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Parasitism of *Argulus japonicus* in cultured and wild fish of Guangdong, China with new record of three hosts

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Abstract This study aimed to demonstrate the ability of Argulus japonicus to infect a wide range of freshwater fishes, as well as to understand the effects of fish origin and host body size on the incidence of A. japonicus. Samples of cultured and wild fish were collected randomly from July 2010 to March 2013, using angling, long-lining, gill-netting, and trapping from rivers and fish farms in Guangdong province, South China. Eight fish species were found to be heavily infected including the common carp, the goldfish, the black carp, the silver carp, the brown trout, the rainbow trout, the mandarin fish, and the perch. Furthermore, the black carp, the brown trout, and the mandarin fish were recorded as new hosts for the first time. During the present study, a total of 2,271 fishes were examined, out of which 712 fishes were found to be infected by a total of 1,443 A. japonicus. Abundance and intensity of A. japonicus infection were significantly influenced by origin of fishes (cultured and wild) and total length (class I, <250 mm; class II, 250–350 mm; and class III, >350 mm) of fish species, whereas varied impacts on prevalence of infection were observed. The correlation between total length of fishes and prevalence of A. japonicus infection was variable, where no significant correlation was observed in the black carp, the silver carp, the mandarin fish, and the perch. In spite of the weak negative correlation between body size of the silver carp and prevalence of infection, A. japonicus was the most abundant and intensive in the silver

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carp. Thus, aquaculturists should pay particular attention to the control of these fish lice due to its host biodiversity.

Introduction

Argulus japonicus has the greatest economic impact of any parasites in cultured fish and is also a threat to wild fish and increase the susceptibility of its host to secondary infections (Bandilla et al. 2006; Walker et al. 2011b). They result in economic losses not only in mortality, but also from treatment expenses and growth reduction during and after the outbreak of disease, and this militate against expansion of aquaculture (Cowx 1992; Omeji et al. 2011). The number of parasites necessary to cause harm to a host varies considerably with species, size of host, and its health status (Carpenter et al. 2001). The relationship between fish and parasites in the natural environment is an important way to understand pathologies in hosts and are also relevant to pisciculture (Tavares-Dias et al. 2000). Direct effects, such as host impairment, can induce indirect consequences (Crowden and Broom 1980; Milinski 1984), greater vulnerability to predators (Arme and Owen 1967), and decreased resistance to environmental stress (Lewis and Hettler 1968).

Host specificity is resulting from vicariance events or a long co-evolutionary history between some parasites and their host (Poulin et al. 2011). Argulid parasites exhibit low host specificity (Walker et al. 2011a); many authors as well have commented on the lack of specificity of argulid parasites, sharing the opinion that individual species from this group can infect a wide range of host species (Kearn 2004; Walker et al. 2004; Alsarakibi et al. 2012).

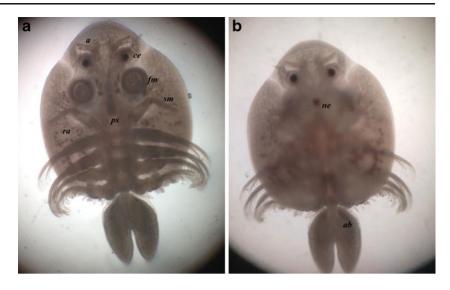
The objectives of current work were to investigate the host biodiversity of *A. japonicus*, as well as to compare the prevalence, abundance, and intensity of *A. japonicus* on wild and cultured fish, in addition to study the relationship between

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Fig. 1 Light micrograph of a female specimen of *A. japonicus*. a dorsal view; *a* antenna, *ce* compined eye, *fin* first maxialla, *sm* second maxilla, *ra* resparatory area, *ps* proboscis. b ventral view; *ne* nupilus eye, *ab* abdomen



host size (total length) and the occurrence of *A. japonicus* in freshwater fish of Guangdong province, China.

Materials and methods

Sample collection

Samples of cultured and wild fish were collected randomly from July 2010 to March 2013, using angling, long-lining, gill-netting, and trapping from rivers and fish farms in Guangdong province, South China. After capture, the fish were immediately labeled, and total length (to the nearest millimeters) was recorded and categorized into three classes (class I, <250 mm; class II, 250–350 mm; and class III, >350 mm). The external surface of each fish was examined thoroughly for ectoparasites using a hand lens. *Argulus* sp. was detected and detached individually from infected fish. *A. japonicus*

specimens were identified according to Wadeh et al. (2008) and counted from each sample separately. Parasite prevalence, abundance, and mean intensity were chosen as comparative parameters based on the definition in Bush et al. (1997).

Statistical analysis

Effect of fish origin (wild and cultured) on abundance and intensity of *A. japonicus* infection was examined using Mann–Whitney *U* test. The difference in parasite prevalence was tested by using Chi-square tests (χ^2). Kruskal–Wallis test was applied to test the difference in infection among host length classes. Spearman's correlation (*r*) was used to examine relationships between host length and prevalence, abundance, and intensity of infection for each fish species. The differences were considered significant at *P*< 0.05. All the statistical tests were performed by using Statistica 10.0 for Windows, StatSoft Inc.

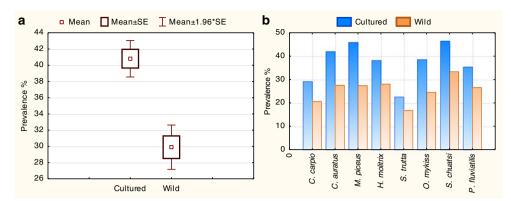
Table 1 Overall prevalence (P%), mean abundance (MA), and mean intensity (MI) of *A. japonicus* (n=1,443) recovered from infected fishes (IF) in Guangdong province, China, during July 2010 to March 2013

Fish species	Common name	EF	IF	n	P (%)	MA±SE	MI±SE
C. carpio	Common carp	425	109	230	25.6a	0.70±0.06a	2.19±0.11a
C. auratus	Goldfish	321	113	183	35.2b	0.64±0.05a	1.57±0.07b
M. piceus	Black carp	219	79	186	36.1b	0.92±0.11b	2.26±0.13a
H. molitrix	Silver carp	168	59	234	35.1b	1.70±0.11c	4.07±0.14c
S. trutta	Brown trout	365	73	144	20.0c	0.48±0.05d	1.97±0.08a
O. mykiss	Rainbow trout	269	91	110	33.8b	0.61±0.07a	1.22±0.06d
S. chuatsi	Mandarin fish	273	115	214	42.1d	$0.80 {\pm} 0.07 b$	1.83±0.10a
P. fluviatilis	Perch	231 2271	73 712	142 1443	31.6b	0.68±0.07a	1.97±0.11a

EF examined fishes

Values with different letters are significant at P < 0.05; values with same letters are insignificant at P > 0.05

Fig. 2 Differences between prevalence of infection in cultured and wild fish (a) and between fish species (b) collected from freshwater of Guangdong province, China, during July 2010 to March 2013



Results

Host biodiversity

Eight fish species, representing four families, encountered A. *japonicus* (Fig. 1) during the sampling period. There were four species of Cyprinidae, the common carp Cyprinus carpio (L. 1758), the goldfish Carassius auratus (L. 1758), the black carp Mylopharyngodon piceus (Richardson 1846), and the silver carp Hypophthalmichthys molitrix (Valenciennes 1844). The brown trout Salmo trutta (L. 1758) and the rainbow trout Oncorhynchus mykiss (Walbaum 1792) represented Salmonidae, the mandarin fish Siniperca chuatsi (Basilewsky 1855), and the perch Perca fluviatilis (L. 1758) represented Percichthyidae and Percidae, respectively. Caught fish species were unevenly presented in our samples, indicating that there are probably greater numbers of some species than others within the resident fish community. Among them, the A. japonicus infection of the black carp M. piceus, the brown trout S. trutta, and the mandarin fish S. chuatsi is the first report.

Parasitism of A. japonicus

A total of 2,271 individual fishes were examined. Seven hundred twelve of these fishes (31.4 %) were infected with a total of 1,443 A. japonicus. The number of fish per species was varied from 168 to 425, a reflection of both relative abundances and differences in distribution of different fish species. Variation in prevalence of infection was observed between fish species examined, where A. japonicus was most prevalent on the mandarin fish S. chuatsi (42.1 %), whereas the prevalence of infection on brown trout S. trutta was the lowest (20 %). Overall, prevalence of infection was varied significantly within fish species examined (χ^2 =66.32, P< 0.01). Furthermore, the highest abundance (1.70 ± 0.11) and the highest intensity (4.07 ± 0.14) were found on the silver carp *H. molitrix*. Abundance ($\chi^2 = 115.277, P < 0.01$) and intensity $(X^2=159.367, P < 0.01)$ of A. japonicus infection was significantly different between fish species captured (Table 1).

Overall, prevalence of infection was significantly higher in cultured fish (n=487, 68.4 %) than in wild fish (n=218, 31.6 %) (Z_{adj} =6.630, P< 0.01) (Fig. 2a). Nevertheless,

Fish species	MA±SE			MI±SE			
	Cultured	Wild	P value	Cultured	Wild	P value	
C. carpio	0.83±0.08	$0.48 {\pm} 0.06$	< 0.01	2.41±0.13	1.87±0.20	< 0.01	
C. auratus	$0.77 {\pm} 0.07$	$0.42 {\pm} 0.05$	< 0.01	1.65 ± 0.09	1.43 ± 0.12	0.14	
M. piceus	1.12 ± 0.13	0.63 ± 0.11	0.03	2.45 ± 0.15	2.15±0.22	0.15	
H. molitrix	$1.84{\pm}0.12$	1.20 ± 0.13	0.01	4.17±0.16	3.73±0.14	0.13	
S. trutta	$0.55 {\pm} 0.06$	$0.35 {\pm} 0.10$	< 0.01	2.13 ± 0.08	1.63 ± 0.16	0.01	
O. mykiss	$0.68 {\pm} 0.09$	$0.40 {\pm} 0.09$	0.02	1.23 ± 0.07	$1.19{\pm}0.09$	0.91	
S. chuatsi	$0.92{\pm}0.09$	$0.54{\pm}0.06$	< 0.01	1.93 ± 0.13	1.60 ± 0.13	0.17	
P. fluviatilis	0.79 ± 0.10	$0.51 {\pm} 0.08$	0.03	2.08±0.12	1.78 ± 0.21	0.20	

Table 2 Mean abundance (MA) and mean intensity (MI) of *A. japonicus* (*n*=1,443) in cultured and wild fishes collected from Guangdong province, China, during July 2010 to March 2013

Differences are significant at P<0.05

Table 3 Spearman's correlation between fish origin and prevalence (P%), abundance (MA), and intensity (MA) of *A. japonicus* (*n*=1443) in Guangdong province, China, during July 2010 to March 2013

Fish species	P%		MA		MI		
	r	P value	r	P value	r	P value	
C. carpio	0.247	0.18	-0.233	0.21	0.202	0.28	
C. auratus	0.847	< 0.01	0.485	< 0.01	-0.184	0.28	
M. piceus	0.644	0.01	0.814	< 0.01	0.633	0.02	
H. molitrix	-0.566	0.07	-0.355	0.28	0.675	0.02	
S. trutta	-0.010	0.68	0.262	0.27	-0.360	0.12	
O. mykiss	0.230	0.39	0.092	0.72	-0.129	0.61	
S. chuatsi	0.456	0.02	-0.428	0.03	-0.295	0.15	
P. fluviatilis	-0.233	0.40	0.336	0.19	0.611	< 0.01	

Differences are significant at P<0.05

prevalence of infection was significantly influenced by fish origin within fish species, with an exception of the silver carp *H. molitrix* which showed insignificant influence. However, *A. japonicus* was most prevalent in cultured (46.2 %) and wild (33.3 %) Mandarin fish *S. chuatsi*, whereas the lowest prevalence was observed in *S. trutta* in both cultured (22.5 %) and wild (16.7 %) fish (Fig. 2b). Mean abundance of cultured fishes (0.89±0.04) was significantly higher than that in wild fishes (0.53±0.04) (Z_{adj} =7.303, P< 0.01); significant differences as well were found on intensity of infection between cultured (2.18±0.06) and wild fishes (1.75±0.08) (Z_{adi} =4.374, P< 0.01).

However, *A. japonicus* was most abundant on cultured (1.84 ± 0.12) and wild (1.20 ± 0.13) silver carp *H. molitrix*, whereas the lowest abundance was observed on *S. trutta* in both cultured (0.55 ± 0.06) and wild (0.35 ± 0.10) fish combined. It is noteworthy that intensity of infection was also the highest in both cultured (4.17 ± 0.16) and wild (3.73 ± 0.14) silver carp *H. molitrix*, whilst the brown trout *O. mykiss* showed the lowest intensity (Table 2). Spearman's correlation coefficient showed that prevalence, mean abundance, and intensity of *A. japonicus* infection were varied within fish species (Table 3).

In the present study, the relationship between total length of fishes and prevalence of infection was varied, generally; smaller-sized fishes were less infected than the larger-sized fishes (class III, 48.0 %; class II, 34.2 %; and class I, 31.7 %) (H=41.033, P<0.01). Moreover, mean abundance and mean intensity of *A. japonicus* infection were significantly varied by host length class, being higher in the length class III compared with class II (H=152.491, P<0.01) and class I (H=130.390, P<0.01), respectively (Fig. 3a and b). It is noteworthy that *A. japonicus* was more prevalent within class I new hosts, the silver carp *H. molitrix* and mandarin fish *S. chuatsi*. Mean parasitological indices (prevalence, abundance, and intensity) within fish species examined are shown in Table 4.

Based on all fishes examined, the prevalence was positively correlated with total host length of six fish species, whilst two new hosts, the silver carp *H. molitrix* and the mandarin fish *S. chuatsi*, showed negative correlation. Moreover, total host length was positively correlated with abundance and intensity of all fish species examined, as well; most correlations were significant as shown in Table 5.

Discussion

A range of different freshwater ecosystems around Guangdong province were investigated during the period studied, and on each sampling trip, several sampling methods were employed to ensure that any bias in fish species caught could not be attributed to sampling technique. Our data showed eight fish species representing four families were heavily infected by *A. japonicus*, indicating this fish louse is a generalist parasite, which is in agreement with many authors who have stated that argulid parasites infect a wide range of freshwater fish hosts (Kearn 2004; Walker et al. 2004, 2011a; Nagasawa 2009; Alsarakibi et al. 2012).

Regardless of fish species variety in Guangdong province, cyprinid were most frequent fish captured, as well as the infected fish during our study. Among more than 40 fish

Fig. 3 Abundance (a) and intensity (b) of *A. japonicus* infection within hosts' length classes of freshwater fishes collected from Guangdong province, China, during July 2010 to March 2013

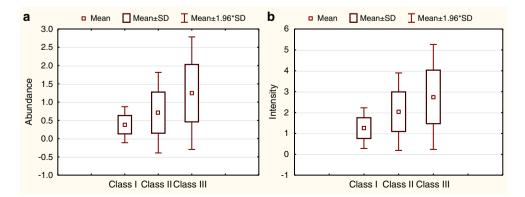


Table 4 Prevalence (P%), mean abundance (MA), and mean intensity (MI) of *A. japonicus* (n=1,443) between length classes of each fish species collected from Guangdong province, China, during July 2010 to March 2013

Fish species	Length classes	P (%)	P value	MA±SE	P value	MI±SE	P value
C. carpio	Class I Class II	24.5 29.9	0.01	$0.25 \pm 0.05 \\ 0.64 \pm 0.05$	<0.01	1.03±0.07 2.22±0.11	< 0.01
	Class III	46.0		$1.66 {\pm} 0.27$		$3.73 {\pm} 0.27$	
C. auratus	Class I Class II	34.8 39.2	< 0.01	$0.37{\pm}0.03$ $0.82{\pm}0.06$	< 0.01	1.09 ± 0.04 2.08 ± 0.10	< 0.01
	Class III	78.3		2.20 ± 0.20		2.90 ± 0.28	
M. piceus	Class I Class II	23.0 37.8	0.02	$0.38 {\pm} 0.04 \\ 0.80 {\pm} 0.08$	< 0.01	1.70±0.15 2.21±0.14	< 0.01
	Class III	52.1		1.41 ± 0.17		$2.86 {\pm} 0.20$	
H. molitrix	Class I Class II	45.8 46.9	0.22	1.13±0.12 1.73±0.17	0.47	2.50 ± 0.29 3.74 ± 0.11	0.02
	Class III	36.7		$1.80 {\pm} 0.15$		$4.80 {\pm} 0.17$	
S. trutta	Class I Class II	14.9 20.6	0.06	$0.21 {\pm} 0.03$ $0.39 {\pm} 0.03$	< 0.01	$1.50 {\pm} 0.16$ $1.83 {\pm} 0.08$	< 0.01
	Class III	34.7		$0.91 {\pm} 0.16$		2.70 ± 0.12	
O. mykiss	Class I Class II	28.2 36.7	< 0.01	0.29 ± 0.04 0.39 ± 0.03	< 0.01	1.08 ± 0.08 1.11 ± 0.05	0.14
	Class III	83.0		$0.97{\pm}0.14$		1.13 ± 0.10	
S. chuatsi	Class I Class II	50.5 39.8	0.15	$0.53 {\pm} 0.06$ $0.60 {\pm} 0.08$	0.02	1.10 ± 0.12 1.48 ± 0.14	< 0.01
	Class III	41.9		0.96 ± 0.10		2.17 ± 0.14	
P. fluviatilis	Class I Class II	30.4 33.9	< 0.01	$0.50 {\pm} 0.07$ $0.64 {\pm} 0.10$	<0.01	1.78 ± 0.22 0.88 ± 0.15	0.04
	Class III	39.7		0.88±0.15		2.33 ± 0.20	

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Differences are significant at P < 0.05

species reported worldwide as host of *A. japonicus*, there are up to 31 species belonged to Cyprinidae (Alsarakibi, unpublished data), thus, it makes sense to say that *A. japonicus* prefer cyprinid. However, the available information indicates that fish responds to parasite infections by activating different innate and adaptive immune mechanism. Studies have been mainly focused on some innate activities, most of which were demonstrated to be involved in the immune response to parasitoses (Alvarez–Pellitero 2008). Generally, fish immune response to *A. japonicus* is varied within species, which is initially restricted to the site of infection but extended to a generalized response throughout the skin as a whole organ at a later stage of the infection (Forlenza et al. 2008). However, it could be attributed to the fact that adult argulids are quite mobile, being able to glide over the surface of their hosts with relative ease using their maxillary suckers (Kearn 2004), demonstrating the ability of *A. japonicus* to infect a wide range of fish species.

The current study presents first record of *A. japonicus* infected the black carp, the brown trout, and the mandarin fish. It is possibly due to either infection transmission between fish hosts in same aquatic system or introduction of *A*.

Table 5 Spearman's correlation
between fish length and preva-
lence (P%), mean abundance
(MA), and mean intensity (MI) of
A. japonicus $(n=1,443)$ in
Guangdong province, China,
during July 2010 to March 2013

P%Fish species Length MA MI range (mm) r P value P value P value r r 210-400 0.377 0.680 < 0.01 C. carpio < 0.010.661 < 0.01C. auratus 120-390 0.353 < 0.010.694 < 0.010.790 < 0.010.01 < 0.01 M. piceus 230-450 0.507 0.783 < 0.010.610 H. molitrix 210-530 -0.249 0.08 0.200 0.690 < 0.01 0.16 S. trutta 220-460 0.442 < 0.01 0.648 < 0.01 0.623 < 0.01 0.085 O. mvkiss 200-460 0.524 < 0.01 0.532 < 0.01 0.52 S. chuatsi 200-530 -0.117 0.31 0.287 0.01 0.464 < 0.01 0.285 P. fluviatilis 190-490 0.183 0.23 0.353 0.02 0.06

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Differences	are	significant	at	
P < 0.05				

japonicus with introduced infected fish, especially under conditions of culture. Three new hosts belonged to three different families. However, comparison of the prevalence of infection between these hosts revealed that prevalence was independently associated with fish species; moreover, abundance and intensity of those three new hosts imply that fish species is not a key factor of *A. japonicus* infection.

Obviously, the prevalence, mean abundance, and mean intensity of A. japonicus were higher in cultured fish than in wild fish, which might strongly be attributed to the fish population in cultured facilities compared with that in wild. In addition, under cultured conditions, the over-stocking, inadequate nutrition, and poor water circulation can cause parasitic outbreaks (Khan 2009). This is consistent with the findings of Taylor et al. (2006, 2009) and McPherson et al. (2012), which concluded that slow stock turnover was an important key in determining the abundance of the Argulus sp., as well as the threshold at which it could survive. Furthermore, the changeable biotic and abiotic factors in the aquatic system may strongly impact on the fish ectoparasite outbreak (Walker et al. 2004; Violante-Gonzalez et al. 2009; Ibrahim 2012; Alsarakibi et al. 2012). In the case of new hosts recorded, A. japonicus infections were higher than expected; it may be attributed to host immune responses.

Parasite abundance can vary by more than one order of magnitude among host populations (Poulin 2006); however, our data on abundance and intensity hint towards a trend of silver carps and black carps being favored as hosts by A. *japonicus*. Notwithstanding this finding, the silver carp has not been considered traditionally as potential hosts of A. japonicus, where only Kimura (1970) and Qizhong and Chenglin (1994) have reported infections of A. japonicus on sliver carp, which were fewer infections as compared with other fish species. For clarification, we offer a plausible suggestion, similar to the host fish and other animals which have adaptive immune mechanisms; this parasite has also the ability to develop offensive mechanisms; this capability could be attributed to genetic changes, where the host selection in this ectoparasite does not depend exclusively on the features of the host/parasite but also on the surrounding conditions. On the other hand, Mikheev et al. (2007) have suggested that the innate ontogenetic shift in host preference maintains the major part of the parasite population on its principal host, ensuring successful reproduction within suitable habitats. Generally, if the parasite and various host fish species were evenly distributed within the water body, the parasite distribution should be related to host population (Walker et al. 2008). However, in our study, A. japonicus distribution within the host community does not appear to be related with the number of available hosts from each fish species.

Only four fish species showed a significant relationship between their prevalence and total host length, while mean abundance and mean intensity of A. japonicus showed a positive significant correlation with total host length in all captured fish species. Generally, parasite loads on fish have been shown to be influenced by host size (Kabata 1981; Grutter 1994). The general trend is that larger individuals have higher physical (ventilation volume) and chemical (mucus) stimuli, which increase their attractiveness to parasites (Lo et al. 1998; Tucker et al. 2002). The reason may be partly related to the host age, older hosts having longer to accumulate parasites (Bush et al. 2003; Zander 2004). However, some fish species appeared to tolerate multiple number of parasites without exhibiting debility, whilst young fish were infected with fewer parasites than older fish, and in addition, prevalence of the infection and mean abundance increased with the host length (Khan 2012). It also may be partly related to the surface area of host as a larger host has a greater surface area, making it easier for parasites to locate and attach to them (Walker et al. 2007). The negative correlation between the total length of silver carp and its prevalence may be the result of host immune responses and likewise of the insignificant correlation between three other fish species and total length. Similar to our finding, Bakke et al. (2002) have reported negative correlation between the population of Gyrodactylus salaries and age of Salmonidae.

Conclusion

A. japonicus infects a wide range of freshwater fish. Cyprinids are preferred species of *A. japonicus*, where silver carps and black carps were the most susceptible. Cultured fish are more infected than wild fish. Furthermore, *A. japonicus* were more abundant in larger-sized fish than smaller-sized fish. Therefore, fish population and body size are important keys in determining the abundance and intensity of *A. japonicus*. The black carp, the brown trout, and the mandarin fish were three new hosts found in the current study. Hence, aquaculturists should pay particular attention to the control of fish lice due to their economic importance.

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