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Geographical distribution of *Metagonimus yokogawai* and *M. miyatai* in Shizuoka Prefecture, Japan, and their site preferences in the sweetfish, *Plecoglossus altivelis*, and hamsters

Hideto Kino [a,*], Tohru Suzuki [a], Hisaji Oishi [b], Saeko Suzuki [b], Shizuko Yamagiwa [b], Mitsuru Ishiguro [b]

[a] *Department of Parasitology, Hamamatsu University School of Medicine, 1-20-1 Handayama, Hamamatsu 431-3192, Japan*
[b] *Shizuoka Health Service Association, 1-3-43 Takyo, Aoi-ku, Shizuoka 421-1292, Japan*

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Abstract

Sweetfish, *Plecoglossus altivelis*, obtained from 18 rivers in Shizuoka Prefecture were examined for metacercarial infection of 2 flukes, *Metagonimus yokogawai* and *Metagonimus miyatai*. The infection rate and density of metacercariae in the fish were higher in eastern and western regions than in central region of the prefecture. After infection of hamsters with metacercariae derived from the scale, 98.7% of the adult worms obtained from the intestine was found to be *M. miyatai*. Conversely, from infection with metacercariae from the flesh, 90.0% of the worms was *M. yokogawai*. Since the worms had no exclusivity in the tissues, we conclude that the flukes have location preference with the former primarily preferring the scale, and the latter the flesh. Fish from two rivers located in adjacent areas in the western region had relatively a higher ratio of *M. yokogawai* in the scale relative to other rivers, suggesting an intraspecific genetic variation due to geographical isolation. On examination of adult worms in the hamster's intestine, *M. yokogawai* was mainly located towards the anterior part of the intestine, unlike *M. miyatai*, suggesting that in mammalian host too, the parasites have site preference.

Keywords: *Metagonimus yokogawai*; *Metagonimus miyatai*; *Plecoglossus altivelis*; Metacercaria; Site preference

1. Introduction

Since the first time *Metagonimus yokogawai* was isolated and described in Taiwan [1], many studies have been carried out on *Metagonimus* flukes and metagonimiasis in Japan and Korea [2–4]. Among the studies, several variations in the species have been described mainly from a morphological point of view. For instance, *M. takahashii* had earlier been classified as a subspecies of *M. yokogawai.* However, later studies have characterized several morphological differences between the two such as egg size as well as the relative position of the two testes and the uterus, and therefore it is currently recognized as a distinct species [5]. Miyata [6,7] described a variation of the parasite with a similar arrangement of the testis and uterus to *M. yokogawai*, and which had been recognized as a part of the species for a long time. Saito [8] stressed the validity of the parasite as a distinct species. He noted that unlike *M. takahashii*, the parasite's vitelline gland does not cross the right testis. Later, Saito et al. [9] described it as a new species, *Metagonimus miyatai*. Thus in the genus *Metagonimus*, there exist 3 parasite species that can parasitize humans namely *M. takahashii*, *M. miyatai* and *M. yokogawai*, although the latter was previously thought to be the only one.

In addition to morphological differences, the parasites also differ in their specificity to the second intermediate host. In Japan, whereas *M. yokogawai* is known to parasitize the sweetfish, *Plecoglossus altivelis*, and the dace, *Tribolodon hakonensis*, *M. takahashii* prefers the carp, *Cyprinus carpio*, and the crucian carp, *Carassius carassius*. However, *M. miyatai* can parasitize a lot of freshwater fish species including the sweetfish [2]. Site preferences of metacercariae in the fishes and of adult worms in final hosts are also said to differ among the species. While metacercariae of *M. yokogawai* prefer both the

* Corresponding author. Tel.: +81 53 435 2338; fax: +81 53 435 2337.
E-mail address: kino624@hama-med.ac.jp (H. Kino).

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flesh and the scale, those of both *M. takahashii* and *M. miyatai* prefer only the scale [9]. In dogs, while adult worms of *M. yokogawai* and *M. takahashii* tend to parasitize the upper part of the small intestine, those of *M. miyatai* parasitize the lower part [8,9]. It has been suggested that the site preference of adult worms could be attributed to species specific traits, but not to individual difference of the host or duration of infection [2,8].

In the sweetfish, the common fish host of *M. yokogawai* and *M. miyatai*, it is not unusual to find a co-infection with metacercariae of both species. Many epidemiological surveys reported before the description of *M. miyatai* but classified the parasite species as *M. yokogawai* [2,10] and since many of the studies on prevalence and distribution only examined the scale of the sweetfish, the results could have possibly included those of *M. miyatai*. The present study aims to clarify the geographical distribution of both *M. yokogawai* and *M. miyatai* in Shizuoka Prefecture, Japan, and parasites' site preference by metacercariae and adult worms in the sweetfish and hamsters, respectively.

2. Materials and methods

2.1. Collection and processing of metacercariae

The fresh sweetfish were collected from 18 rivers in Shizuoka Prefecture (Fig. 1) through the Fishery Cooperative of each river between August and September of 2003 to 2005, except for the collection from Kano River which was done in December 2002. Among the 18 rivers surveyed, 6 are located in the eastern region, 4 in the central region and 8 in the western region of the prefecture. Atago River, Ai River and Ohchiise River, which lie in the western region, are tributaries of Tenryu River, and Kurumegi River is an upper tributary of Miyakoda River. For each river, a total of 10 to 49 individuals were examined. After body weight and body length were determined, the scale and the flesh of one side (either left or right) of the individual fish were separately removed. The scales and minced flesh were separately digested with artificial digestive fluid (0.1% pepsin solution with 0.7% hydrochloric acid) for 3 to 4 h at 37 °C. The sediments were sieved with a nylon mesh with an opening of 80 μm and debris retaining metacercariae on the mesh was washed out into a cup with saline solution. For each sample, the metacercariae in the saline were counted and collected under a dissecting microscope.

2.2. Infection of hamsters

To identify the adult parasite of each of the species using the morphological differences, the metacercariae derived from each part of all fish individuals obtained from the same location were mixed and orally inoculated to a 5-week old female Syrian hamster (Japan SLC Inc.) using a stomach tube. A total of 58 hamsters were used for the infection with metacercariae derived from both the scale and the flesh. The hamsters were handled and maintained according to the guidelines provided in the *Guide for the Care and Use of Laboratory Animals* of Hamamatsu University School of Medicine, Japan. The number of metacercariae inoculated per hamster varied from 3 to 584 depending on the metacercarial yield from each river. To recover the adult worms, the hamster was sacrificed with an

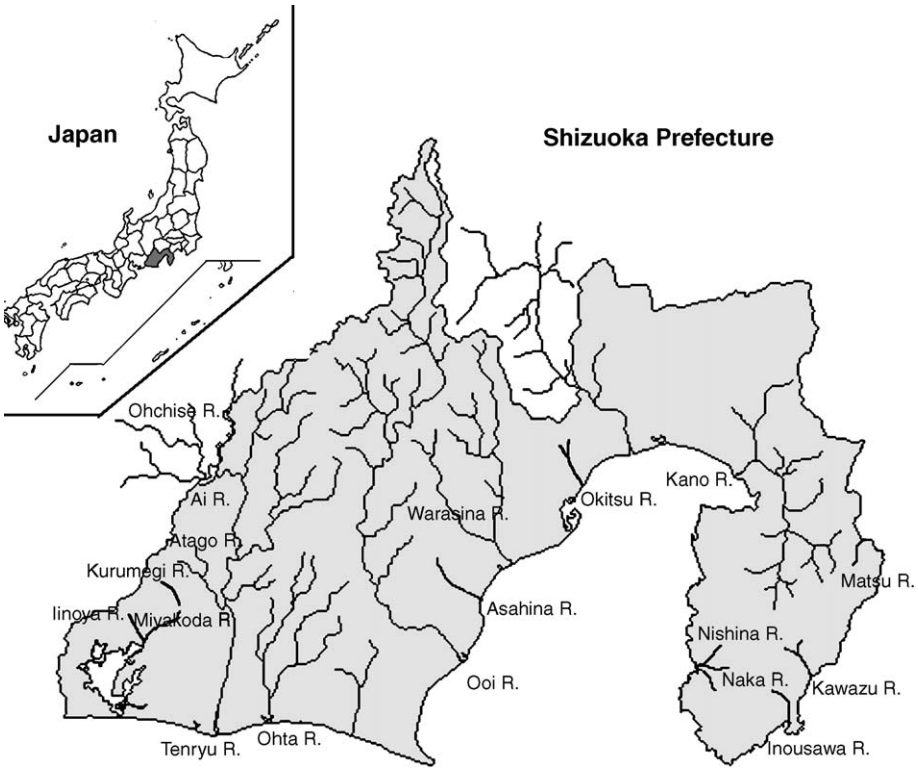

Fig. 1. A map of Shizuoka Prefecture showing location of the rivers surveyed.