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Short communication

Distribution of *Anisakis* species larvae from fishes of the Japanese waters ☆

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ABSTRACT

Human anisakiasis is caused by the consumption of raw, marinated or undercooked fish and squid infected with nematodes of the genus *Anisakis* Dujardin, 1845. In view of food safety, this study was carried out to examine the distribution of *Anisakis* species in marine fishes within Japanese waters. Seven fish species from six localities were collected and examined for *Anisakis* infection. Morphological and molecular (ITS region and *mt*DNA *cox*2 gene) characterization revealed the presence of two, among the three sibling species of *Anisakis simplex*, viz. *A. simplex* sensu stricto (s.s.) and *A. pegreffii*. Distribution data were collated with the results from the previous researches to better understand *Anisakis* distribution in Japanese waters. Distributions of *Anisakis* species were found to be locality-specific rather than host-specific, particularly between the two major species, *A. simplex* s.s. and *A. pegreffii*. *Anisakis simplex* s.s. is mainly found in fishes from northern Japan to Pacific sides, whereas *A. pegreffii* is in fishes from the Sea of Japan to East China Sea sides.
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There are many studies on the identification of third-stage *Anisakis* larvae (L3) infecting marine fishes in Japanese waters. However, most of the identifications were carried out morphologically and were only limited to the genus level (*Anisakis* sp.) [1] or type groupings (*Anisakis* type I and *Anisakis* type II groups) [2–4]. With the recent application of molecular techniques for the identification of *Anisakis* species in Japan, five species belonging to *Anisakis* type I group (*A. simplex* sensu stricto [s.s.], *A. pegreffii*, *A. simplex* C, *A. typica* and *A. ziphidarum*), two species belonging to *Anisakis* type II group (*A. brevispiculata* and *A. physeteris*), and hybrid genotypes of *A. simplex* s.s. and *A. pegreffii* have so far been reported [5–12]. Recently, it was suggested that anisakiasis in Japan is mainly caused by *A. simplex* s.s. because its larvae penetrate the muscle of fish at a higher rate than do those of *A. pegreffii* [12,13]. Considering the zoonotic implication of *Anisakis* spp., it is worthwhile to understand their distribution in marine fishes in Japanese waters. Such information is useful for government agencies responsible for human food safety in setting up future guidelines to reduce incidences of human anisakiasis. In an intensive investigation of *Anisakis* infection in chub mackerel (*Scomber japonicus*) caught in various areas surrounding Japan, *A. simplex* s.s. was found to be the dominant species group in Pacific coast fishes, whereas *A. pegreffii* was dominant in the Sea of Japan [12]. Limited data exist on the geographical distributions of *Anisakis* species in other fish species. In this study, we examined the fishes collected from various locations to obtain data on the distribution of *Anisakis* species in Japanese waters. Furthermore, the present data were combined with those from the previous molecular studies [6,8,9,11,12,14] (Table 1) to determine an overall view of *Anisakis* distribution infecting different fish species within Japanese waters.

In this study, seven fish species from six localities were collected and examined for *Anisakis* infection (Table 1). All collected *Anisakis* spp. were initially identified based on morphological differences [2,14], which were all verified molecularly using PCR-RFLP on the ITS (ITS1-5.8 S rRNA–ITS2) region. Final confirmation of species identification was made after sequencing of the ITS region and *mt*DNA *cox*2 gene [8,11,14–16] (GenBank accession numbers: HQ717429–HQ717432) of the three subsamples of each larval group sharing a similar fragment pattern generated using PCR-RFLP. The data obtained in this study were combined with the previous data [6,8,9,11,12,14], where *Anisakis* species were identified using PCR-RFLP analysis on the ITS region (ITS1-5.8 S rRNA–ITS2), multiplex PCR analysis and sequencing of the ITS region and *mt*DNA *cox*2 gene.

Morphological and molecular approaches have identified majority of *Anisakis* spp. in marine fishes as *A. simplex* s.s. and *A. pegreffii*. Genetically, generated fragment patterns in PCR-RFLP and sequence comparison on the ITS region and *mt*DNA *cox*2 gene coincide with the previous studies [6,8,11,14] revealing that majority of *Anisakis* species in Japanese waters are *A. simplex* s.s. and *A. pegreffii*. After combining the present data with the previously reported results, *Anisakis* distributions were found to be locality-specific rather than host-specific. Regardless of host species, different patterns of geographical distribution for *A. simplex* s.s. and *A. pegreffii* were clearly evident. Fish

Abbreviations: PCR-RFLP, Polymerase chain reaction-restriction fragment length polymorphism; ITS, Internal transcribed spacer; *mt*DNA, Mitochondrial DNA; *cox*2, Cytochrome oxidase subunit 2.

☆ Nucleotide sequence data reported in this paper are available in the GenBank under the accession numbers HQ717429–HQ717432.

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Table 1
Distribution of larval *Anisakis* species in various marine fishes in Japanese waters.

References (Codes for Fig. 1)	Host fishes	Locality	Prevalence	Intensity	No. of host fishes examined	No. of worms examined		No. of worms (percentage)								
								Anisakis type I larvae					*Anisakis* type II larvae			Hybrid genotype
						Morphological	Molecular	*A. simplex* (s.s.)	*A. pegreffii*	*A. simplex C*	*A. typica*	*A. ziphidarum*	*A. brevispiculata*	*A. physeteris*	*A. paggiae-like* species	
Umehara et al. [9] (A)	*Pleurogrammus azonus*	Hokkaido[a]					10	10 (100%)								
	Scomber japonicus	Kyushu[a]					11		10 (90.9%)					1 (9.1%)		
Umehara et al. [8] (B)	*P. azonus*	Hokkaido[a]					20	20 (100%)								
	Theragra chalcogramma	Hokkaido[a]					19	19 (100%)								
	S. japonicus	Hokkaido[a]					16	16 (100%)								
	Hypomesus pretiosus japonicus	Hokkaido[a]					10	10 (100%)								
	S. japonicus	Fukuoka[a]					38		37 (97.4%)							1 (2.6%)
Abe et al. [6] (C)	*Gadus macrocephalus*	Hokkaido[a]					26	24 (92.3%)	2 (7.7%)							
Quiazon et al. [14] (D)	*Oncorhynchus keta*	Chitose River, Hokkaido				101	101	99 (98%)								2 (2%)
	T. chalcogramma	Hokkaido[a]				10	10	10 (100%)								
	T. chalcogramma	Between Aomori–Hokkaido				20	20	18 (90%)								2 (10%)
	G. macrocephalus	Iwate				22	22	10 (45.3%)	7 (31.8%)							5 (22.7%)
	Scomberomorus niphonius	Kyoto				6	6		6 (100%)							
	Scomber australasicus	Kanagawa				8	8	8 (100%)								
	S. australasicus	Miyazaki				5	5	5 (100%)								
	S. japonicus	Kumamoto				67	67		66 (98.5%)							1 (1.5%)
	G. macrocephalus	Between Aomori–Hokkaido				19	19	8 (42.1%)	8 (42.1%)							3 (15.8%)
	G. macrocephalus	Iwate				6	6	6 (100%)								
Quiazon et al. [11] (E)	*T. chalcogramma*	Rausu, Hokkaido				110	110	104 (94.5%)	6 (5.5%)							
	T. chalcogramma	Iwate				100	100	87 (87%)	5 (5%)				3 (3%)		5 (5%)	
Suzuki et al. [12] (F)	*S. japonicus*	Ishikawa[a]					20	3 (15%)	17 (85%)							
		Fukui[a]					12	2 (16.7%)	10 (83.3%)							
		Shimane[a]					28	1 (3.6%)	27 (96.4%)							
		Fukuoka[a]					21		21 (100%)							
		Nagasaki[a]					52		52 (100%)							
		Kochi[a]					17	14 (82.4%)	2 (11.8%)	1 (5.9%)						
		Hyogo[a]					8	7 (87.5%)	1 (12.5%)							
		Mie[a]					21	15 (71.4%)	2 (9.5%)	1 (4.8%)						3 (14.3%)[b]
		Kanagawa[a]					22	19 (86.4%)	2 (9.1%)	1 (4.5%)						
		Iwate[a]					33	28 (84.8%)	4 (12.1%)		1 (3%)					
		Chiba[a]					92	78 (84.8%)	10 (10.9%)	1 (1.1%)						3 (3.3%)[b]
		Miyagi[a]					24	20 (83.3%)	1 (4.2%)	1 (4.2%)	1 (4.2%)	1 (4.2%)				
		Aomori[a]					16	15 (93.8%)	1 (6.2%)							
Present study (G)	*Takifugu poecilonotus*	Shimonoseki, Yamaguchi	25%	1	4	1	1		1 (100%)							
	Arctoscopus japonicus	Fukui	5.6%	1	18	1	1		1 (100%)							
	Hippoglossoides dubius	Fukui	63.2%	1–7	19	28	28		28 (100%)							
	S. australasicus	Tanegashima	44.7%	1–5	47	47	47	32 (68.1%)	15 (31.9%)							
	S. japonicus	Nagasaki	100%	80–120	12	50	50		50 (100%)							
	T. chalcogramma	Rausu, Hokkaido	100%	5–10	12	50	50	50 (100%)								
	G. macrocephalus	Iwate	100%	30–80	5	50	50	50 (100%)								

[a] Exact location is not specified.
[b] Exact total number of worms examined was unclear.

collected from the Sea of Japan and East China Sea were mainly infected with *A. pegreffii,* whereas those collected from the Pacific Ocean were mainly infected with *A. simplex* s.s. (Fig. 1).

The exact localities of fish sampled off Hokkaido were not specified except for Alaska Pollock (*Theragra chalcogramma)*, which was collected off Rausu in the Okhotsk Sea. Almost all chum salmon (*Onchorynchus keta*) migrating upstream from the Sea of Japan to the Chitose River were infected with *A. simplex* s.s. Japanese stock of this fish spends most of its life in the North Pacific including the Bering Sea [17] where they most likely become infected with the nematode.

The combined data on *Anisakis* infection and their distribution in Japan gave an updated *Anisakis* distribution, particularly of the two dominant species, *A. simplex* s.s. and *A. pegreffii*. Although these two species are largely dominant in Japanese waters, a few other species from *Anisakis* type I group (i.e., *A. simplex* C, *A. typica* and *A. ziphidarum*), *Anisakis* type II group (i.e., *A. brevispiculata*, *A. physeteris* and unidentified *Anisakis* sp. genetically close to *A. physeteris* in ITS region but close to *A. paggiae* in *mt*DNA *cox*2 gene) and hybrid genotypes of *A. simplex* s.s. and *A. pegreffii* are also present [12,13]. Due to difficulties in morphological identification of *Anisakis* larvae from fishes caught in Japanese waters, they have been reported as *Anisakis* sp. With the current morphological and molecular results, distribution of *Anisakis* species revealed to be locality-specific and not host-specific. A majority of fishes in Hokkaido are infected with *A. simplex* s.s. and this is the species commonly recovered from patients with anisakiasis, which is possibly the reason why incidence of human infection in this region is high [18]. In southern Japan, fishes examined in eastern and northern Kyushu area were mostly infected with *A. pegreffii*. However, patients with anisakiasis in Kyushu area were mainly diagnosed with *A. simplex* s.s. infections (79 out of 80 patients), while only one patient was infected with *A. pegreffii* [18]. Recently, penetration rates in the muscle of chub mackerel were found to be much lower by larvae of *A. pegreffii* than by those of *A. simplex* s.s. [12], suggesting a lower risk of *A. pegreffii* infection in humans. Unfortunately, such differences on the penetration rate of these two *Anisakis* species have not been closely examined in the present study. In the Kyushu area, sashimi of chub mackerel is frequently consumed. This tradition may further explain the lower risk of *A. pegreffii* infection as compared to *A. simplex* s.s. On the other hand, although hybrid genotypes have not been observed in the samples examined, and have not yet been reported from human patients, the high numbers of these genotypes found generally in that area [9,12,14] should be better investigated if these are representing F1 hybrids or the result of a retained polymorphism at a conserved region of the ITS gene, in either *A. simplex* s.s. or *A. pegreffii.* It is also thus interesting if penetration rate of hybrids is similar with that of *A. simplex* s.s. or *A. pegreffii* [12].

Parasites have also been used or considered to be promising biological tags for discriminating different stocks or populations of marine fishes, and *Anisakis* larvae have been reported as a good tool for stock recognition of fishes in Europe [15,19–21]. From the distributions of *A. simplex* s.s. and *A. pegreffii*, these two nematodes

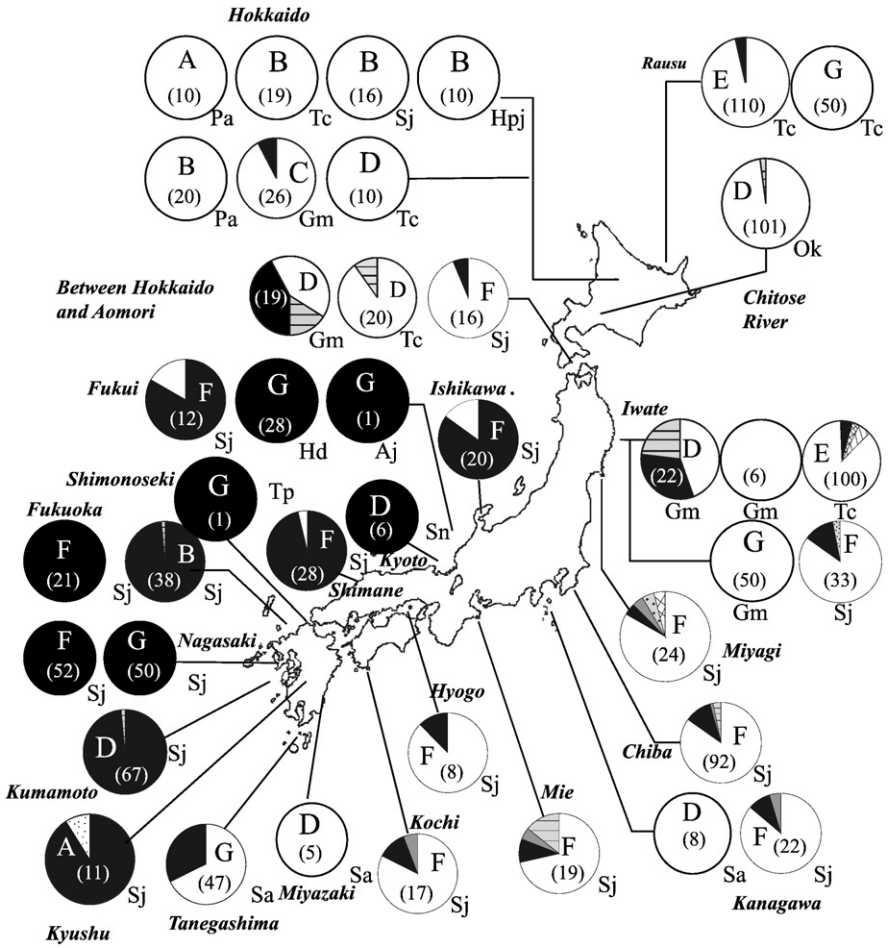


Fig. 1. Distribution and relative proportions of different *Anisakis* species. ○: *A. simplex* s.s.; ●: *A. pegreffii*; ⊘: *Anisakis* sp.; ⊗: *A. brevispiculata*; ✪: *A. physeteris*; ◍: *A. simplex* C; ⊗: *A. ziphidarum*; ⊗: *A. typica*; ⊜: hybrid genotypes. (Letters represent sources of *Anisakis* data from previous reports: A–Umehara et al. [9]; B–Umehara et al. [8]; C–Abe et al. [6]; D–Quiazon et al. [14]; E–Quiazon et al. [11]; F–Suzuki et al. [12]; and G–present study. Numbers of examined worms are in parentheses. *Abbreviations of host names*: Pa – *Pleurogrammus azonus*; Tc – *Theragra chalcogramma*; Sj – *Scomber japonicus*; Sa – *Scomber australasicus*; Sn – *Scomberomorus niphonius*; Tp – *Takifugu poecilonotus*; Hd – *Hippoglossoides dubius*; Aj – *Arctoscopus japonicus*; Gm – *Gadus macrocephalus*; Hpj – *Hypomesus pretiosus japonicus*; and Ok – *Oncorhynchus keta*).

may be good biological markers for differentiating various fishes around Japan, including migratory chub mackerel and blue mackerel (*S. australasicus*). Indicating the origin of wild caught fishes has also recently become mandatory in Japan and validation of these two nematode species as markers may further aid this assessment of geographic origin.

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References

[1] Ishikura H. Gastric anisakiasis in Japan. Epidemiology, diagnosis, treatment. In: Ishikura H, Namiki M, editors. Tokyo: Springer-Verlag; 1989. p. 3–11.
[2] Koyama T, Kobayashi A, Kumada M, Komiya Y. Morphological and taxonomical studies on Anisakinae larvae found in marine fishes and squids. Jpn J Parasitol 1969;18:466–87 (In Japanese with English abstract).
[3] Moravec F, Nagasawa K, Urawa S. Some fish nematodes from freshwaters in Hokkaido, Japan. Folia Parasitol 1985;32:305–16.
[4] Moravec F, Nagasawa K. Observations on some nematodes parasitic in Japanese freshwater fishes. Folia Parasitol 1989;36:127–41.
[5] Mattiucci S, Paggi L, Nascetti G, Ishikura H, Kikuchi K, Sato N, et al. Allozyme and morphological identification of *Anisakis, Contracaecum* and *Pseudoterranova* from Japanese waters (Nematoda, Ascaridoidea). Syst Parasitol 1998;40:81–92.
[6] Abe N, Ohya N, Yanagiguchi R. Molecular characterization of *Anisakis pegreffii* larvae in Pacific cod in Japan. J Helminthol 2005;79:303–6.
[7] Mattiucci S, Nascetti G. Molecular systematics, phylogeny and ecology of anisakid nematodes of the genus *Anisakis* Dujardin, 1845: an update. Parasite 2006;13:99–113.
[8] Umehara A, Kawakami Y, Matsui T, Araki J, Uchida A. Molecular identification of *Anisakis simplex* sensu stricto and *Anisakis pegreffii* (Nematoda: Anisakidae) from fish and cetacean in Japanese waters. Parasitol Int 2006;55:267–71.
[9] Umehara A, Kawakami Y, Araki J, Uchida A. Multiplex PCR for the identification of *Anisakis simplex* sensu stricto, *Anisakis pegreffii* and other anisakid nematodes. Parasitol Int 2008;57:49–53.
[10] Yoshinaga T, Kinami R, Hall KA, Ogawa K. A preliminary study on the infection of anisakid larvae in juvenile greater amberjack *Seriola dumerili* imported from China to Japan as mariculture seedlings. Fish Pathol 2006;41:123–6.
[11] Quiazon KMA, Yoshinaga T, Santos MD, Ogawa K. Identification of larval *Anisakis* spp. (Nematoda: Anisakidae) in Alaska pollock (*Theragra chalcogramma*) in northern Japan using morphological and molecular markers. J Parasitol 2009;95: 1227–32.
[12] Suzuki J, Murata R, Hosaka M, Araki J. Risk factors for *Anisakis* infection and association between the geographic origins of *Scomber japonicus* and anisakid nematodes. Int J Food Microbiol 2010;137:88–93.
[13] Quiazon KMA, Yoshinaga T, Ogawa K . Experimental challenge of *Anisakis simplex* sensu stricto and *Anisakis pegreffii* (Nematoda: Anisakidae) in rainbow trout and olive flounder. Parasitol Int (in print).
[14] Quiazon KMA, Yoshinaga T, Ogawa K, Yukami R. Morphological differences between larvae and *in vitro*-cultured adults of *Anisakis simplex* (sensu stricto) and *Anisakis pegreffii* (Nematoda: Anisakidae). Parasitol Int 2008;57:483–9.
[15] Mattiucci S, Nascetti G. Advances and trends in the molecular systematics of anisakid nematodes, with implications for their evolutionary ecology and host–parasite co-evolutionary processes. Advances in Parasitology. In: Rollinson D, Hay SI, editors. Elsevier Ltd.; 2008. p. 49–137.
[16] Valentini A, Mattiucci S, Bondanelli P, Webb SC, Mignucci-Giannone AA, Colom-Llavina MM, et al. Genetic relationships among *Anisakis* species (Nematoda: Anisakidae) inferred from mitochondrial *cox*2 sequences, and comparison with allozyme data. J Parasitol 2006;92:156–66.
[17] Urawa S, Ueno Y, Ishida Y, Takagi S, Winans G, Davis N. Genetic stock identification of young chum salmon in the North Pacific Ocean and adjacent Seas. (NPAFC Doc. 336). Sapporo: National Salmon Resources Center, Fisheries Agency of Japan; 1998.
[18] Umehara A, Kawakami Y, Araki J, Uchida A. Molecular identification of the etiological agent of the human anisakiasis in Japan. Parasitol Int 2007;56:211–5.
[19] MacKenzie K. Parasites as biological tags in population studies of marine organisms: an update. Parasitology 2002;124:153–63.
[20] MacKenzie K, Campbell N, Mattiucci S, Ramos P, Pinto AL, Abaunza P. Parasites as biological tags for stock identification of Atlantic horse mackerel *Trachurus trachurus* L. Fish Res 2008;89:136–45.
[21] Mattiucci S, Abaunza P, Damiano S, Garcia A, Santos MN, Nascetti G. Distribution of *Anisakis* larvae, identified by genetic markers, and their use for stock characterization of demersal and pelagic fish from European waters: an update. J Helminthol 2007;81: 117–27.