The prevalence of non-indigenous parasitic copepod (*Neoergasilus japonicus*) spreads with fishes of pet trade in Kerman, Iran

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Abstract Ergasilids are copepods living in the river mouth of freshwaters and parasitic on Teleost fish family in both natural and artificial environments. So far, 5 species of the copepod have been discovered that belong to the genus *Neoergasilus*. This copepod is most likely to be disseminated through aquarium trades, aquaculture and over-nutrition, or construction of sand carrying water. The females of Ergasilidae are external parasites attaching to the anal and dorsal fins and sometimes to gills and nasal cavities of fish living in freshwaters. In total, 552 pieces of ornamental fish (301 males and 251 females) with length of 5–10 cm from fish (*Poecilia sphenops*) species (Singapore, Sandy, Dirigible and scorpion's tail) were collected from ornamental fish stores in different regions of Kerman, Iran during 1 year in 2012–2013 and tested in order to examine *Neoergasilus japonicus* infestation. From 188 adult females *Neoergasilus japonicus* specimens recorded on the fish host, 8 (4.26 %) were on the anal, 120 (63.83 %) on the dorsal, 10 (5.32 %), on the pectoral, 45 (23.94 %) on the pelvic, and 5 (2.66 %) on the caudal fins. In this study, the prevalence of parasitic copepod infestation from Dec. to May was 26.31, 27.69, 26.19, 14, 18.75, and 7.5 %, respectively. There was no significant difference between infestation prevalence in indigenous and non-native fishes ($P = 0.18$). There were significant differences between different months of year in the prevalence and intensity of *Neoergasilus japonicus* ($P < 0.05$). There was significant difference between frequency distribution of *Neoergasilus japonicus* infestation in different organs ($P < 0.05$). The male fish infestation (16.3 %) was significantly higher than

female fish infestation (5.6 %) ($P < 0.05$). Considering that the *Neoergasilus japonicus* was first observed in native and nonnative ornamental fish in Kerman, further studies should be conducted on the copepod infestation in stores supplying ornamental fish in other parts of Iran to make more accurate judgments.

Keywords Copepod parasite · *Neoergasilus japonicus* · Aquarium fish · Kerman

Introduction

Ergasilids are copepods living in the river mouth of freshwaters and parasitic on Teleost fish family in both natural and artificial environments (Nagasawa and Inoue 2012). *Neoergasilus japonicus* is a member of Ergasilidae family, a copepod that is usually classified under Poecilostomatoida order (Huys and Boxshall 1991; Abdelhalim et al. 1993). This copepod was first discovered by Yin (1956), and its species as *Neoergasilus japonicus* was determined by Harada (1930), (Harada 1930 and Yin 1956). So far, 5 species of the copepod have been discovered that belong to the genus *Neoergasilus* (Walter and Boxshall 2009). *Neoergasilus japonicus* has been largely recorded in different species of freshwater fish in Eastern Europe and North America (Hudson and Bowen 2002). It has been also recorded in Hungary (Ponyi and Molnar 1969), Czechoslovakia (Hanek 1968), Britain (Mugridge et al. 1982), France (Lescher-Moutoué 1979), and Finland (Tuuha et al. 1992). The female copepod is an external parasite attaching to the anal and dorsal fins and sometimes to gills and nasal cavities of fish living in freshwaters (Hayden and Rogers 1998; Hudson and Bowen 2002; Baud et al. 2004). This genus can produce at least two generations per year

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showing that free-living adults have seasonal reproduction during summer and fall (Tuuha et al. 1992). The fish examined in this study were of one species and were abundant in all aquariums of Kerman. Most of the fish were brought to Iran from Southeastern Asian countries, such as China and Thailand. This import resulted in entering new parasitic agents to Iran and rapid spread of these agents to other fish, and might lead to much loss if they are not prevented and treated. The study was conducted to detect, report, and describe a new nonnative species and its prevalence and evaluate risks of this copepod for ornamental fish, especially *Poecilia sphenops*, in a family of aquarium fish in Kerman (southeast), Iran.

Materials and methods

In total, 552 pieces of ornamental fish (301 males and 251 females) with length of 5–10 cm from indigenous and imported fish species (*Poecilia sphenops*) (Singapore, Sandy, Dirigible and scorpion's tail) were collected from the ornamental fish store in 10 stores in different regions of Kerman, Iran during 1 year in 2012–2013 and tested in order to examine *Neoergasilus japonicus* infestation. In the ornamental fish stores, the fish were caught alive using a manual net, placed into a plastic containers containing water separately, and delivered to School of Veterinary Medicine, Shahid Bahonar University of Kerman. Then, the fish were killed and their fins, gills and skin were examined by a microscope (4x, Olympus Tokyo Japan). The parasitic samples were removed from above parts and placed in 70 % ethanol. Then, they were made transparent using lactophenol and examined using an optical microscope (40–100×, Olympus Tokyo Japan). Finally, they were detected using Bauer's identification guide (Bauer 1987).

Statistical evaluation

Statistical analysis was undertaken using the Chi square test. Data was analyzed by SPSS software, version 16 and $P < 0.05$ was accepted as statistically significant.

Results

In total, 552 pieces of ornamental fish species (*Poecilia sphenops*) were examined for *Neoergasilus japonicus* infestation. 201 pieces (36.41 %) and 351 pieces (63.58 %) were indigenous and non-native respectively (Fig. 1). 18 pieces of indigenous fish (9 %) and 45 pieces of non-native fish (12.8 %) were infested by *Neoergasilus japonicus*. There was no significant difference between infestation prevalence in indigenous and non-native fishes ($P = 0.18$).

In total, 63 pieces (11.41 %) of ornamental fishes were infested.

Parasitic copepods were only detected between Dec. 2012 to May 2013. In general, 188 parasitic copepods were detected from the fishes, which all have been identified as *Neoergasilus japonicus* (Fig. 2).

One hundred and eighty-eight adult female *Neoergasilus japonicus* specimens were detected on the fishes. 8 (4.26 %) were on the anal, 120 (63.83 %) on the dorsal, 10 (5.32 %), on the pectoral, 45 (23.94 %) on the pelvic and 5 (2.66 %) on the caudal fins. But, there was no parasitic

Fig. 1 A studied ornamental fish (*Poecilia sphenops*)

Fig. 2 Adult female *Neoergasilus japonicus*

copepod on gills and skin, and most of the parasitic copepods were on the dorsal fins (Fig. 3).

The highest and the least frequency of *Neoergasilus japonicus* were observed on dorsal fins (63.83 %) and gills (0 %) as well as skin (0 %), respectively. There was significant difference between frequency distribution of *Neoergasilus Japonicus* infestation in different organs ($P < 0.05$). Table 1 shows monthly spread of *Neoergasilus japonicus* in different limbs of the fishes.

In this study, the prevalence of parasitic copepod infestation from Dec. 2012 to May 2013 was 26.31, 27.69, 26.19, 14, 18.75, and 7.5 %, respectively. Table 2 indicates number of examined fish, number of infested fish, infestation prevalence, mean infestation severity, and mean frequency of parasitic copepods in each month. There were significant differences between different

months of year in the prevalence and intensity of *Neoergasilus japonicus* ($P < 0.05$) (Table 2). This study also examined monthly prevalence of *Neoergasilus japonicus* infestation in Kerman's ornamental fish in terms of fish's sex. Based on the results, maximum (100 %) and minimum (0 %) prevalence of infestation in male fish happened in May 2013 and Jun. to Nov. 2013, respectively. In female fish, maximum (42.30 %) and minimum (0 %) prevalence of infestation happened in Jan. 2013 and May to Nov. 2013, respectively (Fig. 4). 251 and 301 fishes were female and male respectively. 49 male fishes and 14 female fishes were infested. The male fish infestation (16.3 %) was significantly higher than female fishes infestation (5.6 %) ($P < 0.05$).

Species description

Family: Ergasilidae

Genus: *Neoergasilus* (Yin 1956)

Species: *Neoergasilus japonicus* (Harada 1930)

The entire length of *Neoergasilus japonicus*, from its cephalosome to the uropods, was 751 μm . The body of this copepod consists of 4 thoracic segments, and a reduced fifth segment. Its first antenna has 6 segments, and the second antenna has 4 segments with a short spine on the inner margin of the second segment. The third segment is recurved, and the fourth segment has obviously a sharp claw. In mouth parts of the copepod, located ventrally mandible is 2-segmented with a curved apical segment. The first maxilla has an unsegmented lobe with two setae on the posterior margin and on medial margin, and the second maxilla consists of two segments. Both rami of legs 1–3 are three-articulated. The first article of the exopodite has a thin denticulation on the outer margin and a strong

Fig. 3 *Neoergasilus japonicus* on the dorsal fin (4 $\times$ zoom)

Table 1 Attachment sites of *Neoergasilus japonicus* in ornamental fish (*Poecilia sphenops*) in different months of the year

Month	Anal fins	Dorsal fins	Pectoral fins	Pelvic fins	Caudal fins	Gills and skin
Dec. 2012	3	76	1	0	2	0
Jan. 2013	0	18	1	14	3	0
Feb. 2013	4	15	2	8	0	0
Mar. 2013	1	0	3	8	0	0
Apr. 2013	0	10	3	5	0	0
May 2013	0	1	0	5	0	0
Jun. 2013	0	0	0	0	0	0
Jul. 2013	0	0	0	0	0	0
Aug. 2013	0	0	0	0	0	0
Sep. 2013	0	0	0	0	0	0
Oct. 2013	0	0	0	0	0	0
Nov. 2013	0	0	0	0	0	0
Total	8	120	10	45	5	0
(%)	4.26	63.83	5.32	23.94	2.66	0

Table 2 *Neoergasilus japonicus* infestation indexes in Kerman's ornamental fish (*Poecilia sphenops*)

Month	EFN	IFN	P (%)	TPN	MI	MA
Dec. 2012	57	15	26.31	82	5.46	1.43
Jan. 2013	65	18	27.69	36	2	0.55
Feb. 2013	42	11	26.19	29	2.63	0.69
Mar. 2013	50	7	14	17	2.42	0.34
Apr. 2013	48	9	18.75	18	2	0.37
May. 2013	40	3	7.5	6	2	0.15
Jun. 2013	30	0	0	0	0	0
Jul. 2013	44	0	0	0	0	0
Aug. 2013	25	0	0	0	0	0
Sep. 2013	55	0	0	0	0	0
Oct. 2013	52	0	0	0	0	0
Nov. 2013	44	0	0	0	0	0

EFN examined fish number, IFN infected fish number, P prevalence, TPN total parasite number, MI mean intensity, MA mean abundance

Fig. 4 Monthly prevalence (%) of *Neoergasilus japonicus* infestation in Kerman's ornamental fish (*Poecilia sphenops*) in terms of fish's sex

spine accompanied by a short cuticular process. The second article has a thin denticulation on the outer margin and a spine enlarged to form a plate, characteristic of the genus, longer than article. The third article, similar in both sexes, is armed with two spines and five setae. The endopodite is three-articulated: one inner seta on the first article, one inner seta on the second article, two spines and four setae on the third article. The one-articulated rami of the fifth leg has 3 apical setae of which one is short, and the two other setae are long.

Discussions

The studied copepod in Iran was first recorded in Kerman (Southeast of Iran). Most of the infested fishes were non-native and imported from Southeast Asian countries. The

copepods detected in this study were *Neoergasilus japonicus* based on the morphological features. There were reports about this copepod in the world. Species of the genus *Neoergasilus* apparently originated in eastern Asia. Of the 5 species within this genus, *N. japonicus* was described from Taiwan (Harada 1930), *N. longispinosus* from China (Yin 1956), *N. squaliobarbi* from the Amur River basin in the eastern part of Russia (Dogiel and Akhmerov 1952), *N. ferozepurensis* and *N. notopteri* from India (Kumari et al. 1988). In this study, one species of fish (*Poecilia sphenops*) was examined for *Neoergasilus japonicus* infestation. According to the results, only 63 fishes were infested with the copepod, and there was no significant difference between ornamental fish imported to Iran and native cultured fish in the frequency of infestation. It may be related to the globalization of aquaculture and inadequate quarantine standards to have facilitated the introduction of parasites into new environments; this has harmed native aquatic organisms (Soylu and Soylu 2012). Various studies have been conducted on *Neoergasilus japonicus* infestation and its transmission to different species of fish in different parts of the world. Soylu and Soylu (2012) in Turkey, Knopf and Holker (2005) in Germany, and Beyer et al. (2005) in England respectively reported cases of infestation with *Neoergasilus japonicus* in red carp, Minnow fish (*Rutilus rutilus*), and sunfish (*Leucaspis delineatus*) (Beyer et al. 2005; Knopf and Holker 2005; Soylu and Soylu 2012).

According to theory Soylu and Soylu (2012), Knopf and Holker (2005) and our study, the routes of *N. japonicus* invasion have been linked to unintentional releases from sources such as the worldwide live fish trade and ballast water introduction. Also it has been introduced into other countries, transported on exotic fish hosts associated with aquaculture, and it is found only in freshwater (Knopf and

Holker 2005). Also this parasite has great swimming capabilities exhibits a fast life cycle (21 days at 20 °C) and high fecundity as a female can produce an average 1425 eggs at 20 °C during their life (Beyer et al. 2005). Regarding the *N. japonicus* infestation in ornamental fish and the studies performed in different parts of the world, it can be concluded that *N. japonicus* can simply cause changes in hosts living in fresh water systems. Moreover, the copepod can infest a broad spectrum of fish even in geographically limited areas and also can use both the native and nonnative fish as the host, and this capability is one of the reasons of its spread all over the world (Kim and Choi 2003; El-Rashidy and Boxshall 2009; Suarez-Morales et al. 2010; Nagasawa and Inoue 2012). All the removed copepods in this study were female because adult males and larva live freely, and only females are parasitic (Kabata 1979). Moreover, the above similarity existed in other Ergasilids that were only fertile female parasites and could infest the host fish (Hayden and Rogers 1998; Hudson and Bowen 2002). In this study, *N. japonicus* was detected in all fins, mainly in dorsal fins, with frequency of 63.83 %. However, there was no copepod in gills and skin (Table 1). In contrast to typical ergasilids, *N. japonicus* are mainly attached to the host's fins, and are able to move from one host to another (Ponyi and Molnar 1969). The following site preferences have been reported by others: dorsal fins and base of the anal fins (Hayden and Rogers 1998); dorsal, pelvic, anal, and caudal fins (Hudson and Bowen 2002); anal fin Only (Knopf and Holker 2005); and occasionally on the gills (Baud et al. 2004). In this study, 11.41 % of the fish were infested with the copepod, and totally, 188 copepods, which were fewer than those in other countries, were removed from the fish. Mugridge et al. (1982) found 50–250 parasites/fish in British ponds and suggested that the reduced growth rate of roach may be caused by *N. japonicus* (Mugridge et al. 1982). Urawa et al. (1991) reported 500–700 parasites/fish from ponds in Japan (Urawa et al. 1991). Also Ponyi and Molnar (1969) noted severe infestations of *N. japonicus* in Hungary but provided no details on the intensity or effects (Ponyi and Molnar 1969). In the present study, *N. japonicus* was detected between Dec. 2012 and May 2013, that higher and lower prevalence was in Jan. (27.69 %) and Jun. to Nov. (0 %) respectively. Therefore, the frequency of infestation in cold months of the year was higher than that in warm months. Soyly and Soyly (2012) found *N. japonicus* between June and November, as maximum and minimum prevalence of infestation was related to July and June, respectively. That the reason of the disagreement is due to the relation of fish migration to deeper waters after November with thermal structure and reduction of food chain in the lake (Soyly and Soyly 2012). Furthermore, a study on *ergasilid* copepods of roach in Central Finland did not reveal a regular seasonal

pattern of occurrence, indicating that *N. japonicus* might reproduce, or recruit fish hosts not only at higher temperatures, but also in autumn and winter (Tuuha et al. 1992). This theory conforms to results of the present study. Finally, it should be noted that the tropical aquarium fish trade comprises a large part of aquaculture trade in the world, and this include the countless ornamental fish exported from Southeast Asian countries (Evans and Lester 2001). Iran also is an importer of tropical aquarium fish, and the number of these imported fish considerably increases every year. Sometimes, some parasitic infestations may enter the domestic aquarium system along with the imported fish, which may result in much economical loss if it is not controlled and treated. Furthermore, the infested fish make many problems in native fish if they are released in the ecosystem of the importing country. In this respect, it is obvious that the random identification of newly emerging parasites can reduce their adverse effects in the importing countries, and imported ornamental fish should be quarantined in order to inhibit the transmission of pathogens. Therefore, importing countries, such as Iran, should examine the imported ornamental fish in terms of different pathogens, if any, and treat them before distribution in other cities. As the *N. japonicus* entered Iran from South-east Asia and was first observed in native and nonnative ornamental fish in Kerman, further studies should be conducted on the copepod infestation in stores supplying ornamental fish in other parts of Iran to make more accurate judgments. Moreover, the import of these fish to Iran should be controlled in order to avoid the incidence of infestation in native ornamental fish through management, treatment, and quarantine methods.

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Conflict of interest The authors declare that they have no conflict of interest.

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