Exotic freshwater planarians currently known from Japan

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ABSTRACT. Biogeographical and taxonomic information on the four non-indigenous freshwater planarians of Japan is reviewed, viz. *Dugesia austroasiatica* Kawakatsu, 1985, *Girardia tigrina* (Girard, 1850), *G. dorotocephala* (Woodworth, 1897), and *Rhodax evelinae*? Marcus, 1947. The occurrence of *Girardia dorotocephala* in Japan is unequivocally demonstrated. New karyological data are presented for populations of *D. austroasiatica* (chromosome complement: 2x=16, 3x=24), *G. tigrina* (2x=16, 3x=24), and *G. dorotocephala* (2x=16). The following factors may have facilitated the introduction and subsequent geographical spread of exotic freshwater triclads in Japan: popularization of domestic tropical fish cultures, and culture of exotic aquatic animals for food.

KEY WORDS: Tricladida, freshwater, exotic, introduced, Japan

INTRODUCTION

The purpose of the present paper is to review the scattered information available in the literature on non-indigenous freshwater planarians in Japan, and to present new karyological and taxonomic data on some of these species. Furthermore, we discuss possible factors that may have facilitated the introduction and subsequent geographical spread of exotic freshwater triclads in Japan.

MATERIALS AND METHODS

Samples of four species of non-indigenous freshwater planarians were obtained from more than 20 stations (Fig. 1). Collection data for each station will be given below under the species accounts. Both living and preserved specimens were used for the morphological observations. External characters used for tentative identification of the species are shown in Kawakatsu et al. (2009, fig. 2; see also Kawakatsu et al. 2007b, c). For the preservation of planarians 70% ethanol was used in most cases, while Bouin's fluid was used in some cases. Serial sections were made at intervals of 7-8µm and were stained with Delafield's haematoxylin and erythrosin or Mallory-Cason. Reconstructions of the copulatory apparatus were obtained using a camera lucida attached to a compound microscope. Some of the histological material examined for this paper is deposited in the Zoological Museum Amsterdam (ZMA). Preparations of chromosomes were obtained by the squash method of Yamamoto: (1) animals were cut transversally at the basal level of the auricles and were kept for 2 days in a covered Petri dish filled with tap water; (2) the regenerating tissues were treated with a solution of 10^{-6} M colchicine for about 30 minutes; (3) tissues were soaked in 45% acetic acid prior to staining with aceto-orcein (cf. Oki et al. 1980).

SPECIES ACCOUNTS

Dugesia austroasiatica Kawakatsu, 1985

Material examined and distribution: Honshû: Stations 1, 3, 8, 12, 13, and 14 (the only naturalized population). Kyûshû: Station 7 (Fig.1).

Morphology. A rather small, slender, and pigmented species (ca. 12 mm long and 1.5 mm wide in large, sexually mature specimens) inhabiting warm waters. Head subtriangular with a pair of bluntly pointed auricles; with two eyes, each surrounded by a narrow non-pigmented ocular area; the distance between the pigment cups is slightly smaller than one-third of the width of the head at the level of eyes (Fig. 2) (cf. Kawakatsu et al. 2009).

Karyology. Idiograms of *D. austroasiatica* from the Saga locality (St. 7) and the Chiba (Isumi) locality (St. 8) were reported earlier (Fig. 3; cf. Kawakatsu et al. 1986, 1993a). Five new idiograms of populations from 3 localities (Stations 12, 13, and 14) are presented in Fig. 4.

Discussion. For additional data, including locality information, see Hirao et al. (1970) and Kawakatsu et al. 1985 (for stations 1 and 3); Kawakatsu et al. (1986b) (station 7); Kawakatsuet al. (1993a) (station 8); Kawakatsu et al. (2007c) (stations 12 and 13); Kawakatsu et al. (2007b, c) (station 14).

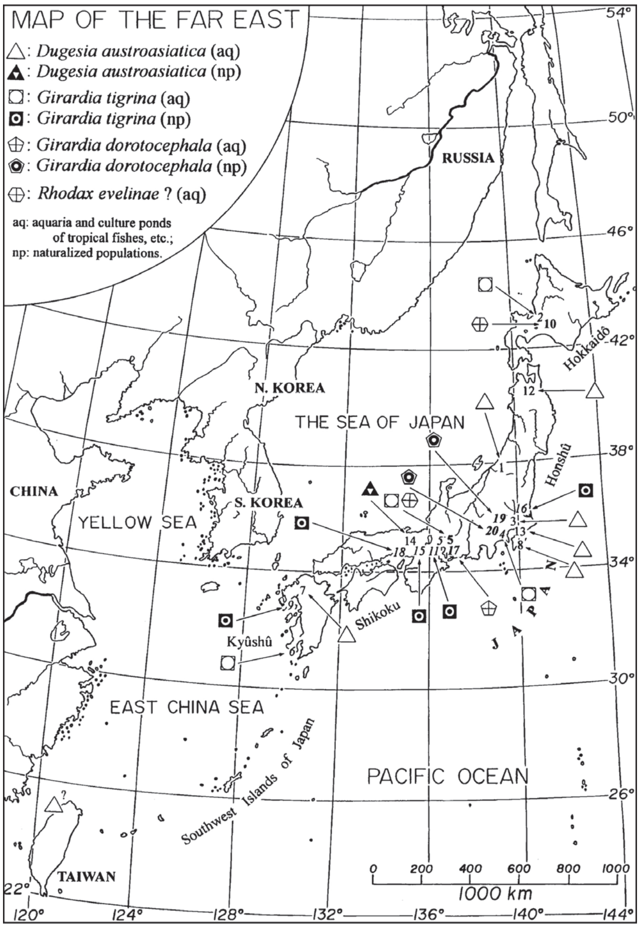

Fig. 1. – Map of the Japanese Islands, showing the geographical distribution of 4 species of exotic freshwater planarians from 20 stations:
1. Aquarium in Nîgata City; 2. Aquarium in Sapporo City; 3. Aquarium of Inokashira Park, Mitaka City; 4. Aquarium in Yokohama City; 5. Aquarium in Nagoya City; 6. Culture ponds of West Australian crayfish in Ibusuki City; 7. Culture ponds of tropical fishes in Saga City; 8. Domestic aquaria in Isumi City; 9. Urakami-gawa River, Nagasaki City; 10. An aquarium in Sapporo City; 11. Lake Biwa-ko in Moriyama City; 12. Domestic aquarium in Aomori City; 13. Domestic aquarium in Tôkyô; 14. Mizoro-ga-ike Pond in Kyôto City; 15. Kamo-gawa River in Kyôto City; 16. Shallow waters in lowland areas in the vicinity of Mitsukaidô City; 17. Tropical fish cultre tank of the Hekinan Sea Side Aquarium in Hekinan City; 18. Muko-gawa River in Amagasaki City; 19. Lower stream of the Asa-kawa River, a tributary of the Tama-gawa River, Hino City (3 localities); 20. Midstream of the Sagami-gawa River and its tributaries, Sagamihara City (3 localities).

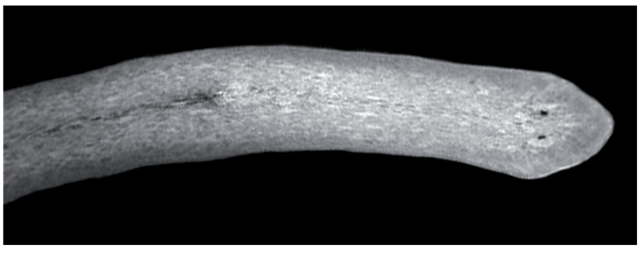

Fig. 2. – External features of *Dugesia austroasiatica.*

Girardia tigrina (Girard, 1850)

Material examined and distribution: Hokkaidô: Station 2. Honshû: Stations 4, 5, 11, 15, 16, and18. Kyûshû: Stations 6 and 9 (Fig. 1).

Naturalized populations concern Stations 11, 15, 16, 18, and 9. Recently, a new naturalized population of *G. tigrina* was found in Kagamihara City (Gifu Pref.), about 50 km N of Station 5.

Morphology. Living, asexual specimens are approximately 10 mm long and 1 mm wide. Head equilateral-triangular, with a pair of broad, short auricles. Two eyes are conspicuous, each surrounded by a large, non-pigmented ocular area. The distance between the eyes is 1/4th-1/5th of the width of the head at level of the auricles. Ground colour of the dorsal surface pale brown with numerous whitish and yellowish pigment spots. Small masses of irregularly arranged blackish and yellowish brown pigment granules are conspicuous on the surface of the pharynx (Fig. 5).

Since sexual specimens of this *Girardia* species were not available, its tentative identification is based only on external features (cf. Kawakatsu et al. 2009). However, external morphology of the *Girardia* species from Japan is very similar to that of specimens of *G. tigrina* from the U.S.A., Mexico, Brazil, and Uruguay (cf. Kawakatsu et al. 1981b, 1982, 1983, 1986a, 1992).

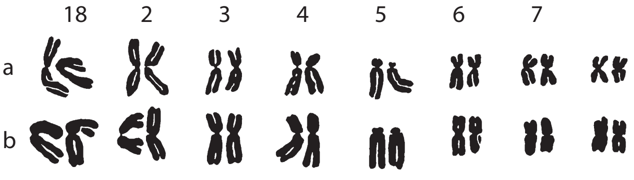

Fig. 3. – *Dugesia austroasiatica*. Two idiograms of population from Station 8, as reported earlier (after Kawakatsu et al., 1993a). a and b: 2x = 16, with a karyotype of 2m + 2m + 2m + 2m/2sm + 2st + 2m + 2m +2m.

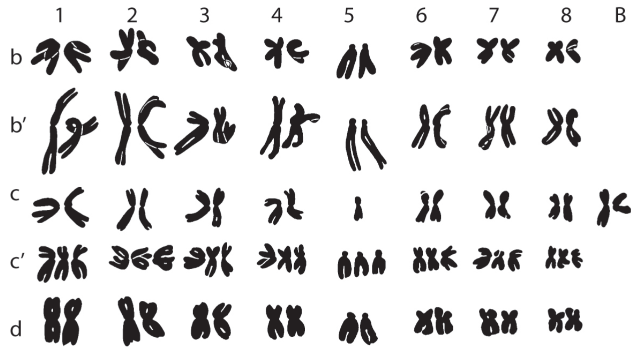

Fig. 4. – *Dugesia austroasiatica*. Five new idiograms of populations from Stations 12 (b and b'), 13 (c and c'), and 14 (d); b and b' and c and c' found in somatic cells of a single specimen, respectively.
b and b': 2x = 16, with a karyotype of 2m + 2m + 2m + 2m/2sm + 2st + 2m + 2m +2m. c: 2x-1 + 2LB = 16-1 + 2LB, with a karyotype of 2m + 2m + 2m + 2m + 1st + 2m + 2m + 2m + 2LB. c': 3x=24, with a karyotype of 3m + 3m + 3m + 3m + 3st + 3m + 3m +3 m. d: 2x=16, with a karyotype of 2m+2m+2m+2m+2st+2m+2m+2m.

Karyology. Three new idiograms of *G. tigrina* from Lake Biwa-ko (St. 11) are given in Fig. 6. The diploid karyotype is 2x=16, with 8 pairs of metacentric chromosomes. Triploid karyotypes also occurred. Usually, diploid and triploid karyotypes were found in different specimens.

Judging from the idiograms of *G. tigrina* from Japan published earlier, the 6th (or the 7th) pair of chromosomes were submetacentric in some cases, in contrast to the results published in the present paper (cf. Kawakatsu et al. 1985, 1993b, 2007b, 2008; Tamura et al. 1985; Oki et al. 1995; Chinone et al. 2008). A similar situation was encountered in Brazilian *G. tigrina* (Kawakatsu et al. 1981b, 1986; see also Benya et al. 2007). Italian *G. tigrina* has a karyotype of 7 pairs of metacentric chromosomes and one pair of submetacentric chromosomes (Fig. 7) (Benazzi 1970).

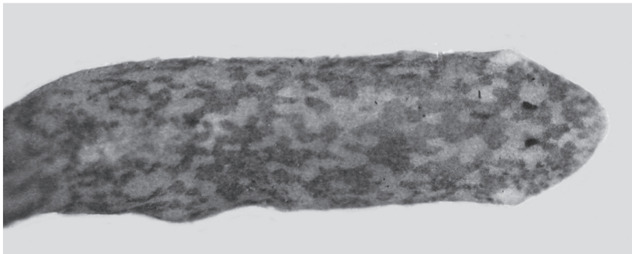

Fig. 5. – External features of *Girardia tigrina*.

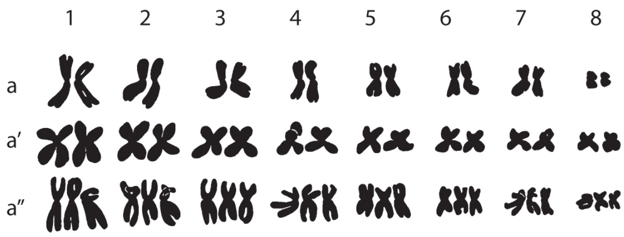

Fig. 6. – *Girardia tigrina*. Three new idiograms of population from Lake Biwa-ko (Station 11). a and a': 2x = 16, with a karyotype of 2m + 2m + 2m + 2m + 2m + 2m + 2m + 2m. a'': 3x=24, with a karyotype of 3m + 3m + 3m + 3m + 3m + 3m + 3m + 3m. Diploid and triploid karyotypes were found in different specimens.

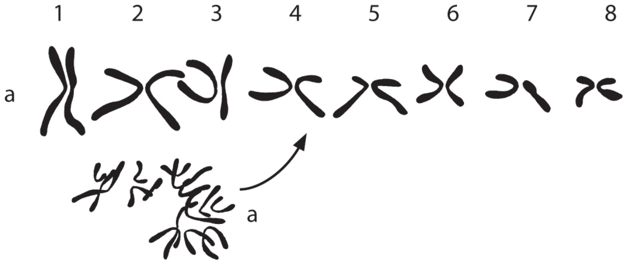

Fig. 7. – Metaphasic plates and idiograms of *Girardia tigrina* from Turin, Italy (idiograms reconstructed by Kawakatsu, based on data in Benazzi 1970).

Discussion. For the distribution of *G. tigrina*, Kenk (1974: 28) wrote: "North America, Mexico, Brazil, also introduced to Europe (widely distributed, including the British Isles), Israel, and Japan". Later, *G. tigrina* was reported from several localities in Brazil, Uruguay, and additional localities in Japan (Kawakatsu et al. 1981a, b, 1982, 1983, 1985, 1986a, 2007b, c, 2008; Chinone et al., 2008). For additional data, including locality information, see Hirao et al. (1970), Kawakatsu et al. (2007c) (station 2); Kawakatsu et al. (1985) (stations 4 and 5); Tamura et al. (1985) (station 6); Kawakatsu et al. (1993b) (station 9); Nishino et al. (2002) (station 11); Kawakatsu et al. (2007b, c) (staion 15); Chinone et al. (2008) (station 16); Tanaka (2008) (station 18).

Girardia dorotocephala (Woodworth, 1897)

Material examined and distribution: Honshû: Stations 17, 19 (3 localities), and 20 (3 localities) (Fig. 1). Naturalized populations occur at stations 19 and 20.

Morphology. Living sexually mature specimens from the Hino (Station 19) and Sagamihara (Station 20) populations measure 12 -18 mm x 1.5 -2.5 mm. The large head is of a broad, triangular form with a pair of long and pointed tentacles. Two eyes, each surrounded by conspicuous, non-pigmented ocular areas, are located slightly anterior to the level of the base of the auricles (Kawakatsu et al. 2007b, 2008, 2009).

Ground colour of the dorsal surface uniform brown, with the central region of the body being uniformly grey, interspersed with many small, clear spots (Fig. 8).

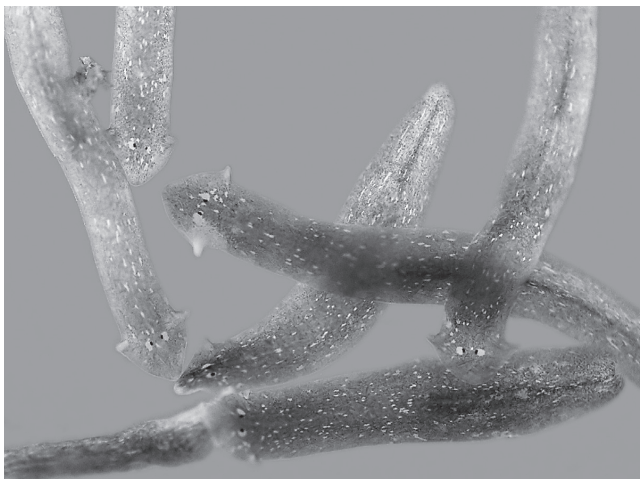

Fig. 8. – External features of *Girardia dorotocephala*.

The pharynx shows a brownish pigmentation; its outer musculature is provided with a third, extra layer of longitudinal muscle fibres, a feature that is characteristic for this species (Kawakatsu & Mitchell 1981). Testes ventral, throughout the body. For copulatory apparatus, see Fig. 9. Specimens from Japan have a short, conical and symmetrical penis papilla and a large, spherical penis bulb with spacious bulbar cavities.

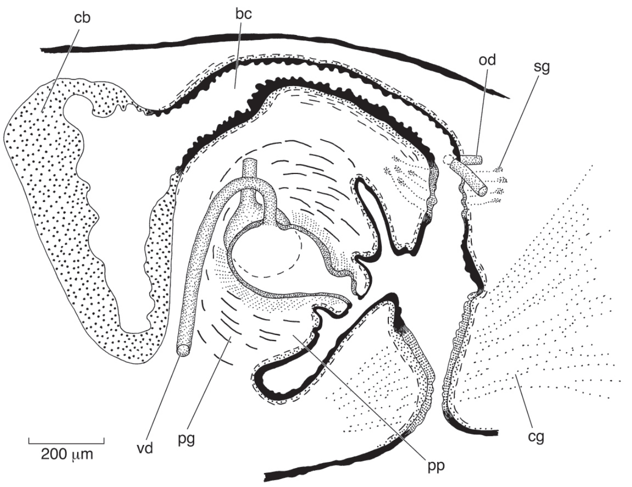

Fig. 9. – *Girardia dorotocephala.* ZMA V.Pl. 6813.1 (Station 20). Sagittal reconstruction of the copulatory apparatus. Abbreviations: bc, bursal canal; cb, copulatory bursa; cg, cement glands; od, oviduct; pg, penis glands; pp, penis papilla; sg, shell glands; vd, vas deferens.

Karyology. Three sets of idiograms of *G. dorotocephala* from three localities in Japan are given in Fig. 10. The karyotype of *G. dorotocephala* is 2x=16, with 8 pairs of metacentric chromosomes (Kawakatsu et al. 2007b, 2008). The diploid karyotype of *G. dorotocephala* resembles that of *G. tigrina*.

G. dorotocephala specimens from South Virginia, U.S.A. showed a diploid karyotype of 6 pairs of metacentric chromosomes and 2 pairs of submetacentric elements (Fig. 11) (Benazzi 1970).

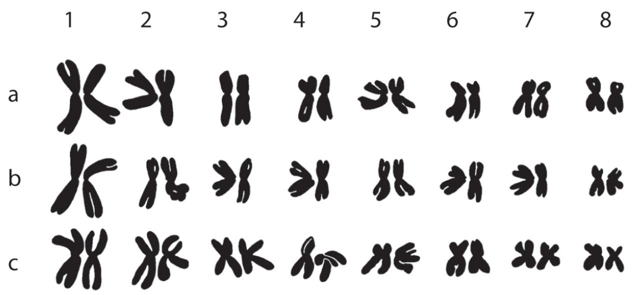

Fig. 10. – *Girardia dorotocephala.* Three idiograms for populations from Stations 17 (a), 19 (b), and 20 (c). a: 2x=16, with a karyotype of 2m + 2m + 2m + 2m + 2m + 2sm + 2sm + 2m. b and c: 2x=16, with a karyotype of 2m + 2m + 2m + 2m + 2m + 2m + 2m + 2m.

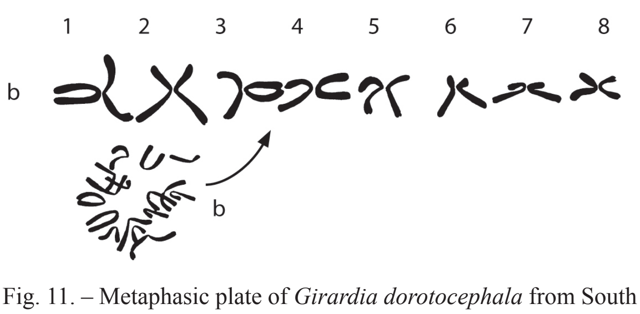

Fig. 11. – Metaphasic plate of *Girardia dorotocephala* from South Virginia, U.S.A. (idiograms reconstructed by Kawakatsu, based on data in BENAZZI 1970).

Discussion. The histological and morphological characteristics of the Japanese animals are in conformity with those of the North American animals (cf. Kawakatsu & Mitchell 1981). The native distribution of *G. dorotocephala* concerns North America, southern Canada, and Mexico (Kenk 1974). The species has been introduced into Hawaii (Kawakatsu et al. 1984). The occurrence of this North American species in Japan was noted promptly by Kawakatsu et al. (2007b, 2008). The present paper supports these earlier and preliminary identifications by providing a reconstruction of the copulatory apparatus of the Japanese representatives of *G. dorotocephala*. For additional data, including locality information, see Kawakatsu et al. 2007b, c, 2008) (station 17).

Rhodax evelinae? Marcus, 1946

Material examined and distribution: *R. evelinae*? has been reported from two stations (Fig. 1). Honshû: Nagoya City (Station 5). Hokkaidô; Sapporo City (Station 10) (cf. Kawakatsu et al. 1985, 1995).

Morphology. Living, asexual specimens of the Nagoya (Station 5) and Sapporo (Station 10) populations measure 3-5 mm x 0.4-0.5 mm. Head rounded, with a gentle swelling on either side. Eyes situated close together, each surrounded by a non-pigmented ocular area. Ground colour of the dorsal surface pale brown. Numerous, small, darkish pigments present at the pharyngeal region. Ventral surface pale. A slightly thi-ckened adhesive region present at the anteroventral end of the body (Fig. 12).

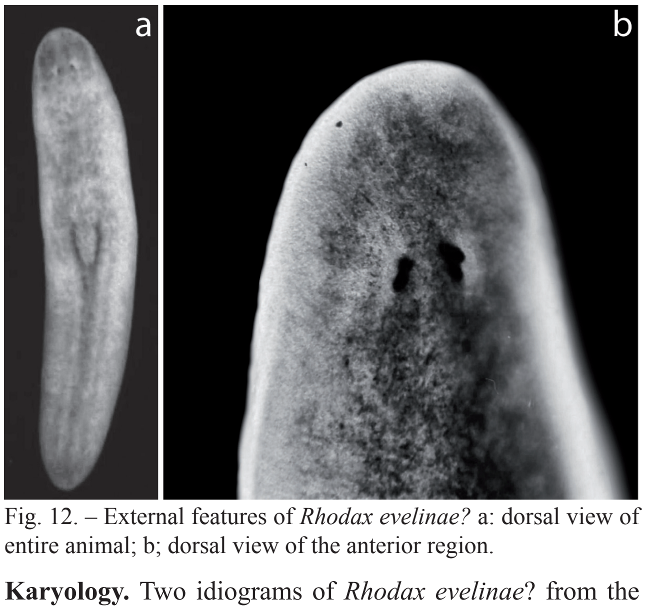

Fig. 12. – External features of *Rhodax evelinae?* a: dorsal view of entire animal; b; dorsal view of the anterior region.

Karyology. Two idiograms of *Rhodax evelinae*? from the Sapporo locality are shown in Fig. 13. The karyotype of asexual specimens of *Rhodax evelinae*? is 3x=24, with four pairs of metacentric chromosomes (1st, 2nd, 3rd, 6th) and four pairs of submetacentric chromosomes (4th, 5th, 7th, 8th pairs). A few B-chromosomes are also present. Idiograms **a** and **a'** concern two different somatic cells of a single specimen. see

Fig. 13. – *Rhodax evelinae*? Two idiograms of a population from Station 10 (a and a'), as reported by Kawakatsu et al. 1995); a and a' were found in somatic cells of a single specimen. a: 3x=24, with a karyotype of 3m + 3m + 3m + 3sm + 3sm + 3m + 3sm + 3sm. a': 3x + 1LB + 1SB =25 + 1SB, with a karyotype of 3m + 3m + 3m + 3sm + 3sm + 3m + 3sm + 3sm + 1LB + 1SB.

For idiograms of *R. evelinae*? from the Nagoya population, Kawakatsu et al. (1985) and Oki et al. (1995).

Discussion. *Rhodax evelinae*? was found only in aquaria for tropical fish culture (water temperature ≥ 20° C). In Brazil *Rhodax evelinae* Marcus, 1946 inhabits dirty ponds, rivers and clear-water brooks near the city of São Paulo (Marcus 1946). Recently, immature or asexual *Rhodax* sp. specimens have been found also throughout Rio Grande do Sul, southern Brazil, mainly in drainage ditches of rice fields but also in other types of wetlands. (A. M. Leal-Zanchet, pers. com.).

For additional data, including locality information, see Kawakatsu et al. (1985) (station 5) and Kawakatsu et al. (1995) (station 10).

GENERAL DISCUSSION

The following factors may have facilitated the introduction and subsequent geographical spread of exotic freshwater triclads in Japan:

(1) Popularization of domestic tropical fish cultures

After the 1960's various kinds of tropical fishes and aquatic plants have been imported increasingly in Japan from regions such as Hong Kong, Singapore, Australia, Europe (Germany and The Netherlands), South and East Africa, North America, South America (especially Peru and Brazil; cf. Kawakatsu et al. 1995). Various exotic species of shrimps, crabs, crayfishes, newts, frogs, and tortoises are now common in tropical fish stores and pet shops in Japan. Those freshwater pet organisms were frequently discharged into Japanese waters, where they now run out of control (artificial removal of exotic harmful organisms is prohibited by the Invasive Alien Species Act) (cf. Kawakatsu et al. 2007b, c, 2008).

The naturalized population of *D. austroasiatica* is recorded only from Kyôto (Mizoro-ga-ike Pond) in Central Japan. Since this presumably Southeast Asian species is an inhabitant of warm waters, an increase of its naturalized populations in Japan is expected to be minimal. In contrast, the naturalized populations of the two North American *Girardia* species, *G. tigrina* and *G. dorotocephala*, may increase rapidly in water systems of lowland areas in Southern and Central Japan. Under natural conditions in Southern and Central Japan these animals may propagate asexually by fission (*G. tigrina*) or sexually by cocoon-laying (*G. dorotocephala*). The collector of *G. dorotocephala* at the Hino locality observed many cocoons of this species in the field (cf. Kawakatsu et al. 2008).

(2) Culture of exotic aquatic animals as food

Case 1. In the middle of 1984 many specimens of *Dugesia austroasiatica* were collected from culture ponds of Tilapia fishes (*Oreochromis niloticus* (Linnaeus, 1758) from Africa) in Saga City, Kyûshû (St. 7) (cf. Kawakatsu et al. 1986b). Water temperature was ≥15°C, even in mid-winter. Many specimens of exotic planarians were attached to roots of the water hyacinth [*Eichhornia crassipes* (Martius) Solm, 1883] grown in these culture ponds.

Case 2. Cherax tenuimanus (Smith, 1912), an edible West Australian crayfish, was cultured at the Ibusuki Branch of the Kagoshima Prefectural Fisheries Experimental Station, Ibusuki City, Kyûshû. The culture ponds were fed by underground water, with temperatures ≥15°C, even in mid-winter. In the spring of 1985 many specimens of *G. tigrina* were collected from the body surface of a host crayfish (Station 6; cf. Tamura et al. 1985; Oki et al. 1995). Many specimens of *Temnosewellia minor* (Haswell, 1887) were also found on the body surface and gill chamber of these crayfishes(cf. Tamura et al. 1985; Kawakatsu et al. 2007a).

(3) Importation of live specimens of Chinese freshwater shrimps

Niwa & Ohtaka (2006) reported the occurrence of many specimens of the Chinese symbiotic branchiobdellidan, *Holtodrilus truncatus* (Liang, 1963) on shrimps from the Sugo River (Yumesaki River system), Honshû, Central Japan since 2003. The authors considered these shrimps (probably *Caridina denticulata* (De Haan, 1849) or a closely related species) to have been introduced from China for use as live baits in recreational fishing. A temnocephlid species, *Scutariella japonica* (Matjašič, 1990), has been recorded from Japanese shrimps [*Caridina denticulata* (De Haan, 1849)] at the same locality of *H. truncatus* in the Sugo River. At present, exotic planarians have not been recorded from the Sugo River locality. However, this kind of commercial activity may potentially accelerate the arrival of exotic freshwater planarians in Japan.

ACKNOWLEDGEMENTS

Kawakatsu and Yamamoto are indebted to the following Japanese lady and gentlemen for supplying the live and preserved planarians used in our chromosomal and taxonomic studies: Dr. Machiko Nishino (Ôtsu), Dr. Akifumi Ohtaka (Hirosaki), Dr. Shigeo Chinone (Iwai), Messers. Gen-Yû Sasaki (Tôkyô), Hitoshi Murayama (Nagaoka), Motoyasu Masuda (Hekinan), Yôichi Kimura (Aomori), Daisaburô Tsuruda (Hino), and Tomoyuki Kimura (Sagamihara). Mr. J. van Arkel (University of Amsterdam) is thanked for the digital rendering of the figures.

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