Identification of Blood Flukes Infecting Tiger Puffer Takifugu rubripes

Kazuo Ogawa and Shengfa Liu

1Meguro Parasitological Museum, Tokyo 153-0064, Japan
2State Key Laboratory of Cellular Biology, Innovation Center for Cell Signaling Network, School of Life Sciences, Xiamen University, Xiamen 361102, Fujian Province, P. R. China

(Received February 25, 2017)

ABSTRACT—Blood flukes infecting tiger puffer Takifugu rubripes were re-examined taxonomically. Psettarium sp. TPC Ogawa et al., 2007, Psettarium sp. TPJ Ogawa et al., 2007 and P. japonicum sensu Yamaguti, 1938 were analysed, together with the type specimens of P. sinense (Liu, 1997), originally recorded in Takifugu oblongus as Paradeontacylix sinensis Liu, 1997. Psettarium sp. TPC, represented by blood flukes from T. rubripes cultured in Dalian, China and those collected from T. rubripes imported from China and cultured in Kagawa Prefecture, Japan in 2005, were synonymized with P. sinense. Psettarium sp. TPJ, collected in 1993 from T. rubripes caught in Wakasa Bay, Fukui Prefecture and maintained in a cage for several months, was different from the congeners in the combination of having a large body size (larger than 7 mm long), a single testis extending posterior to the ovary, presence of anterior and posterior vasa deferentia, and is described here as P. wakasaense n. sp. A single museum specimen of P. japonicum sensu Yamaguti, 1938 collected from T. rubripes off Shanghai was morphologically distinguishable from most similar P. sinense in the dextral ovary, the female pore opening anterior to the cirrus pouch and absence of the testis on dextral side of the ovary, and is proposed here as P. yamagutii n. sp.

Key words: Psettarium, blood fluke, Takifugu rubripes, tiger puffer, Takifugu oblongus

Blood flukes are the most important digenean parasites in marine fish/shark aquaculture due to their high pathogenicity (Bullard and Overstreet, 2002; Shirakashi and Ogawa, 2016). At least nine species of blood flukes are known as harmful to cultured marine fish (Ogawa, 2015; Ogawa et al., 2015), and mass mortalities have been recorded worldwide: amberjacks, Seriola spp. in Japan and Europe caused by four Paradeontacylix spp., bluefin tunas, Thunnus spp. in Japan, Australia and Europe by three Cardicola spp. and tiger puffer Takifugu rubripes in China and Japan by two Psettarium spp. (Ogawa, 2015).

Tiger puffer, a highly valuable fish cultured in Japan and China, is often heavily infected with blood flukes. The causative parasite was identified as Psettarium sp. TPJ Ogawa et al., 2007 in Japan and as P. sinense (Liu, 1997) (Liu et al., 2006; Wang et al., 2008) or Psettarium sp. TPC Ogawa et al., 2007 in China. A redescription of P. sinense is provided using the type material (Liu, 1997), material collected previously by the first author (as Psettarium sp. TPC) and new material Psettarium sp. TPC from China. We concluded that Psettarium sp. TPC is a synonym of P. sinense, as the measurements of the blood flukes from cultured pufferfish were within the ranges of those collected from T. oblongus. We also re-examined specimens of Psettarium sp. TPJ Ogawa et al., 2007 collected in 1993 from T. rubripes caught in Wakasa Bay, Fukui Prefecture and maintained in a cage for several months and a museum specimen collected from T. rubripes (as Fugu rubripes) caught off Shanghai (cited as “East China Sea”), which was identified as P. japonicum by Yamaguti (1938). We propose Psettarium wakasaense n. sp. for Psettarium sp. TPJ and Psettarium yamagutii n. sp. for P. japonicum sensu Yamaguti, 1938.

Materials and Methods

Specimens used in this study were 1) type specimens of Paradeontacylix sinensis collected from lattice baasop, Takifugu oblongus (as Fugu oblongus), deposited at Xiamen University, China (n=3, stained with carmine), 2) Psettarium sp. TPC collected from tiger puffer, Takifugu rubripes, cultured in Dalian, China (n=7) or imported from China and cultured in Kagawa Prefecture,
Japan (n=6), 3) *Psettarium* sp. TPJ, collected from tiger puffer, captured in Wakasa Bay, Fukui Prefecture and maintained for several months in a cage (n=15) and 4) a voucher specimen of *Psettarium japonicum* collected off Shanghai, China by Dr. S. Yamaguti, deposited at Meguro Parasitological Museum, Tokyo, M. P. M. Coll. No. 22806 (stained with Heidenhain’s iron hematoxylin (HH)). The method of preparing permanent slide specimens for *Psettarium* sp. TPC and *Psettarium* TPJ were described in Ogawa et al. (2007); the worms were flattened, fixed in AFA and stained with HH or alum carmine, dehydrated and mounted in Canada balsam. The same method was applied to the newly collected specimens from Dailian. Measurements were made using a calibrated ocular micrometer or digital photo equipment (DS-F11 and DS-L2, Nikon), and given in micrometers as the range plus the mean in parentheses. When measurements of body parts were not available from all specimens in the groups 1)–3), numbers of specimens used are given in parentheses after measurement data. All figures were drawn using a drawing tube.

**Results**

Family Aporocotylidae Odner, 1912
Genus *Psettarium* Goto & Ozaki, 1930
Synonyms: *Plehnia* Goto & Ozaki, 1929; *Psettarioides* Lebedev & Parukhin, 1972


Material examined: 16 whole-mounted specimens, consisting of 13 from cultured *T. rubripes* (six from Kagawa and seven from Dailian) [TR] and three type specimens from *T. oblongus* in Xiamen, Fujian Province [TO].

Redescription (Figs. 1 & 2): Body smooth, dorsoventrally

---

**Fig. 1.** *Psettarium sinense.* Entire body of a paratype, ventral view (A) and a specimen from Kagawa Prefecture, ventral view (B); photographs of rows of body spines of a paratype (C) and a specimen from Kagawa Prefecture (D); anterior body of a paratype, ventral view (E) and a specimen from Kagawa Prefecture, ventral view (F). Scale bars: 1 mm for A and B, 0.01 mm for C and D and 0.05 mm for E and F.
blood flukes from tiger puffer

flattened, lanceolate, narrowing at level of male termina-

lia, forming an indistinct notch or dent sinistrally, TR

4,320–5,880 (5,034) long, 770–1,170 (954) wide, TO

3,500–4,560 (4,140) long, 660–1,190 (870) wide, TR

4.28–6.33 (5.33) times, TO 3.86–5.74 (4.97) times lon-
ger than wide. Anterior end armed with 5 rows of min-

te spines (n=7 for TR, n=3 for TO). Notch, TR 270–

424 (362) or 5.8%–9.1% of body length from posterior

body end (n=12), TO 288–349 (328) or 6.3%–8.8% of

body length from posterior body end. Tegumental

spines on both lateral margins ventrally, TR in 340–530

(457) rows, TO in about 510 rows (n=1) on sinistral side

of body; same size throughout the body, TR 7–10 long

(n=32), 7–13 spines per row (n=33), TO 8–9 long (n=8),

8–11 spines per row (n=5), reduced numbers of spines

in anterior and posterior few rows. Nerve commissure

TR 117–194 (145), TO 113–225 (155) from anterior end.

Mouth opening ventro-subterminally, TR 7–24 (16)

(n=11), TO 11–14 (12) from anterior end. Oral sucker

weakly developed, TR 15–26 (20) long (n=12), 20–38

(27) wide (n=12), TO 22–34 (27) long, 36–49 (41) wide.

Oesophagus sinuous, surrounded by gland cells poste-
rrior to nerve commissure, TR 810–1,292 (1,030) long or

17.0%–24.1% of body length, 34–70 (45) wide, TO 910–

1,010 (943) long or 20.9%–26.0% of body length, 34–112 (68) wide. Caeca H-like; anterior caeca

unequal, TR 113–395 (246) long (n=26), TO 152–440

(288) long (n=6); posterior caeca unequal, TR 920–

2,360 (1,759) long (n=23), TO 770–2,200 (1,433) long

(n=6); TR 4.1–14.6 times, TO 3.6–6.6 times longer than

anterior pair.

Testis single, inter-caecal, with margins extending

anteriorly beyond anterior caeca, TR 296–961 (526) or

5.5%–18.8%, TO 330–736 (542) or 9.4%–16.1% from

anterior body end, postero-dextrally to near posterior

end or even extending to sinistral side, postero-sinistrally
to level of female genital pore, laterally beyond caecal

margins. Two vasa deferentia present. Anterior vas
deferens medial, running posteriorly, ventral to ovary

and uterus, united with posterior vas deferens, leading to
cirrus pouch. Posterior vas deferens emerging from

postero-dextral portion of testis at level of female genital

Fig. 2. Psettarium sinense, ventral view. Posterior body of a paratype (A) and a specimen from Kagawa Prefecture (B); Terminal
genitalia of a paratype (C). Scale bars: 0.2 mm for A and B: 0.1 mm for C.
pore, running transversely to join anterior vas deferens. Cirrus pouch curved in middle, widened at proximal and distal ends, TR 103–181 (140) long, TO 113–120 (117) long in straight line, TR 29–72 (54), TO 31–50 (40) in proximal width, TR 34–63 (52), TO 39–55 (47) (n=2) in distal width. Vesicula seminalis slightly curved in middle, tapering distally, TR 84–163 (127) long, TO 105–112 (109) long in straight line, TR 20–66 (47), TO 26–38 (34) in proximal width. Pars prostatica short and straight, TR 13–41 (23) long, 10–19 (13) wide (n=12), TO 14 long, 6 wide (n=1). Cirrus rounded or elliptical, TR 13–33 (23), TO 18–25 (22) (n=2) in diameter. Male genital pore dorsal, TR 23–77 (48), TO 49–66 (56) from sinistral body margin, TR 273–411 (334) or 5.4%–8.3% of body length, TO 294–304 (298) or 7.0%–8.4% of body length (n=2) from posterior end of body.

Ovary rounded, lobed, medial or slightly dextral, concave at postero-dextral margin, TR 209–363 (288) long, 193–406 (298) wide, TO 112–248 (191) long, 225–296 (258) wide. Oviduct emerging from postero-dextral margin of ovary, passing posteriorly, expanding to form oviducal seminal receptacle, TR 12–37 (23), TO 19–22 (20) wide, joining vitelline duct before forming oötype. Oötype, TR 39–78 (56) long, 28–54 (37) wide, TO 45–70 (60) long, 24–42 (34) wide, surrounded by Mehlis’ gland. Vitelline duct, TR 18–98 (44), TO 22–34 (28) in maximum width, passing posteriorly, ventral to oviduct. Uterus sinistral to vitelline duct, ascending, coiling several times, then descending at level of posterior end of ovary, leading to metraterm. Metraterm short, TR 48–89 (64) long (n=11), 25–73 (40) wide (n=12), TO 42–65 (56) long, 39–60 (47) wide, demarked from uterus by a sphincter (n=9 for TR, n=3 for TO). Female genital pore dorsal, sub-median, opening at base of cirrus pouch, TR 50–118 (82), TO 70–94 (83) from sinistral body margin, TR 22–72 (51), TO 54–56 (55) from, antero-dextral to, male genital pore, TR 290–420 (367) or 5.9%–8.6% of body length, TO 292–349 (327) or 6.4%–9.7% of body length from posterior end of body. Vitellarium extending from level between mouth opening and nerve commissure to level of female genital pore sinistrally, to level of oötype dextrally. Uterine eggs ellipsoidal, TR 24–47 long (n=33), 20–35 wide (n=33); TO 30–52 (41) long (n=11), 19–34 (25) wide (n=11). Excretory vesicle, TR 12–30 (21) long, 5–18 (12) wide, 4–21 (10) from posterior end; TO 10–14 (12) long, 5–8 (6) wide, 7–13 (10) from posterior end.

Remarks

We could not find any crucial morphological differences between the type specimens of P. sinense and Psettarium sp. TPC collected in Japan and in China. All measurements were within the range of intra-specific variation. We conclude here that Psettarium sp. TPC is a synonym of P. sinense. In the original description of P. sinense (Liu, 1997), only one vas deferens identified originating from somewhere anterior to the ovary, i.e. the anterior vas deferens in this redescription, was described, and the testis was distributed up to the level of ovary posteriorly. Re-examination of the three type specimens confirmed presence of the posterior vas deferens and the testis extending to near the posterior end of body on the dextral side, and in one paratype, the testis passing near to the posterior end, even extending to the sinistral side as in the specimen from Kagawa Prefecture (Fig. 2B).

Taxonomic summary

Synonyms: Psettarium sp. TPC Ogawa, Nagano, Akai, Sugita & Hall, 2007

Sites in host: Visceral blood vessels.

Type host, locality and date of collection: Takifugu oblongus (Bloch, 1786), Xiamen, Fujian Province, China (34°14’N, 136°15’E) (type-locality), November 18, 1990.

Other host, localities and dates of collection: Takifugu rubripes (Temminck & Schlegel, 1850), Kagawa Prefecture, Japan on August 5, 2005 and Dailian, China on October 13, 2006.

Specimens deposited: Holotype and two paratypes from T. oblongus, deposited in Xiamen University (No. 19901118-1~3). Six specimens from T. rubripes cultured in Kagawa Prefecture, Japan (deposited at Meguro Parasitological Museum, M. P. M. Coll. No. 21030) and seven specimens from T. rubripes cultured in Dailian, China. (M. P. M. Coll. No. 21031)

Psettarium wakasaense n. sp.

Description (based on 15 specimens) (Figs. 3 & 4): Body smooth, dorsoventrally flattened, narrowing at anterior and posterior ends, forming a notch or a dent sinistrally at level of male terminalia; 7,291–9,990 (8,515) long, 1,032–1,284 (1,135) wide, 6.56–8.60 (7.51) times longer than wide. Head spines absent. Notch indistinct, sinistral, 501–765 (625) or 6.3%–8.4% of body length from posterior end of body (n=10). Segumental spines on both lateral margins ventrally, in 690–900 (787) rows on sinistral side of body; same size throughout the body, 8–11 long (n=30), 21–34 spines per row (n=19), reduced numbers of spines in anterior and posterior few rows. Nerve commissure 194–413 (291) from anterior end. Mouth opening ventro-subterminally, 8–36 (21) from anterior end. Oral sucker weakly developed, 16–32 (21) long (n=13), 19–47 (30) wide (n=14). Oesophagus sinuous, gradually widening towards its posterior end, surrounded by conspicuous gland cells posterior to nerve commissure, 1,134–1,469 (1,300) or 14.0%–17.2% of body length. Caeca H-like; anterior caeca 212–536 long (n=30), unequal; posterior caeca 1,452–4,584 (3,440) long (n=29), 3.83–12.92 times longer than anterior pair (n=29).
Testis single, inter-caecal, with margins extending anteriorly beyond anterior caeca, often anterior to nerve commissure, 101–304 (215) or 1.2%–3.7% from anterior body end, postero-dextrally to level of, or anterior to, oötype, postero-sinistrally to level of, or anterior to, female genital pore, laterally beyond caecal margins. Two vasa deferentia present. Anterior vas deferens medial, running posteriorly, passing sinistral margin of ovary, ventral to uterus, united with posterior vas deferens, leading to cirrus pouch. Posterior vas deferens emerging from postero-dental portion of testis at level of female genital pore, running transversely to join anterior vas deferens. Cirrus pouch curved inward in middle, 138–184 (162) long in straight line, 45–80 (67) in proximal width, 39–98 (78) in distal width. Vesicula seminalis 108–164 (137) long in straight line, 32–75 (58) wide. Prostate gland cells in distal half of cirrus pouch. Pars prostatica straight, 38–54 (45) long, 13–29 (20) wide. Cirrus rounded or elliptical, 31–55 (41) long, 18–37 (25) wide, protruded from cirrus pouch. Male genital pore sinistral, 0–33 (17) from sinistral body margin, 406–644 (517) or 4.8%–6.9% of body length from posterior end of body.

Ovary, rounded, lobed, medial, 284–570 (383) long, 298–436 (380) wide, centrally positioned 900–1,259 (1,032), or 10.3%–14.1% of body length, from posterior end of body. Vitelline duct, 21–34 (27) in maximum width, passing posteriorly, side by side, ventral to Fig. 3. *Psettarium wakasaense* n. sp. Entire body of holotype, ventral view (A); photograph of rows of body spines of a paratype (B); anterior body of holotype, ventral view (C); posterior body of holotype, ventral view (D). Scale bars: 1 mm for A, 0.01 mm for B, 0.2 mm for C and 0.3 mm for D.
Oviduct. Oviduct originating at postero-dextral part of ovary, passing posteriorly, expanding to form oviducal seminal receptacle, 17–33 (24) wide, joining vitelline duct before forming oötype. Oötype medial, 58–98 (78) long, 29–72 (45) wide, surrounded by Mehlis' gland. Uterus post-ovarian, sinistral to vitelline duct, ascending, coiling several times, then descending at level of posterior end of ovary, leading to metraterm. Metraterm short, 55–114 (88) long (n=13), 37–97 (64) wide (n=13), demarked from uterus by a sphincter (n=9). Female genital pore dorsal, 90–154 (121) (n=14) from sinistral body margin, antero-dextral to cirrus pouch, 106–218 (143) (n=14) from male genital pore, 581–760 (644) or 6.6%–8.2% of body length from posterior end of body. Vitellarium extending from level between mouth opening and nerve commissure to level of female genital pore sinistrally, to level of oötype dextrally. Uterine eggs ellipsoidal, 23–36 long (n=45), 17–31 wide (n=45). Excretory vesicle, 15–38 (26) long (n=15), 8–14 (10) wide from posterior end.

Remarks

Morphologically, *P. wakasaense* n. sp. is most similar to *P. sinense*. *P. wakasaense*, *P. japonicum* and *P. sinense* are unique in the genus by possessing a single, reticulate testis extending to posterior to the ovary and possessing two vasa differentia. *P. wakasaense* can be differentiated from *P. japonicum* and *P. sinense* by the absence of a clear sinistro-lateral protuberance and by the larger size (7.29–9.99 mm vs. 4.32–5.88 mm), absence of head spines (vs. 5 spine rows in *P. sinense*) and the larger number of tegumental spines (690–900 rows, 21–34 spines/row vs. 340–530 rows, 7–13 spines/row), respectively.

**Taxonomic summary**

*Synonym:* *Psettarium* sp. TPJ Ogawa, Nagano, Akai, Sugita & Hall, 2007

Sites in host: Visceral blood vessels.

Type host, locality and date of collection: *Takifugu rubripes* (Temminck & Schlegel, 1850), Wakasa Bay, Fukui Prefecture, Japan (35°30'N, 135°43'E), August 26, 1993

Specimen deposition: Holotype and 14 paratypes (deposited at Meguro Parasitological Museum, M. P. M. Coll. No. 21032).

Etymology: The species name refers to Wakasa Bay, Japan, where the host fish was caught and maintained.

*Psettarium yamagutii* n. sp.

Description (based on a single specimen) (Fig. 5):

Body smooth, dorsoventrally flattened, narrowing at anterior and posterior ends, 5,550 long, 962 wide; 5.77 times longer than wide. Head spines not clearly observed. Notch very weakly formed, sinistral at level of male terminalia, 327 from posterior body end or 5.9% of body length. Tegumental spines on both lateral margins ventrally, approximately 580 rows on sinistral side of body; 7–10 long (n=6), number of spines per row not
Blood flukes from tiger puffer

Fig. 5. Pseutarium yamagutii n. sp., dorsal view. Entire body (A), posterior body (B) and terminal genitalia (C). Scale bars: 1 mm for A, 0.2 mm for B and 0.1 mm for C.

determined. Nerve commissure 295 from anterior end. Mouth opening ventro-subterminally, 13 from anterior end. Oral sucker weakly developed, 12 long, 21 wide. Oesophagus almost straight, surrounded by conspicuous gland cells, 1,260 long, 22.7% of body length. Caeca H-shaped; anterior caeca 470–520 long; posterior caeca 2,860–3,120 long, 5.50–6.64 times longer than anterior pair.

Testis single, massive, inter-caecal, with margins extending anteriorly beyond anterior caeca, 215 or 3.9% of body length from anterior body end, postero-dextrally passing sinistral to ovary, extending to posterior end, postero-sinistrally to level of male terminalia, laterally beyond caecal margins. Two vasa deferentia present. Anterior vas deferens medial, running posteriorly, ventral to uterus, united with posterior vas deferens, leading to cirrus pouch. Posterior vas deferens emerging from postero-dextral portion of testis, running transversely to join anterior vas deferens. Cirrus pouch strongly curved in middle, 126 long in straight line, 48 in proximal width, expanded to 82 wide to contain prostate gland cells. Vesicula seminalis straight, tapering distally, 97 long, 44 wide. Pars prostatica slightly widening toward cirrus, 37 long, 31 wide. Cirrus spherical, 45 in diameter. Male genital pore dorsal, 47 from sinistral body margin, 317 or 5.7% of body length from posterior end of body.

Ovary, an inverted triangular in shape, lobed, dextral, ventral to vas deferens, 254 long, 367 wide. Oviduct emerging on sinistral side of ovary, passing posteriorly, expanding to form oviducal seminal receptacle, 46 wide, joining vitelline duct before forming oötype.
Vitelline duct, 41 in maximum width, passing posteriorly, ventral to oviduct. Oötype, 82 long, 68 wide, surrounded by Mehlis’ gland. Uterus sinistral to vitelline duct, ascending, coiling several times, then descending at level of anterior end of ovary, leading to metraterm. Metraterm short, 77 long, 50 wide, demarked from ovary (medial in the ovary, leading to metraterm.

Female genital pore dorsal, submedian, 224 from sinistral body margin, 186 from male uterus by a sphincter. Female genital pore sinistrally, to level of oötype dextrally. Excretory vesicle 20 long, 9 wide, 25 from posterior end. Vitellarium extending from level between mouth opening and nerve commissure to level of female genital pore sinistrally, to level of oötype dextrally. Uterine eggs ellipsoidal, 38–40 long, 28–32 wide. Excretory vesicle 20 long, 9 wide, 25 from posterior end.

**Remarks**

Yamaguti (1938) made no detailed description of his specimen except for the body size and the cirrus pouch. Examination of his specimen revealed that it is clearly different from *P. japonicum* sensu stricto in the position and a relative size of the ovary, distribution of the testis, indistinctive protuberance at the level of male terminalia, and the position of the female pore. *P. yamagutii* may be most similar to *P. sinense*, but can be easily differentiated by the following morphological features: position of the ovary (medial in *P. sinense* vs. dextral in *P. yamagutii*), distribution of the uterus (mostly post-ovarian vs. extending to the level of anterior end of the ovary), opening of the female pore (at the base of the cirrus pouch vs. anterior to the cirrus pouch and more centrally positioned), distribution of the testis (extending to both sides of the ovary vs. non-existent on dextral side of the ovary).

**Taxonomic summary**

Synonym: *Psettarium japonicum* sensu Yamaguti, 1938

Type host, locality and date: *Takifugu rubripes* (Temminck & Schlegel, 1850), off Shanghai (cited as “East China Sea”), China, May 1, 1935

Specimen deposition: Holotype at Meguro Parasitological Museum, M. P. M. Coll. No. 22806.

Etymology: The species name refers to Dr. Satyu Yamaguti, who described this parasite as *P. japonicum* in 1938.

**Discussion**

The male gonad of the genus *Psettarium* has been defined by many authors, in which there have been different interpretations on the testis/testes. Goto and Ozaki (1929) described *P. japonicum* (as *Plehnia japonica* Goto & Ozaki, 1929), the type species of the genus, had a single testis occupying the dorsal side of body from near the posterior end of body to near esophagus. Yamaguti (1953), however, defined testes as diffuse, uncountable with indistinct boundaries, followed by Yamaguti (1958) and Yamaguti (1971), whereas Smith (2002) and Bullard and Overstreet (2006) defined it as a single entity, as in the original description of the type species by Goto and Ozaki (1929). In fact, *P. wakasaense* n. sp. has an extensive, diffuse testis, which could be misinterpreted as numerous testes with indistinct boundaries. Recently, Yong et al. (2016) described three new species of *Psettarium*, in which they emended the generic definition that *Psettarium* has one or more testes to include *P. ogawai*, a species with 16–22 spherical testes.

Yamaguti (1930) described a new blood fluke, *Aporocotyle odhneri*, collected from the blood of the intestinal wall of *Takifugu porphyreus* (as *Sphaeroides borealis*) caught in the Peter The Great Bay, Russia, which was later transferred to *Paradeontacylix* by McIntosh (1934) as it had two to three rows of testes, a medi ally positioned ovary and a long, coiled, post-ovarian uterus. Following the definition of Yong et al. (2016), no contradiction arises when this parasite is transferred to *Psettarium*. Host of *P. odhneri* belongs to the genus *Takifugu*, from which the type and some other *Psettarium* species have been recorded. Since the original description was based on a single specimen, which had been lost, re-examination based on new material is required on the taxonomy of this less known blood fluke.

In this paper we propose *P. wakasaense* n. sp. for *Psettarium* sp. TPJ and *P. yamagutii* n. sp. for *P. japonicum* sensu Yamaguti, 1938, and synonymize *Psettarium* sp. TPC as *P. sinense*. Thus, the genus *Psettarium* now comprises 12 species, nine of them from tetraodontid fish (Table 1). They have different types of male genital system, which can be divided into three groups: four species, i.e. *P. japonicum*, *P. sinense*, *P. wakasaense* n. sp. and *P. yamagutii* n. sp., all from *Takifugu* spp., have a single, extensive and reticular testis, four species from *Arothron* spp., viz. *P. pulchellum*, *P. ogawai*, *P. jimbaranense* and *P. nolani*, have a degenerate posterior testis beside anterior testis/testes and the remaining four species, viz. *P. tropicum*, *P. rachycentri*, *P. pseudupenei* and *P. antichum*, have pre-ovarian testis/testes but no posterior one. Redescription of the type species, *P. japonicum* infecting *Takifugu pardalis* is required for accurate definition of this genus, which is in progress by one of the present authors.

Among the *Psettarium* species, only *P. sinense* and *P. pulchellum* were recorded from more than one species of host (Table 1), suggesting high host specificity of this group of blood flukes. As for their host species, three *Psettarium* species have been recorded from *T. rubripes*, while two species each were collected from *R. canadum* and *A. reticularis*, implying that *Psettarium* is a rich and diversified group of digeneans.

Since tiger puffer is extensively cultured both in
### Table 1. A list of species of *Psettarium* Goto & Ozaki, 1930

<table>
<thead>
<tr>
<th>Species</th>
<th>Host</th>
<th>Site</th>
<th>Locality</th>
<th>Other records</th>
<th>Synonyms</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Psettarium japonicum</em> (Goto &amp; Ozaki, 1929)</td>
<td><em>Spheroides pardalis</em> (Tetraodontidae)</td>
<td>intestine</td>
<td>Inland Sea, Japan</td>
<td>small intestine of <em>Spheroides pardalis</em> from Hamajima, Mie Pref., Japan (Yamaguti, 1951)</td>
<td><em>Plehnia japonica</em> Goto &amp; Ozaki, 1929</td>
</tr>
<tr>
<td><em>Psettarium anthicum</em> Bullard &amp; Overstreet, 2006</td>
<td><em>Rachycentron canadum</em> (Rachycentridae)</td>
<td>heart (myocardium, atrial wall)</td>
<td>Northern Gulf of Mexico</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Psettarium pulchellum</em> Yong, Cutmore, Bray, Miller, Semarariana, Palm &amp; Cribb, 2016</td>
<td><em>Arothron manilensis de Procé</em>, <em>Tylerus spinoisinus</em> Regan (Tetraodontidae)</td>
<td>A. manilensis in gills; <em>T. spinoisinus</em>: unknown, but adult worms found in body wash</td>
<td>Jimbaran, southern Bali, Indonesia</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Psettarium ogawai</em> Yong, Cutmore, Bray, Miller, Semarariana, Palm &amp; Cribb, 2016</td>
<td><em>Arothron reticularis</em> Bloch &amp; Schneider (Tetraodontidae)</td>
<td>gill arches and gill filaments</td>
<td>Jimbaran, southern Bali, Indonesia</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Psettarium jimbaranense</em> Yong, Cutmore, Bray, Miller, Semarariana, Palm &amp; Cribb, 2016</td>
<td><em>Arothron reticularis</em> Bloch &amp; Schneider (Tetraodontidae)</td>
<td>gill arches and gill filaments</td>
<td>Jimbaran, southern Bali, Indonesia</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Psettarium wakasaense</em> n. sp.</td>
<td><em>Takifugu rubripes</em> (Tetraodontidae)</td>
<td>visceral blood vessels</td>
<td>Wakasa Bay, Fukui Pref., Japan</td>
<td></td>
<td><em>Psettarium</em> sp. TPJ Ogawa, Nagano, Akai, Sugita &amp; Hall, 2007</td>
</tr>
<tr>
<td><em>Psettarium yamagutii</em> n. sp.</td>
<td><em>Spheroides rubripes</em> (=<em>Takifugu rubripes</em>) (Tetraodontidae)</td>
<td>small intestine</td>
<td>off Shanghai (as “East China Sea”)</td>
<td></td>
<td><em>Psettarium japonicum</em> sensu Yamaguti, 1938</td>
</tr>
</tbody>
</table>
Japan and China and blood fluke infection is a serious threat to the culture industry, it is necessary to accurately identify the causative species among the three species described here. Morphologically, *P. wakasaense* is easily distinguished from the other two species by the larger body size (7.29–9.99 mm vs. 4.32–5.88 mm in *P. sinense* and 5.55 mm in *P. yamagutii*); it is rather difficult to separate *P. sinense* from *P. yamagutii* without confirming their male and female genital systems. For the moment, *P. wakasaense* and *P. yamagutii* have been recorded only from Japanese and Chinese waters, respectively. There is a risk of expanding geographical distributions for these species due to international trade of live host fish. Other than morphological identification, it is practically desirable to obtain DNA sequence data for the species discrimination of blood flukes infecting tiger puffer.

Acknowledgments

We express our sincere gratitude to Mr. Akihiro Sugita, Fukui Prefectural Fisheries Experimental Station, for providing help in collecting specimens of *P. wakasaense* n. sp. and to Mr. Taizo Nagano and Ms. Noriko Akai, Kagawa Prefectural Fisheries Experimental Station and Mr. Xueshong Meng, Dalian Tianzheng Industry Co., Ltd., Dalian, China for providing help in collecting specimens of *Psettarium* sp. TPC.

References


