[Research Note]

Limited distribution of *Caenorhabditis japonica* in JapanToyoshi Yoshiga¹

Caenorhabditis japonica forms a species-specific and female host-biased phoretic association with the shield bug *Parastrachia japonensis*. To determine the distribution of *C. japonica*, nematodes from *P. japonensis* were surveyed in islands ranging from Kyushu to Okinawa of Japan. *C. japonica* was isolated from all 6 sampled populations of *P. japonensis* from Kyushu, Amami Oshima, and Tokunoshima Islands, but not from those from Okinawa Island. The limited distribution of *C. japonica* in these islands is suggestive of the origin of *C. japonica* and its limited phoretic association with *P. japonensis* in this region. Nematol. Res. 48(2), 71–73. (2018)

Key words: ecology, *Parastrachia japonensis*, phorecy, phoretic association

INTRODUCTION

The nematode *Caenorhabditis japonica* forms a phoretic association with the shield bug *Parastrachia japonensis* (Kiontke *et al.*, 2002; Yoshiga *et al.*, 2013). *P. japonensis* is a specialist insect herbivore on drupes of *Schoepfia jasminodora*; therefore, its life cycle is synchronized to that of the tree such that the insect's reproductive period coincides with drupe availability (Tachikawa and Schaefer, 1985). In turn, since *C. japonica* is a specialist phoront of *P. japonensis*, its reproductive period is synchronized to that of its host (Yoshiga *et al.*, 2013; Li *et al.*, 2014; Okumura *et al.*, 2013a, 2014). *C. japonica* spends most of the year in a quiescent state, latched onto the adult females of *P. japonensis*, which are in a reproductive diapause, spending this period aggregating with males on leaves of evergreen plants. After mating, *P. japonensis* females make nests in the leaf litter. During the reproductive period of *P. japonensis*, which extends

from spring to early summer, *C. japonica* resumes its mobility, leaves the host, and propagates within the host's nests, feeding on dead nymphs and drupes provided by the mother insect to her offspring (Okumura *et al.*, 2013b). Dauer larvae of *C. japonica* latch onto host nymphs of both sexes, but later form an adult female-biased phoretic association after adult maturation.

Because *C. japonica* is a specialist phoront of *P. japonensis*, its distribution is limited by the distribution of the host. The phoretic association of *C. japonica* with *P. japonensis* in Kyushu, Shikoku, and Amami Oshima Islands has been described previously (Yoshiga *et al.*, 2013; Li *et al.*, 2014); however, there is no information on *C. japonica* in other regions of the *P. japonensis* distribution which ranges to the Yunnan Province of China (Schaefer *et al.*, 1991). In this study, the distribution of *C. japonica* and its phoretic association with *P. japonensis* were examined mainly in the Ryukyu Islands of Japan.

MATERIALS AND METHODS**Sampling sites:**

C. japonica identified on a single *P. japonensis* population per each locality were surveyed from Kyushu, Amami Oshima, Tokunoshima, Okinawa, and Ishigaki Islands mainly from May to July, when *P. japonensis* nymphs emerge (Table 1). Nymphs are relatively easy to find because they aggregate and feed on fallen drupes on the ground around the host tree of *P. japonensis*. However, adults aggregate on different evergreen plants and often enter into the soil for summer and winter hibernation, making it difficult to find adults. In addition, active dauer larvae of *C. japonica* are usually latched onto host nymphs and are easily washed out from the nymphs, while those on adults are slightly desiccated and inactive under the scutellum under the developed wings and between segments (Tanaka *et al.*, 2010; Yoshiga *et al.*, 2013; Yoshiga, personal observation), making it easier to collect the nematodes from insect nymphs than from adults.

Nematode sampling:

C. japonica individuals associated with *P. japonensis* were collected at each site where *P. japonensis* was observed as follows. Insect nymphs were placed individually inside a 2-ml centrifuge tube containing tap water and gently swirled for 10 to 20 seconds to detach the nematodes from their hosts. Nymphs were released soon after washing, and the

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tubes containing nematodes were then brought back to the laboratory, where the numbers of nematodes in the tubes were counted under a stereomicroscope. In the preliminary experiments, the efficacy of isolating nematodes from nymphs was confirmed (Yoshiga, unpublished data). This method effectively isolates nematodes from the insect without sacrificing the insects, which are especially rare in the southern parts of Japan. In total, 7 to 24 insect nymphs were collected from each sample (Table 1). To confirm the absence of the nematodes on nymphs in Okinawa, three nymphs and an additional two adult females of *P. japonensis* from Okinawa Island were individually dissected and soaked in tap water in a 6-cm petri dish. Some of the nematodes were grown on nematode growth medium (Sulston and Hodgkin, 1988) or dog food agar medium (Hara *et al.* 1981) pre-seeded with *Escherichia coli* OP50, and the nematode species were identified morphologically.

RESULTS AND DISCUSSION

Dauer larvae of *C. japonica* were collected from all nymphs obtained from all surveyed *P. japonensis* populations, with the exception of one nymph from Isen (Tokunoshima Island) and a mean of 9 to 18.2 dauer larvae per nymph (range, 0–45; $n = 120$) were collected per site from Kyushu, Amami Oshima, and Tokunoshima Islands (Table 1; Fig. 1). No nematodes, however, were isolated from either nymphs or adult females from two *P. japonensis* populations from Okinawa Island ($n = 64$). An additional *P. japonensis* survey was conducted at Ishigaki Island, located approximately 400 km west-southwest of Okinawa

Fig. 1. Distribution of *C. japonica*. The percentage of hosts carrying *C. japonica* in each *P. japonensis* population is indicated. n, number of insects examined.

Table 1. Sampling locality of *Parastrachia japonensis* and detection of *Caenorhabditis japonica*

Locality (City, Prefecture)	% of insects with nematodes (Number of insects examined)	Stage of Insect	Mean number of nematodes/insect (Range)	Date (D/M/Y)
Kanzaki, Saga	100 (21)	5th nymph	18.2 (12–26)	16/6/2012
Kurume, Fukuoka	100 (24)	5th nymph	no data	25/6/2018
Koshi, Kumamoto	100 (20)	5th nymph	no data	4/6/2012
Nobeoka, Miyazaki	100 (21)	5th nymph	9 (2–22)	21/6/2012
Kirishima, Kagoshima	100 (7)	5th nymph	16.1 (10–33)	6/7/2013
Tatsugo, Kagoshima	100 (7)	5th nymph	no data	25/6/2005
Isen, Kagoshima	95 (20)	5th nymph	14.9 (0–45)	23/6/2018
Kunigami, Okinawa	0 (20)	5th nymph	0	14/6/2014
Nago, Okinawa	0 (12)	2nd nymph	0	22/5/2013
Nago, Okinawa	0 (1)	Adult female	0	?/3/2013
Nago, Okinawa	0 (1)	Adult female	0	22/5/2013
Nago, Okinawa	0 (10)	5th nymph	0	18/6/2013
Nago, Okinawa	0 (20)	5th nymph	0	13/6/2014

Island, in June 2014. *P. japonensis* had previously been reported from Ishigaki Island (Matsunaga, unpublished data), but it was not found in the 2014 survey despite the presence of its host trees and drupes. There is no information available on the presence of *P. japonensis* in other regions of the Ryukyu Islands in Japan. *P. japonensis* had previously been reported from China, but no information on its current distribution in China is available.

In the previous study, *C. japonica* was found to have over three times less polymorphism than other outbreeding *Caenorhabditis* species, and its specialist host association is suggestive to contribute to the lower genetic variation (Li *et al.*, 2014). Here, *C. japonica* was only isolated from *P. japonensis* populations within a limited area: from the islands at latitudes between Tokunoshima and Kyushu Islands in Japan (Fig. 1). The limited distribution might also contribute to the lower genetic variation. Conversely, the possibility of the presence of *C. japonica* in areas west of Tokunoshima Island, such as Taiwan and China, cannot be excluded at this time, but its low nucleotide variation in the previous study might be indicative of its origin and a limited distribution of *C. japonica* in this region.

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