish	The highest No. of parasites in infected fish	Mean intensity No.
Hysterothylacium Aduncum	1	2	1.16
Allocreadium brachirusi	1	15	2.03
Lepocreadioides zebrini	1	17	4.65
Proteocephalus Sp	1	4	1.5

Discussion

In the present study, prevalence of the infection by Trematodes according to Abdi (2010) was examined, the highest prevalence by trematodes was in spring and the lowest was in autumn. These results are caused by the climate of the Persian Gulf, having hot weather in summer and autumn which makes parasites unable to live in this climate, but in spring the weather is very suitable for parasites.

Probably the highest prevalence of infection by nematodes was in spring and the lowest prevalence was in summer in which the climate is unsuitable for parasites. Fischer and Kelso (1990) have reported that parasite loads increased

quickly in spring, and nearly all juvenile fishes over 25 mm long supported a parasite fauna by *Proteocephalus* (Cestoda). In the present study parasite load was in winter by proteocephalus. There was little infection of Gobid species with Proteocephalus, and the prevalence was lower than present studies (Kvach, 2005), especially in winter which the prevalence in our study was highest and in the summer parasites were not found. The prevalence and intensity of the helminthes' infection from the intestine and body cavity of the fish examined in summer did not increase in larger fish. The intermediate hosts of *H. aduncum* have been known to be marine molluscs, annelids and arthropods (Yoshinaga, 1987). Pure infections with Trematodes were common but similar infections with nematodes and cestodes were less frequent (Saoud, 1986). Prevalence and mean intensity of *Hysterothylacium aduncum* in the present study were higher than the mentioned result in Szostakowska and Sulgostowska (2001) from southern Baltic in Herring fishes, but the maximum prevalence was in spring for this parasite and both studies were similar in this matter. Szostakowska and Sulgostowska (2001) reported the high prevalence of *H. aduncum* infection in Baltic herring *Clupea haerengus* from the southern Baltic Sea in spring. *Hysterothylacium aduncum* was found in all seasons with a high prevalence and mean intensity in Flounder fish from the Gulf of Gdansk (Chibani and Rokicki, 2004), but in the present study the prevalence was lower than Chibani, the lowest prevalence and intensity in both cycles was in summer which was similar to our study.

Hysterothylacium aduncum is a parasite that is not very host-specific in either the larval or adult stages (Chibani and Rokicki, 2004). Berenice et al. (2009) reported *Acanthogalea gibsoni* (Digenea, Yamaguti, 1936, finds and describes two new species of the Lepocreadidae family from marine fish. *L. zebrini* from Zebrias zebrinus, is distinguished from the closely related Lepocreadium by the position of the genital poreiidae (from *Balistes vetula),* one of the genus of the Lepocreadiidae family, were captured from surrounding waters in the state of Rio de Janeiro. The prevalence was lower than the present study. Dyer et al .(1998) reported *Lepocreadium trulla* from Marine Fish, *Ocyrus chysurus* with a mean intensity lower than the present study in Paraguera of Puerto Rico. Members of Lepocreadiidae are recognizable as worms with widely distributed vitelline folicles, aspinous tegument, usually with a distinct external seminal vesicle and a typically I–shaped excretory vesicle (Bray 2005).The influence of diet preferences in macroparasite infection levels of 7 Soleidae along the Portuguese coast has been examined by Marques et al. (2006) and these authors found that most of the

variation of prevalence and mean abundance was related to the type and quantity of food ingested as well as habitat use.The excretory vesicle winding between the testes seem to relate it more to the genus *Lepocreadioides* (Yamaguti, 1936). *Lepocreadioides* however, has a cirrus sac and a genital pore far to the left and far anterior. *L. zebrini* (Yamaguti, 1936) and *L. branchiostegi* (Yamaguti, 1937) both have excretory vesicles extending anterior to the acetabulum, but in *L. indicum* (Srivastava, 1941) the vesicle extends only to the ovary (Manter, 1954).

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