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Study of *Argulus spp*. infestation rate in Goldfish, *Carassius auratus* (Linnaeus, 1758) in Iran

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Abstract. Members of the genus *Argulus*, or fish lice, are common parasites of fresh water fish. The present study aimed to investigate necessarily the rate of infestation by argulids and determine the infesting species in goldfish in order to control the invasion of parasite in native and cultured fish population. For the present study, 1200 live goldfish were collected randomly from 10 ornamental fish farms from different areas of Iran in summer 2010 and were investigated macroscopically and microscopically. The number of parasites in every infested fish was numerated and identified by the morphologic key characteristics under light microscope as alive and as fixed in 70% ethyl alcohol. The results showed that 19.17% of sampled goldfish were infested with *Argulus coregoni* (Thorell, 1864), *Argulus japonicus* (Thiele, 1900), and *Argulus foliaceus* (Linnaeus, 1758). The most frequent infestation was with *A. japonicus* (12.5 %), the least frequent was with *A. coregoni* (1.6%), and the frequency of *A. foliaceus* infestation was 5%. Moreover, in this study, multi-cellular and unicellular parasites such as *Gyrodactylus spp*, *Dactylogyrus spp, Trichodina spp* and *Ichthyophthirius multifiliis* (Fouquet, 1876), were observed in infested fish.
Keywords: *Argulus*, infestation, goldfish, Iran.

Introduction. Members of the Branchiura subclass are parasitic Crustacea which are mainly found in freshwater fish containing four genera: *Argulus*, *Dolops*, *Chonopeltis*, and *Dipteropeltis* (Møller 2009). *Argulus* is a well-known and very commonly found genus (Møller 2009; Møller & Olesen 2010). Most species of the genus *Argulus* are 5-10 mm in size and found nearly worldwide (Bykhovskaya-Pavlovskaya et al 1962; Bauer 1991). *Argulus* is generally recognizable through its dorso-ventrally flattened body-form which is covered by a large chitinous carapace. Cephalothorax, thorax and abdomen are 3 parts of its body (Fryer 1982; Soulsby 1982; Lester & Roubal 1995). *Argulus* have a direct life cycle using the fish as hosts (Abele 1982; Shimura 1983; Mikheev et al 2001). Argulids repeatedly attach and detach from their hosts and cause the tissue damage and stress in infested fish. They potentially act as vectors and spread pathogens between fish and this is one of the main worries for fish producers (Cusack & Cone 1986).

Presently, different ornamental fish farms in Iran are breeding, rearing, and importing aquarium fish and their number is increasing. One of the important ways of transmission of parasitic infestations is importation of fish from countries which are infested with these parasites. A great number of different species of ornamental fish including different varieties of goldfish are imported annually from Southeast Asian countries (China, Malaysia, Thailand, and Singapore) to Iran and other countries. Uncontrolled importation of live fish can lead to transmission of *Argulus* and other parasites to the native fish and can cause economic loss. Also, their transmission can cause serious environmental losses to valuable native fish. In previous studies carried out on goldfish infestations in Iran, isolated genera have been identified (Ebrahimzade

Mousavi 2003; Meshgi et al 2006; Mehdizadeh Mood 2009; Noaman 2010). The key objective of this paper is to provide accurate knowledge concerning the infestation rate of *Argulus spp*. in goldfish in Iran.

Materials and methods. **Sampling and isolation of parasites**. About 1200 goldfish were collected randomly from 10 ornamental fish farms from different areas of Iran in summer of 2010. All of these fish were imported to Iran from southeast countries of Asia. Then, they were transported alive to the laboratory of Fish Disease, University of Tehran, Iran. At the outset, fish were examined macroscopically for their health and physical characteristics, presence or absence of abnormal clinical signs, presence or absence of *Argulus* and other Arthropods. Then, they were investigated using stereo microscope. Having observed in different parts of fish body surface, the *Argulus* were isolated by forceps and inspected alive and as fixed in 70% ethyl alcohol under light microscope. Then fish samples characteristics, number and species of isolated *Argulus* from every fish were recorded. In order to investigate the presence of other ectoparasites and the possible larval phases of *Argulus*, wet mount were provided from skin, gills, and different fins of fish. Then the macroscopical and microscopical photos were snapped by Sony camera, SSC-DC80P model.

Identification of infesting species. Identification and morphometric characteristics were conducted according particularly to Bykhovskaya-Pavlovskaya et al (1962) and Rushton-Mellor (1994).

Results. The study showed that of 1200 investigated goldfish, 230 specimens were infested with *Argulus spp*. Infestation value is shown in Table 1.

Table 1

Infestation value of *Argulus spp.* on the sampled goldfish

Number of fish investigated	*Number of fish infested*	*Prevalance (%)*
1200	230	19.17

Based on the intensity of infestation, features like faintness and erratic swimming were observed in infested goldfish. Clinical signs also included coetaneous bleeding, fins bleeding, scales losing, fin rot, acute hemorrhagic inflamed skin wounds, and superficial mucus expansion. In this study, *A. coregoni, A. japonicus*, and *A. foliaceus* were identified using morphological keys mentioned previously (Table 2).

Table 2

Differences among *Argulus* species considered in this study

Species	*Length of body (mm)*	*Posterior lobes of carapace*	*Urosome*	*Posterior incisure of urosome*
A. foliaceus	6-7	Not extend beyond beginning of urosome	Round lobes is covered marginally with small spines	Not reach center
A. japonicus	4-8	Extend beyond level of the middle of urosome, carapace covers forth leg	Round lobes (though more pointed than *A. foliaceus)* is covered marginally with small spines	Reach center
A. coregoni	12	Not extend beyond beginning of urosome	Acuminate lobes without any small spines	Reach beyond center

According to table 2, *A. coregoni* is obviously characterized with its extremely sharp lobes not having urosome marginal spines and their whole body length is more than 10 mm. In *A. foliaceus* urosome with rounded lobes is covered marginally with small spines and in *A. japonicus* carapace is more pointed than *A. foliaceus* and covers forth leg (Fig. 1 A, B, C, D, E, F).

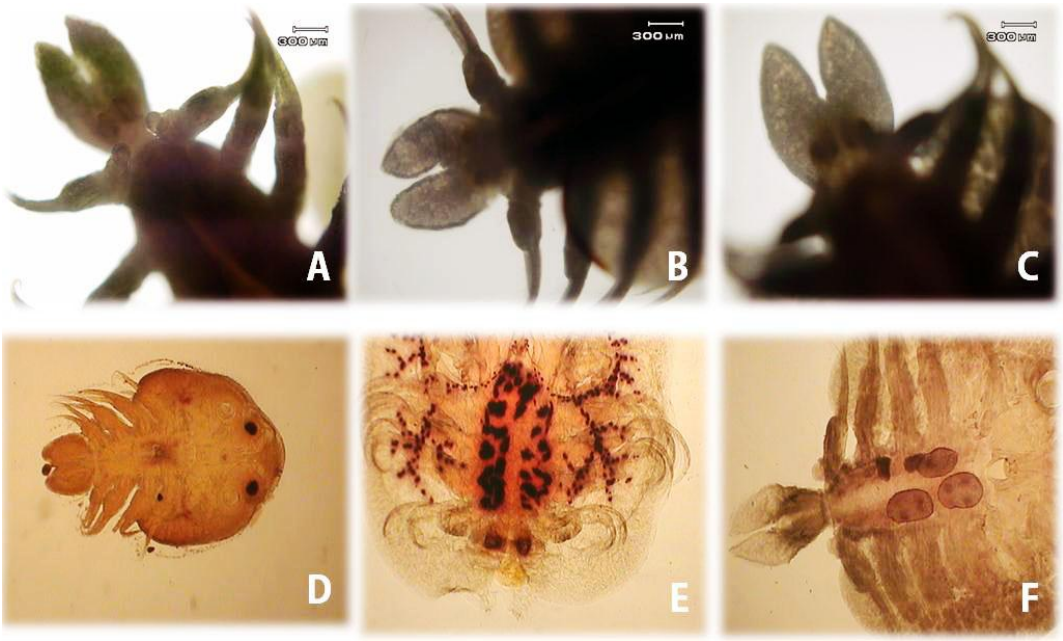

Figure 1. Light micrograph of *Argulus* spp. A, F; *A. coregoni*, B, D; *A. foliaceus*, C, E; *A. japonicus.* A, B, C as fixed in 70% ethyl alcohol, D, E, F as stained.

Table 3 shows the number of infested fish, the most number of *Argulus* species isolated from infested goldfish, and the prevalence of infestation in every species.

Table 3

Infestation values of *Argulus spp.* on the infested goldfish

Argulus sp.	*Maximum number in infested fish*	*Number of infested fish*	*Prevalence(%) in infested fish*
A.foliaceus	2	60	26.09%
A.japonicus	More than 200	150	65.22%
A.coregoni	1	20	8.69%

Infestation value of infested goldfish and prevalence of infestation of three *Argulus* species in investigated goldfish have been shown in figure 2 and 3. According to figure 3, the most frequent infestation was with *A. japonicus* (12.5 %), the least was with *A. coregoni* (1.6%), and the frequency of *A. foliaceus* infestation was 5%. Meanwhile, multi-cellular and unicellular ectoparasites such as *Gyrodactylus spp*, *Dactylogyrus spp*. *Trichodina spp* and *Ichthyophthirius multifiliis* were identified in sampled goldfish besides *Argulus*.

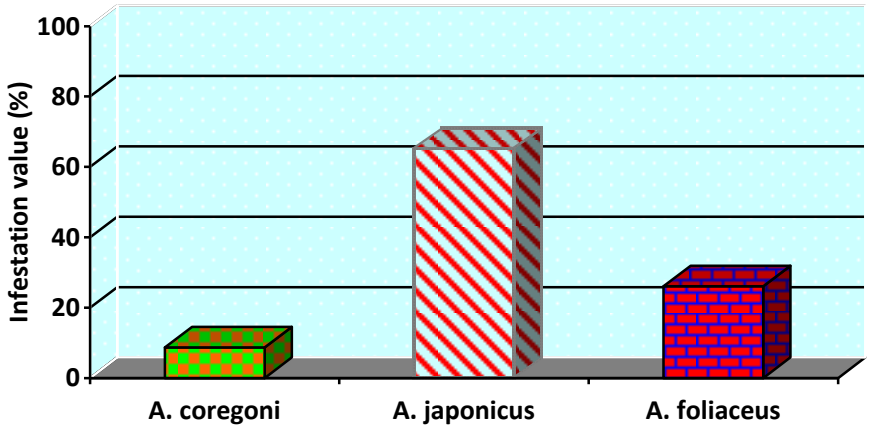

Figure 2: Infestation value to three species of *Argulus* in infested goldfish

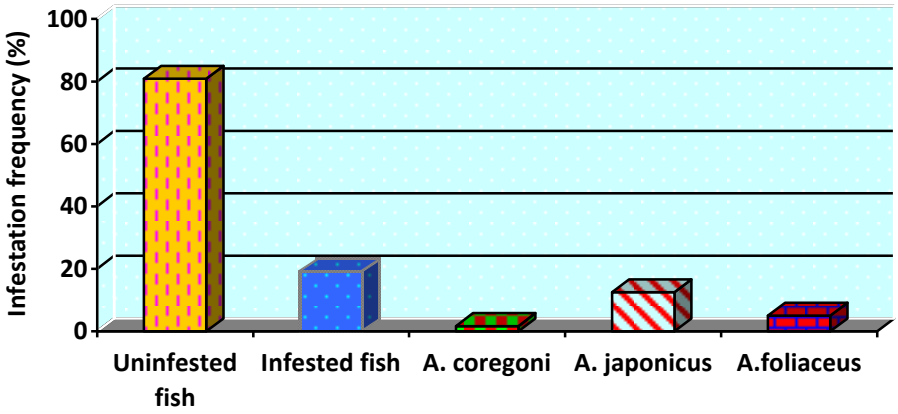

Figure 3: Infestation frequency (%) of *Argulus* species isolated from sampled goldfish

Discussion. The breeding and rearing of ornamental fish has a special position in the world and plays an important role in exchange income of some countries and in occupation. The world business share of these fish which was estimated about $900 million, has given a remarkable insight into it (Meshgi et al 2006). Due to the economic importance of these aquatics, many researchers have noticed the different factors endangering their health. There are about 150 species of aquarium fish in Iran of which, 40 species are bred and reared inside the country (Meshgi et al 2006). Goldfish is one of the major ornamental fish for which more than 100 varieties have been produced with selective hatching and many people are interested in their breeding and rearing. Besides their breeding in different fish farms in Iran, different species of goldfish are imported annually from Southeast Asian countries. Studies carried out on goldfish and koi confirms the *Argulus* as the most prevalent parasite (Noga 2010).

Argulids are good swimmers. Adults and larvae can easily migrate among many hosts. So, *Argulus* can induce morbidity and mortality in cultured fish populations (Yamaguti 1963; Kabata 1985; Benz et al 2001). Also, this ectoparasitic specie is widely adaptable and can live in marine, brackish, and freshwater habitats. It lives not only on fish, but also on amphibians (Geldiay & Balık 1974). According to the presented issues, it is clear that *Argulus* can act as a potential risk factor for natural ecosystems and native fish population of the countries.

In Iran, *Argulus spp*. were reported from different hosts between 1984 and 2010 (Azadikhah et al 2009; Behrouzfar et al 2009; Jaameei et al 2009; Mehdizadeh, 2009; Mokhayer 2006; Mokhayer & Ebrahimzadeh Mousavi 2009; Mosafer et al 2009). In present study in Iran, 3 species of *Argulus* was isolated from goldfish. Clinical signs and

behaviors observed in infested fish were in accordance with the cases reported by Tokşen (2006), Yildiz & Kumantas (2002) and Noaman (2010). In most cases, erratic swimming, flashing, scratching, mucus increase and poor growth as well as blood spots in the surface of their body and fins were observed based on the intensity of infestation and the size of infested fish (Yıldız & Kumantas 2002; Tokşen 2006; Noaman et al 2010).

According to our results, the mean number of *A. foliaceous* from each infested goldfish was 1-2, while Yıldız & Kumantas (2002) and Noaman (2010) reported the isolation of at least 3 of that specie in infested goldfish. This difference can be relevant to the stages of infestation and life cycle of the parasite (Yıldız & Kumantas 2002; Noaman et al 2010).

A. coregoni was the largest isolated species and as the infestation with this species is more common in trout, so it was the minimum prevalent species in goldfish (Soltani, 2006). Our study showed that the maximum infestation value is due to *A. japonicus,* which corresponds with goldfish being the host for *A. japonicus* (Noga 2010). The probability of infestation with this species increases with the prevalence of this parasite in Southeast Asia, the importation of fish to Iran from these regions, and ecological concordance.

Argulus japonicus is an opportunist (Shafir & Oldewage 1992; Avenant-Oldewage 2001). Infestation with this parasite can reach severe proportions in a very short time which result in catastrophic fish deaths (Kruger et al 1983; Menezes et al 1990; Northcott et al 1997; Avenant-Oldewage 2001; Taylor et al 2006). Importation of ornamental fish is carried out in many countries without any special management and strict quarantine. So, in the case of any infestations, diseases come into the country through these infested fish, especially parasitic infestations, that threatens native fish and aquaculture industry of that country. One of the important issues related to parasitic infestations of ornamental fish is the infestation transmission from imported fish to native fish and their habitation as natives in new region. Therefore, imported fish should be examined for their health and for parasitic infestations in order to prevent the burst of new parasitic fauna to different countries and stop direct economic losses caused by mortality derived from infestations appeared in relocation.

Conclusions. Poor controls of importation of fish will cause the relocation of parasites with direct life cycles between different countries and will lead to the increase of infestations. Hence, quarantine of ornamental fish and determination of infested cases will influence the infestation decline. It will in turn inhibit the outbreak of secondary infestations and sometimes fatal bacterial, fungal, and viral infections.

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