

**Redescription of *Heterobothrium tetrodonis* (Goto, 1894)
(Monogenea: Diclidophoridae) and Other Related New Species from
Puffers of the Genus *Takifugu* (Teleostei: Tetraodontidae)**

KAZUO OGAWA

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Abstract

Heterobothrium tetrodonis (Goto, 1894) Cerfontaine, 1895 (Monogenea: Diclidophoridae) is redescribed from specimens newly found on the gills of puffer *Takifugu pardalis* in Japan. Three closely related new species are also described: *H. okamotoi* sp. nov. from the branchial cavity wall of *T. rubripes*; *H. yamagutii* sp. nov. from the gills of *T. xanthopterus*; and *H. shinagawai* sp. nov. from the gills of *T. xanthopterus*, all in Japan. They are compared with their most similar species. *H. bychowskyi* nom. nov. is proposed for *H. tetrodonis* sensu Bychowsky, Mamaev et Nagibina, 1976 from the gills of *T. alboplumbeus* and *T.* sp. from the Yellow Sea.

Key words: monogeneans, *Heterobothrium*, taxonomy, new species, *Takifugu*

Goto (1894) described a new species of monogenean *Diclidophora tetrodonis*, now *Heterobothrium tetrodonis* (Goto, 1894) Cerfontaine, 1895 in the family Diclidophoridae Cerfontaine, 1895, from the gills of puffers “*Tetrodon*” spp. (“Kogome”-fugu and “Kōyose”-fugu given as local names) from Hagi, western Honshu, on the coast of the Sea of Japan. Since then, however, taxonomy of *H. tetrodonis* has been complicated and confused, partly because of the difficulty in specifying the host puffers in his paper and partly because of his simplified description.

The name “Kōyose”-fugu is commonly used for *Takifugu pardalis* (standard Japanese name: Higan-fugu), but it is not certain what the scientific name is for “Kogome”-fugu, the name applied only in Hagi district. Local people there used to call coarsely ground rice “Kogome”, the material for rice cake, and accordingly, “Kogome”-fugu is considered to refer to a puffer with “Kogome”-like small white spots on the body. This external feature of “Kogome”-fugu

and the geographical distribution of Japanese puffers suggest that “Kogome”-fugu is either *T. poecilnotus* (standard Japanese name: Komon-fugu), *T. vermicularis* (Shosai-fugu) or *T. niphobles* (Kusa-fugu). No redescription has so far been made of *H. tetrodonis* collected from these original hosts.

Goto’s original description is simple without measurements except for body length. This makes it difficult to compare it with the redescription of the parasite as *H. tetrodonis* on the branchial cavity wall of *T. rubripes* from the Inland Sea of Japan (Okamoto, 1963), on the gills of *Spheroides xanthopterus* (= *T. x.*) from Sagami Bay, Japan (Yamaguti, 1958) and on the gills of *S. alboplumbeus* (= *T. a.*) and *Spheroides* sp. (= *Takifugu* sp.) from the Yellow Sea (Bychowsky *et al.*, 1976). They differ from one another in morphology and/or site of infection on the hosts.

This paper deals with a redescription of *H. tetrodonis* and three other related new species of *Heterobothrium* on the basis of specimens newly obtained from *Takifugu* spp. and discusses taxonomy of the “*H. tetrodonis* species group”.

Department of Fisheries, Faculty of Agriculture, The University of Tokyo, Yayoi, Bunkyo-ku, Tokyo 113, Japan.

小川和夫 (東京大学農学部水産学科)

Materials and Methods

Puffers of the genus *Takifugu* (Teleostei: Tetraodontidae) from more than ten different localities in Japan were examined for heterobothriid monogeneans during 1986–1991. Freshly collected monogeneans were flattened between a cover slip and a slide glass, fixed with AFA or 70% alcohol, stained with Heidenhain's iron hematoxylin or alum carmine, and mounted in Canada balsam.

Measurements are given in millimeters. Figures were drawn with the aid of a camera lucida. All the parasite specimens are deposited at the Meguro Parasitological Museum (MPM), Tokyo.

Results

Heterobothrium tetrodonis (Goto, 1894)
Cerfontaine, 1895 (Fig. 1)

Synonym. *Diclidophora tetrodonis* Goto, 1894, pp. 213–215, pl. X, figs. 1–4
nec *H. tetrodonis* sensu Yamaguti, 1958, p. 79
nec *H. tetrodonis* sensu Okamoto, 1963, pp. 22–25, figs. 6–8; Okamoto et Ogasawara, 1965, pp. 42–43.
nec *H. tetrodonis* sensu Bychowsky, Mamaev et Nagibina, 1976, pp. 32–33, fig. 1

Host and site of infection. On the gills of *Takifugu pardalis* (standard Japanese name: Higan-fugu).

Localities, dates of collection and specimens. Hagi, Yamaguchi Prefecture, Japan, May 1986 (one mature specimen, neotype, M.P.M. Coll. No. 19553); Misaki, Kanagawa Pref., March 1989 (one mature specimen, No. 19554); Iki Island, Nagasaki Pref., June, 1989 (four mature specimens, No. 19555).

Redescription. Body 14.3–17.8 long with maximum width of 3.35–4.58 at level of ovary. Body proper fusiform, separated from opisthohaptor by a long isthmus which is 2.4–4.8 long or 17–27% of the body length. Opisthohaptor 2.1–2.7 long by 2.2–3.4 wide, provided with four pairs of short-stalked clamps which are uniform in skeleton. Anterior jaw of clamp con-

sisting of an inverted U-shaped peripheral sclerite with inwardly curved distal ends and a median sclerite, both fused with each other to form a continuous skeleton. Median sclerite curved almost at a right angle midway, fused distally with arm of peripheral sclerite and proximally with curved distal end of peripheral sclerite to form a loop. Loop accompanied with a slightly sclerotized membrane. Posterior jaw composed of two pairs of peripheral sclerites and a median sclerite. Muscular pads lying along peripheral sclerites. 1st pair of clamps (posteriormost) 0.22–0.30 long by 0.23–0.27 wide; 2nd 0.29–0.39 long by 0.28–0.33 wide; 3rd 0.33–0.39 long by 0.31–0.34 wide; 4th, rotated 180° from the other pairs, 0.30–0.39 long by 0.26–0.32 wide.

Mouth opening subterminally. A pair of oral suckers 0.214–0.247 long by 0.132–0.167 wide, folded in middle apparently with a clamping function, situated on both sides of mouth cavity. Pharynx elliptical, 0.270–0.400 long by 0.194–0.275 wide. Esophagus short, bifurcating at level of genital pore. Intestines with numerous branches on both sides, descending and entering into opisthohaptor.

Testes 34–56 in number, postovarian in interintestinal field. Vas deferens ascending in median field, dorsal to uterus, leading into ejaculatory bulb of 0.105–0.153 long by 0.090–0.125 wide. Copulatory organ muscular, 0.150–0.165 in diameter, provided with a corona of 10–13 (usually 12) hooks.

Ovary winding with its distal end directed backward, situated at middle of body. Oviduct descending obliquely to left, branching off genito-intestinal canal, and after joining with vitelline duct, turning its direction anteriorly, leading into ootype, which is situated in the space between the ovary and testes. Uterus ascending in median line, leading into genital atrium. Vagina and receptaculum seminis absent. Vitellaria extending from level of genital pore to posterior end of testes. Eggs 0.160–0.210 long by 0.070–0.094 wide with a long filament of about 1 long at both ends.

Remarks. This redescription coincides quite well with Goto's description in main specific features (body shape, position of testes, etc.). A

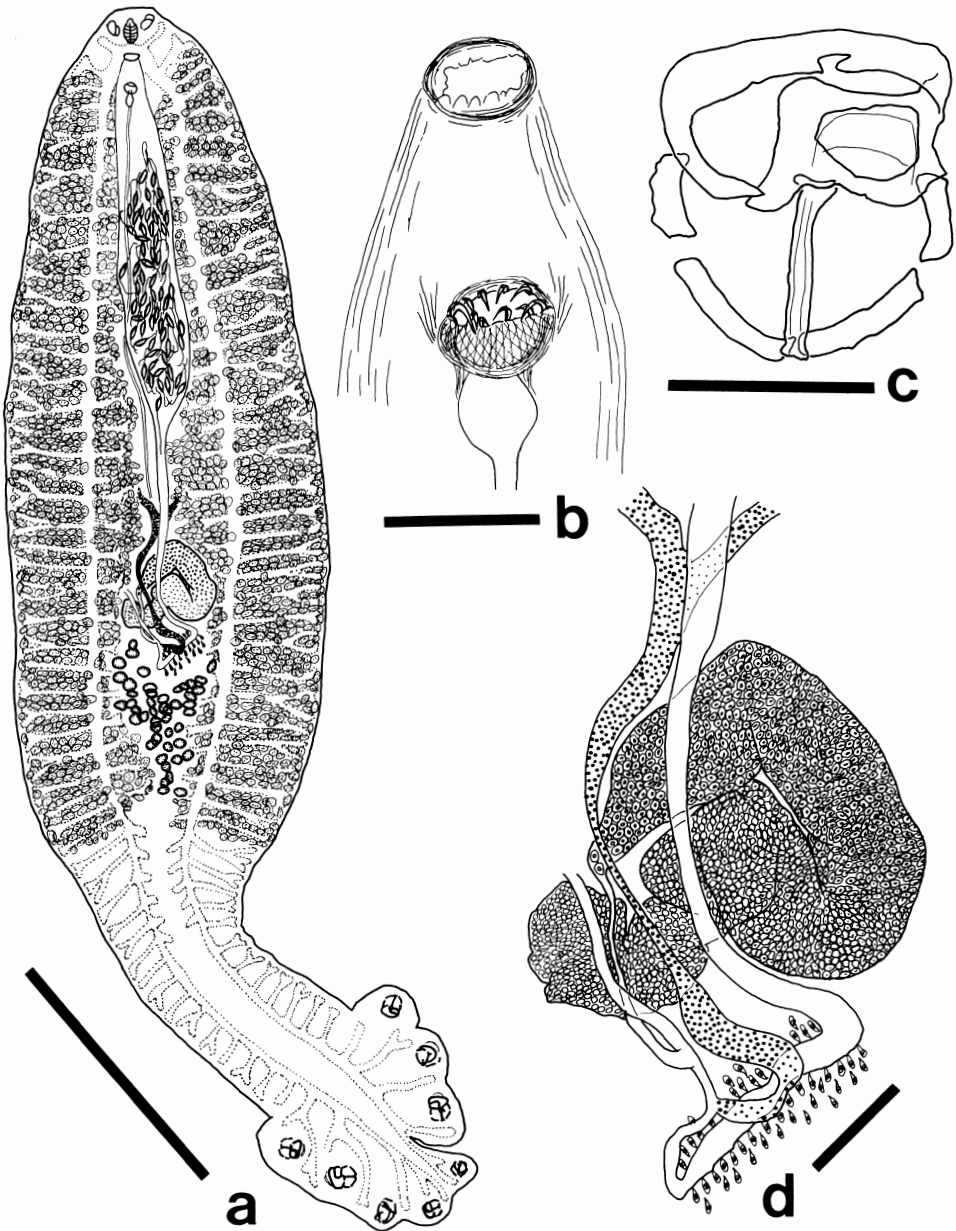


Fig. 1 *Heterobothrium tetradonis* (Goto, 1894) Cerfontaine, 1895, neotype, from *Takifugu pardalis*. a, entire worm, ventral view (scale: 3 mm); b, copulatory organ and genital opening, ventral view (scale: 0.2 mm); c, clamp (scale: 0.2 mm); d, ovarian complex, ventral view (scale: 0.3 mm).

slight difference might be found in the number of testes: 34–56 in the former vs. 29 in the latter (counted from Goto's figure). However, it would show the range of specific variation in the number of testes, because the former includes a specimen

on the same host species from the same locality as the latter. Since the type specimens must have been lost, the specimen (M.P.M. Coll. No. 19553) collected from *T. pardalis*, one of the probable original hosts, at Hagi, Yamaguchi

Pref., the type locality, is designated as the neotype.

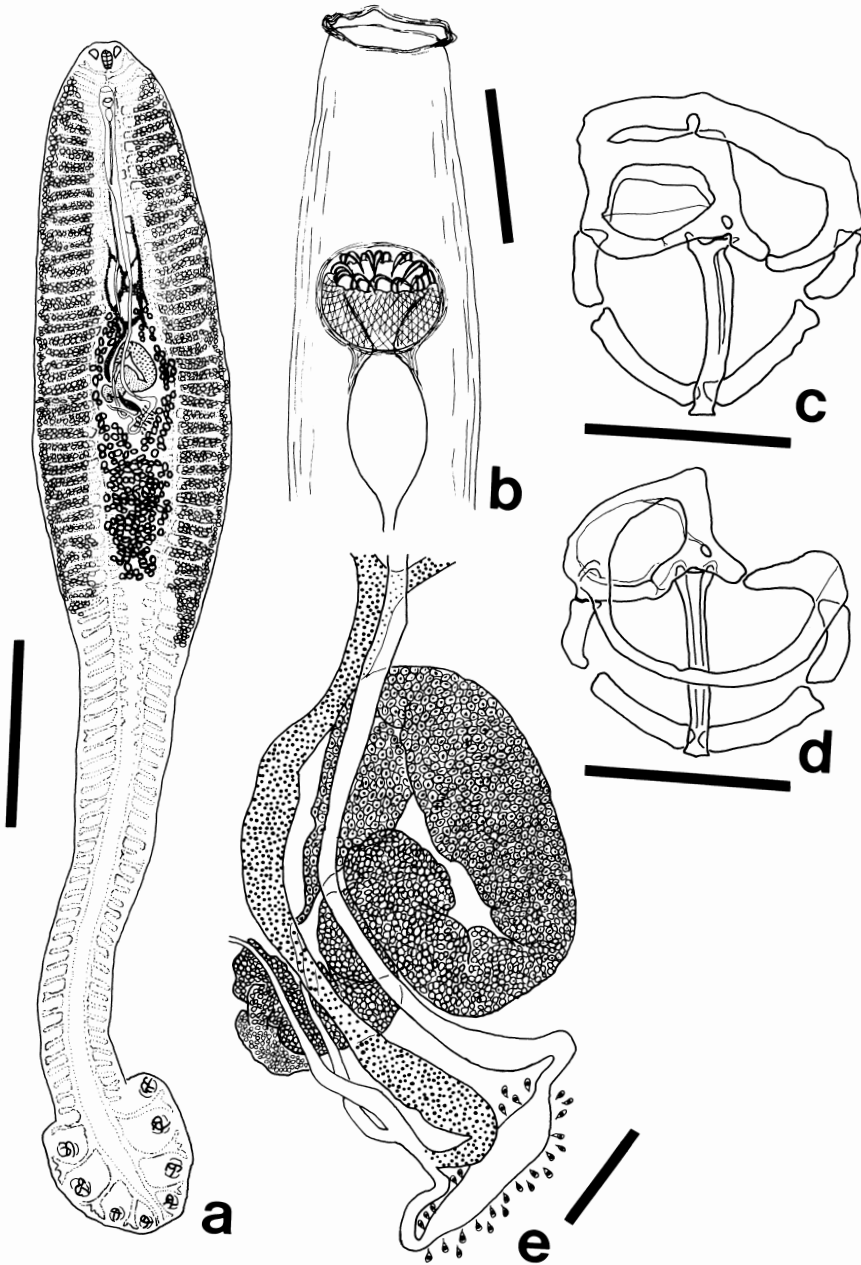


Fig. 2 *Heterobothrium okamotoi* sp. nov. from *Takifugu rubripes*. a, entire worm, holotype, ventral view (scale: 3 mm); b, copulatory organ and genital opening, paratype, ventral view (scale: 0.2 mm); c, "open" clamp, paratype (scale: 0.2 mm); d, "closed" clamp, paratype (scale: 0.2 mm); e, ovarian complex, holotype, ventral view (scale: 0.3mm).

Heterobothrium okamotoi sp. nov.
(Fig. 2)

Synonym. *Heterobothrium tetrodonis* sensu Okamoto, 1963, pp. 22–25, figs. 6–8; Okamoto et Ogasawara, 1965, pp. 42–43 (nec Goto, 1894)

Host and site of infection. On the branchial cavity wall of *Takifugu rubripes* (standard Japanese name: Tora-fugu).

Locality, dates of collection and specimens. Naru Island, Nagasaki Pref., Japan, Dec. 1985 and May 1987 (holotype and 19 paratypes, mature, M.P.M. Coll. No. 19556).

Description. Body fusiform, 12.4–23.1 long with maximum width of 2.0–4.2 at level of ovary or ootype. Isthmus between body proper and opisthohaptor long, 3.82–8.60 long or 25–40% of body length. Opisthohaptor 1.5–3.2 long by 1.9–2.9 wide. Clamp skeleton as in *H. tetrodonis*, usually in open state. 1st pair of clamps 0.26–0.29 long by 0.20–0.26 wide; 2nd 0.30–0.37 long by 0.25–0.34 wide; 3rd, largest, 0.33–0.41 long by 0.25–0.33 wide; 4th, 180° rotated from the other pairs, 0.30–0.36 long by 0.23–0.29 wide.

Mouth opening subterminally. Oral suckers 0.217–0.258 long by 0.143–0.184 wide on both sides of mouth cavity. Pharynx 0.312–0.428 long by 0.237–0.300 wide. Esophagus short, bifurcating at level of genital pore. Intestine with numerous side branches, extending to opisthohaptor.

Testes 150–240 in number, distributed in front of ovary to well behind postovarian region in interintestinal field. Ejaculatory bulb 0.140–0.241 long by 0.092–0.142 wide. Copulatory organ 0.169–0.198 in diameter, with 11–13 (usually 12) hooks.

Ovary about anterior one-third of body. Ovarian complex similar to that of *H. tetrodonis*. Vitellaria extending from level of genital pore to a little behind testes.

Remarks. This species is different from the most similar *H. tetrodonis* sensu stricto in more testes and their distribution extending anterior to the ovary. It was observed that immature worms moved from the gills to the branchial cavity wall

for maturation (Ogawa and Inouye, unpublished). The opisthohaptor and part of the isthmus was usually embedded in the wall, provoking a marked host reaction at the attachment site.

The specific name, *okamotoi*, has been taken in honor of the late Dr. T. Okamoto, who studied in detail the parasitic disease of cultured tiger puffer (*Tora-fugu*) caused by this species.

Heterobothrium yamagutii sp. nov.
(Fig. 3)

Synonyms. *Heterobothrium tetrodonis* sensu Yamaguti, 1958, p. 79 (nec Goto, 1894)
Heterobothrium sp. Bychowsky, Mamaev et Nagibina, 1976, p. 34, fig. 2

Host and site of infection. On the gills of *Takifugu xanthopterus* (standard Japanese name: Shima-fugu)

Locality, date of collection and specimens. Mogi, Nagasaki Pref., May 1991 (holotype and nine paratypes, mature, M.P.M. Coll. No. 19557).

Description. Body slender, 7.4–14.4 long with maximum width of 2.1–3.5 at level of ovary. No distinct isthmus present. Opisthohaptor 1.8–2.6 long by 2.3–3.2 wide, with four pairs of short-stalked clamps of almost same size; 1st pair of clamps 0.45–0.54 long by 0.31–0.42 wide; 2nd, 0.49–0.53 long by 0.36–0.46 wide; 3rd, 0.45–0.55 long by 0.35–0.47 wide; 4th, 180° rotated from the other pairs, 0.44–0.55 long by 0.32–0.44 wide.

Mouth opening subterminally. A pair of oral suckers 0.285–0.320 long by 0.190–0.250 wide on both sides of mouth cavity. Pharynx barrel-shaped, 0.310–0.355 long by 0.295–0.315 wide. Esophagus short, soon bifurcating anterior to genital pore. Intestines with numerous branches on both sides, reaching opisthohaptor.

Testes 100–160 in number, in interintestinal field from level of ovary to near end of body proper. Ejaculatory bulb transversely wide, 0.120–0.150 × 0.130–0.210 in size. Copulatory organ 0.126–0.155 in diameter, armed with a corona of 10–12 hooks.

Ovary bending deeply in middle, situated in

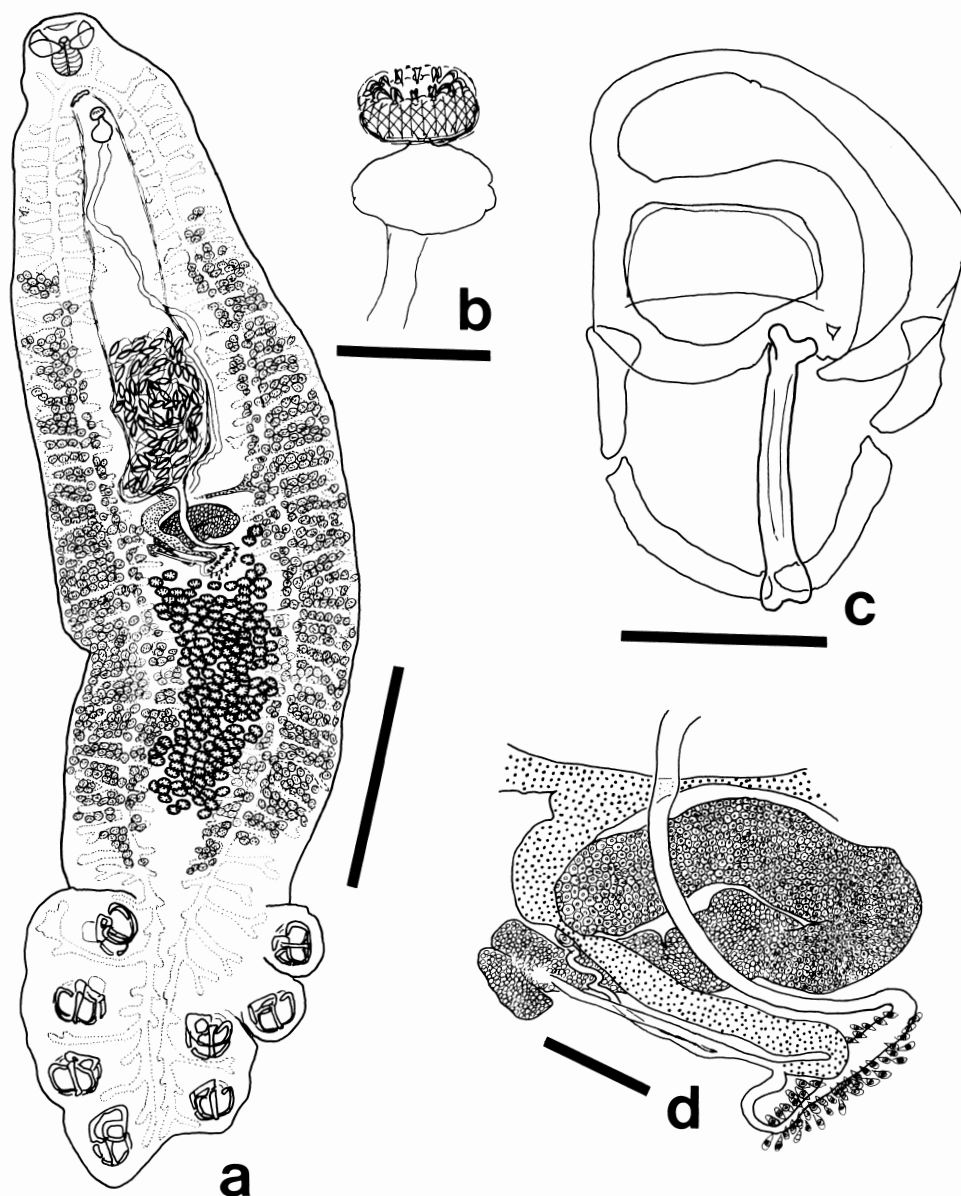


Fig. 3 *Heterobothrium yamagutii* sp. nov. from *Takifugu xanthopterus*. a, entire worm, holotype, ventral view (scale: 2 mm); b, copulatory organ, paratype, ventral view (scale: 0.2 mm); c, clamp, paratype (scale: 0.2 mm); d, ovarian complex, paratype, ventral view (scale: 0.3 mm).

central body. Vagina and receptaculum seminis absent. Vitellaria distributed on both sides of body from well behind genital pore to end of body proper.

Remarks. This species is easily distinguishable from *H. tetrodonis* sensu stricto and *H.*

okamotoi sp. nov. by the absence of isthmus between the body proper and the opisthohaptor. It most resembles *H. tonkinense* Bychowsky, Mamaev et Nagibina, 1976 from which it is separated by more genital hooks and larger body size.

Yamaguti (1958) briefly described this species under the name of *H. tetradonis*, and Bychowsky *et al.* (1976), as *Heterobothrium* sp. The only significant difference between these descriptions and the present one exists in the number of testes: 25–30 (Bychowsky *et al.*, 1976) vs. 100–160. Yamaguti's specimens (M.P.M. Coll. No. 22305) have not been used for the present description because of rather poorly-stained conditions. It was not possible to give the number of testes accurately. It seems likely that many of the testes were overlooked by the Russian authors.

The specific name, *yamagutii*, has been dedicated to the late Dr. S. Yamaguti, who first found this species.

Heterobothrium shinagawai sp. nov.
(Fig. 4)

Host and site of infection. On the gills of *Takifugu xanthopterus* (standard Japanese name: Shima-fugu)

Locality, date of collection and specimen. Hagi, Yamaguchi Pref., April 1987 (one specimen, holotype, mature, M.P.M. Coll. No. 19558).

Description. Body 10.4 long with maximum width of 3.4 at level of ovary, rather stout without isthmus. Opisthohaptor large, 3.9 long by 4.0 wide. Clamps massive and large, 0.69–0.74 long by 0.46–0.60 wide, first pair being smallest.

Mouth opening subterminally. Oral suckers 0.233 long by 0.205 wide. Pharynx 0.350 long by 0.327 wide. Esophagus short, bifurcating anterior to genital pore. Intestine with numerous side branches, descending and entering deep into opisthohaptor.

Testes about 90 in number, in interintestinal field from level of ovary to near end of body proper. Vas deferens leading into ejaculatory bulb, 0.154 long by 0.143 wide. Copulatory organ 0.120 in diameter, with a corona of 12 hooks.

Ovary situated in middle of body. Vagina and receptaculum seminis absent. Vitellaria distributed on both sides of body from level of genital pore to anterior end of opisthohaptor.

Remarks. This species is distinct from the

most related *H. yamagutii* sp. nov. on the gills of the same host species in larger clamps, larger opisthohaptor and fewer testes.

The specific name, *shinagawai*, has been dedicated to Mr. S. Shinagawa, Hagi, Yamaguchi Pref., who collaborated with the author in this study.

Heterobothrium bychowskyi nom. nov.

Synonym. *Heterobothrium tetradonis*: Bychowsky, Mamaev et Nagibina, 1976, pp. 32–33, fig. 1 (nec Goto, 1894)

Discussion

In the present survey, a total of 242 individuals of “Kōyose”-fugu or *T. pardalis* collected from seven prefectures were examined for *Heterobothrium*, and only six specimens of *H. tetradonis* were recovered. As discussed above, “Kogome”-fugu is probably either *T. poecilonotus*, *T. vermicularis* or *T. niphobles*. No *H. tetradonis* was found on 120 *T. poecilonotus* from four prefectures, 120 *T. vermicularis* from four prefectures or 60 *T. niphobles* from five prefectures. Nor was it found on more than 100 individuals of other species of *Takifugu* from different localities in Japan.

H. tetradonis has a long isthmus between the body proper and the opisthohaptor, as was pointed out in the original description (Goto, 1894). *T. poecilonotus* and *T. vermicularis* were rather commonly parasitized by *H. praeorchis* Bychowsky, Mamaev et Nagibina, 1976 and an unidentified species of *Heterobothrium*, respectively (Ogawa, unpublished). Both species have no isthmus, and can easily be distinguished from *H. tetradonis* sensu stricto by the body shape. Consequently, it seems unlikely that Goto found other *Heterobothrium* species with short or no isthmus on the gills of *Takifugu* spp. (“Kōyose”-fugu = *T. pardalis* and “Kogome”-fugu) and erroneously identified them as *H. tetradonis*. The scientific name for “Kogome-fugu” still remains unknown.

Bychowsky *et al.* (1976) described *H. tetradonis* from the gills of *S. albobumbeus*

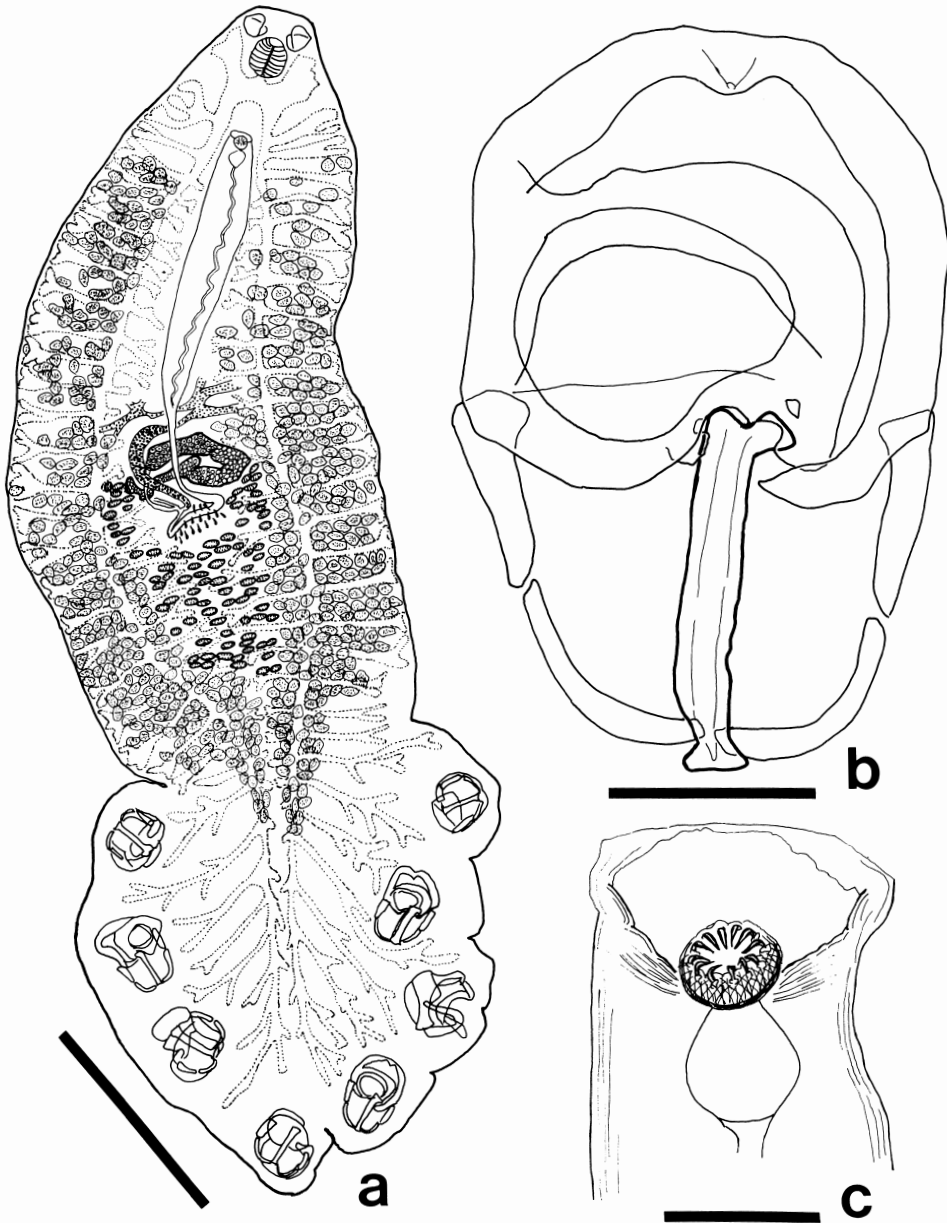


Fig. 4 *Heterobothrium shinagawai* sp. nov., holotype, from *Takifugu xanthopterus*. a, entire worm, ventral view (scale: 2 mm); b, clamp (scale: 0.2 mm); c, copulatory organ and genital opening, ventral view (scale: 0.2 mm).

(= *T. a.*) and *Spheroides* sp. (= *Takifugu* sp.) from the Yellow Sea. Based on their description and the specimens deposited in the Academy of Sciences of the U.S.S.R., their species is distinguished from *H. tetrodonis* sensu stricto by

the distribution of the testes, which extend to the level of the ovary or anterior to it, while in *H. tetrodonis* sensu stricto, the testes are limited to the postovarian region. It also appears similar to *H. okamotoi* sp. nov., but is distinct from the

latter in smaller body size and fewer testes. Therefore, *H. bychowskyi* nom. nov. is proposed here for *H. tetradonis* sensu Bychowsky, Mamaev et Nagibina, 1976.

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