養殖ヤマメから得tetraonchus属の2新種について

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Two New Species of the Genus *Tetraonchus* (Monogenea: Tetraonchidae) from Cultured *Oncorhynchus masou*

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Two new species of the genus *Tetraonchus* (Monogenea: Tetraonchidae) were found parasitic on the gills of masu salmon, *Oncorhynchus masou* cultured in freshwater in Yamanashi Prefecture, Japan. The specific names, *T. awakurai* n.sp. and *T. oncorhynchi* n.sp. were given to them. *T. awakurai* identical with *T.*sp. of AWAKURA (1966), resembles *T.*sp. 1 of ERGENS (1971) and *T. gvosdevi* (SPASSKY et ROYTMAN, 1960), but differs from the latter two species in the shape of the cirrus. *T. oncorhynchi* is different from all other species in the shape of the cirrus.

In adult *T. awakurai*, the marginal hooks are usually absent, although they exist in eight pairs initially and juvenile *T. awakurai* has their remnants and/or the full marginal hooks. So it is presumed that they disappear during the course of the development of the parasite.

The present species are characterized by the presence of a single intestine and the absence of the vagina. These primitive morphological characteristics indicate that they occupy a particular systematical position in the monogeneans.

In our country, tetraonchids have been known to be parasitic on the gills of salmonid fish cultured in freshwater, but have been unidentified until now, though AWAKURA (1966)[1] reported *Tetraonchus* sp. from cultured *Oncorhynchus masou* in Hokkaido. We have studied tetraonchids from *O. masou* (Japanese common name "Yamame") and found two species of *Tetraonchus*; one is identical with *T.* sp. of AWAKURA (1966) and another has not yet been reported. Two new species, *T. awakurai* n. sp. and *T. oncorhynchi* n. sp. are described in this paper.

MIZELLE and CRANE (1975)[2] presented a list of seventeen species of *Tetraonchus* including two unidentified species reported by ERGENS (1971),[3] but did not refer to *T.* sp. of AWAKURA (1966). ARTHER *et al.* (1976)[4] pointed out that *T. rauschi* MIZELLE et WEBB, 1953[5] is a synonym of *T. borealis* (OLSSON, 1893). By taking these theories into consideration, seventeen species of the genus have been known so far. Consequently, the number of *Tetraonchus* species comes to eighteen including two unidentified species and the two new ones reported here.

Tetraonchus species have been usually described referring only to the chitinous parts and not to other structures. In this paper, the other characteristics of the present parasites are also discussed on the basis of their internal morphology.

Materials and methods

More than two hundred tetraonchids were obtained from thirteen masu salmon, *Oncorhynchus masou* and a single rainbow trout, *Salmo gairdneri*. Both species of fishes were 0+ year old and had been cultured in freshwater. Two methods were used for preparing microscopic specimens for observation and measurement. One was the staining methods of OGAWA and EGUSA (1976).[6] Although most structures of the parasites could be observed in this way, the characteristics of only the chitinous parts, the most important key for identifying tetraonchid species, were not clearly observable bacause of specimen thickness. In order to observe the chitinous parts accurately, the method using ammonium picrate-glycerin was employed which was devised by MALMBERG (1957)[7] and later modified by ERGENS (1969)[8] by making a permanent mounting in Canada balsam.

Description and measurement of soft parts of the body, that is, the haptor, pharynx, testis, ovary, receptaculum seminis, prostatic reservoirs and vesicula seminalis were carried out on the stained specimens.

To compare morphologically with *Tetraonchus* sp. of AWAKURA (1966) donated by Dr. AWAKURA and with eight species of *Tetraonchus* from Mongolia donated by Dr. ERGENS, our specimens were examined. The former specimens were

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obtained from *O. masou* in Hokkaido in 1965, fixed in formalin and stained with haematoxylin, and the latter were fixed in ammonium picrate-glycerin.

All measurements were given with the minimum and maximum values in microns in the text, followed by the means in parentheses. Figures were drawn with the aid of a camera lucida.

Results

From the gills of masu salmon, we obtained 219 specimens of *T. awakurai* n. sp. and 22 specimens of *T. oncorhynchi* n. sp. From the gills of a rainbow trout, 32 specimens of *T. awakurai* were found.

Tetraonchus awakurai n. sp.

Host: *Oncorhynchus masou* and *Salmo gairdneri*, both 0+year.

Locality and date: Yamanashi Pref., Sept. 1976.

Specimens: The holotype and some paratypes are deposited in the Meguro Parasitological Museum, M. P. M. Coll. No. 19179, and the other paratypes in the authors' collection.

Description

1) *Chitinous parts*: Ten specimens obtained from *O. masou* were used for description. The various chitinous parts to be measured are shown in Fig. 1. The measurement was made on the basis of ERGENS' idea (1971).[3] The haptoral armature consists of two pairs of anchors, one bar and a pair of "fan-shaped" bars. The marginal hooks are usually absent, and only their domus and sometimes the remnants of the hooks are located at their original sites. Two pairs of the anchors are situated in the center of the opisthaptor. Total length of the ventral and dorsal anchors are all represented by **a** in Fig. 1 (**a**>**b**). The ventral anchor ranges 73–79 μm (76 μm) in total length. The inner root is thinner than the outer one. The inner basal part is 45–51 μm (48 μm) long. The outer root is wide and round and the outer basal part, 43–53 μm (48 μm) has about the same length as the inner basal one. The point is 28–31 μm (30 μm) long. The maximum width of the anchor is 46–53 μm. The dorsal anchors are 69–76 μm (73 μm) long and very similar in shape and size to the ventral ones, but differ from the latter in that it has a wider angle between the inner and the outer root than the latter. The lengths of the inner and outer basal parts are 41–49 μm and 42–49 μm, respectively. The point is 26–29 μm (28 μm) in length and the maximum width of the anchor is 45–54 μm. The ventral bar is mostly V-shaped and is 45–56 μm long by 9–14 μm wide. The "fan-shaped" bars are paired and situated very closely to each other. Each of the pair is 23–25 μm long by 22–28 μm wide. Some ventral and dorsal anchors are different from normal ones in shape, namely their inner and outer roots are somewhat transformed (Figs. 5 and 6). Even if one of a pair of the ventral or the dorsal anchors is in such an unusual shape, the other may remain in the normal shape.

The cirrus, its size being 56–62 μm (60 μm) long by 4–4.5 μm wide, is a simple and almost straight tube. It is gradually widened proximally and becomes narrowed and slightly curved at the

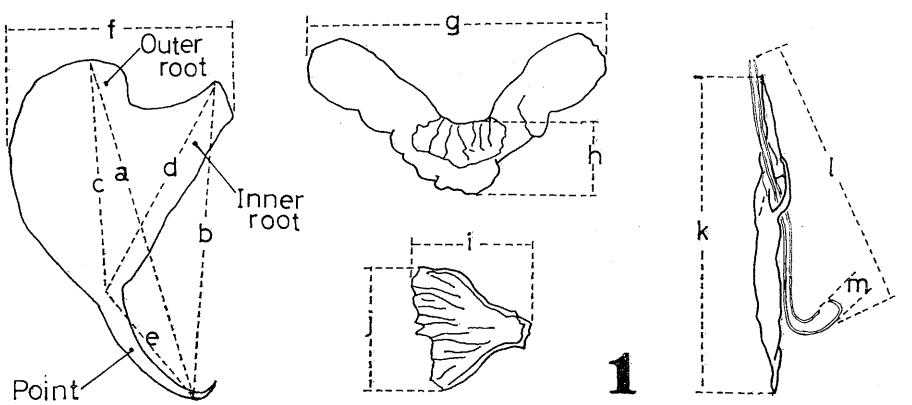


Fig. 1. The chitinous parts to be measured. a, b: Total length of the anchor is represented by a larger value of either a or b. c: Length of the outer basal part. d: Length of the inner basal part. e: Length of the point. f: Maximum width of the anchor. g: Length of the bar. h: Width of the bar. i: Length of the "fan-shaped" bar. j: Width of the "fan-shaped" bar. k: Length of the cirrus accessory. l: Length of the cirrus. m: Maximum width of the cirrus.

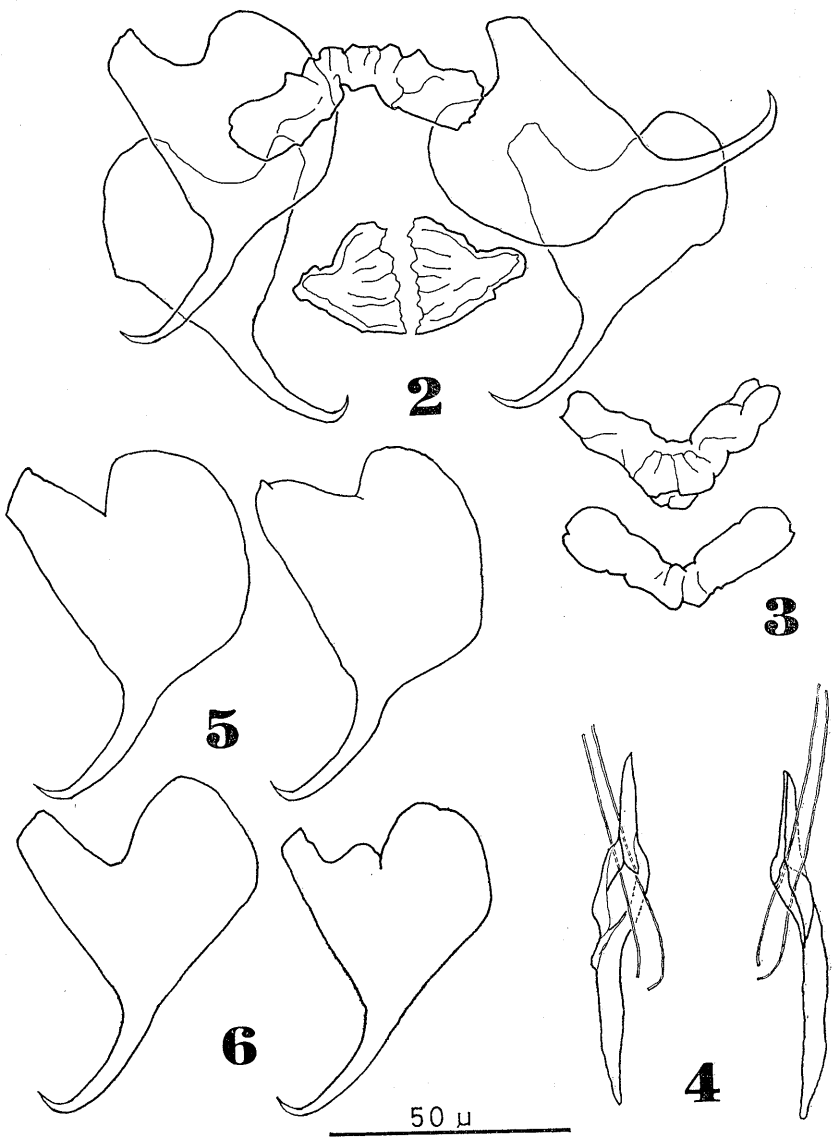
base. There is no supplementary supporting process. The accessory, 69–75 μm (73 μm) long, is pointed at the both ends, and grooved at the distal part. It has a hole approximately at the middle, where the cirrus tube passes.

This species was also obtained from a cultured rainbow trout. Their measurements of the chitinous parts are as follows (Nos. measured: 12): total length of the ventral anchor, 67–75 μm (72 μm); that of the dorsal anchor, 64–70 μm (67 μm); the bar, 42–53 μm long; the "fan-shaped" bar, 15–23 μm long by 14–24 μm wide; the cirrus, 52–62 μm (58 μm) long by 4–4.5 μm wide; the accessory, 58–75 μm (69 μm) long.

2) *General structure*: Ten stained specimens from *O. masou* were examined.

The body is stout, 650–1080 μm long by 250–415 μm wide, and somewhat thick. The opisthaptor, 81–100 μm × 119–161 μm, is well marked off from the body proper.

At the anterior extremity of the body, the head lobes are not well differentiated. The head organs are present where the ducts of sticky glands open. Two pairs of eye spots are situated anterodorsally



Figs. 2–6. Chitinous parts of *T. awakurai* n.sp., all paratypes. 2: An anchor complex, ventral view. 3: Ventral bars. 4: Copulatory organs. 5: Ventral anchors of unusual shape. 6: Dorsal anchors of unusual shape.

to the pharynx.

The mouth opens just anterior to the globular pharynx which is 64–89 μm ×68–96 μm in size. The esophagus is very short. The intestine is a single tube with several expansions like side branches at the anterior part, running through the central part almost to the end of the body proper. The cement glands are present posterior to the end of the intestine.

The testis (83–136 μm×87–171 μm) is rounded with irregular incisions and located just behind the ovary and dorsally to the intestine. The vas deferens arises from the anterior part of the testis, and turns round the intestine from the left side. It emerges ventrally and proceeds forward, dilated to form the vesicula seminalis (41–62 μm long by 19–31 μm wide), which is usually curved and ellipsoidal. The seminal duct reaches the base of the cirrus. There are two prostatic reservoirs at the base of the cirrus; one on the right is 19–36 μm long by 13–21 μm wide and is usually smaller than the other on the left, 26–40 μm long by 15–19 μm wide. They contain coarse granules and are not stained with carmine. The prostate glands are well developed, the cells of which are distributed between the level of the genital pore and that of the anterior part of the ovary, and even extend to the right lateral margin of the worm body at the level of the receptaculum seminis. Each duct of two prostatic reservoirs opens to the base of the cirrus together with the seminal duct.

The flask-shaped ovary is situated on the right side of the parasite and originates dorsally to the intestine. Its anterior part is slightly curved to the right, and the anterior extremity where the oviduct starts, is on the right of the intestine. The oviduct runs backwards, soon turns its course inwards and it is dilated to form the receptaculum seminis at the anteroventral side of the ovary. The receptaculum seminis receives the common vitelline duct approximately on the median line posterior to the ootype, leading into the latter. The MEHLIS' glands are located around the posterior end of the ootype. The uterus is not recognized. The size of the receptaculum seminis varies 50–100 μm long or more by 14–31 μm wide. The vagina is absent. Sperms are often observed in the ootype. They reach the receptaculum seminis by way of

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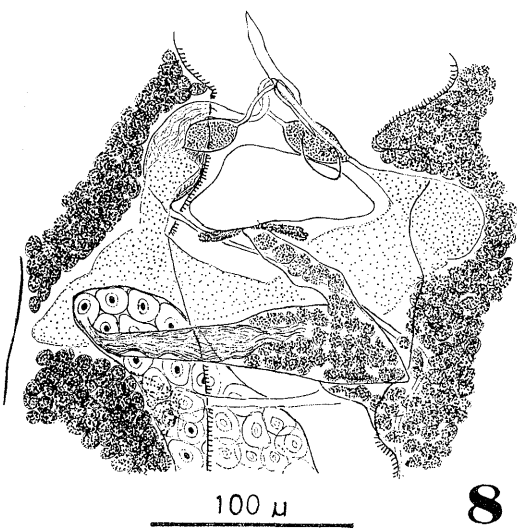

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Figs. 7–8. The entire worm and the terminal genitalia of *T. awakurai* n. sp. 7: Entire worm of holotype, ventral view. 8: Terminal genitalia of paratype, ventral view.

the common vitelline duct. The vitellaria extend over from the level of the posterior end of the pharynx to near the end of the intestine on both sides of the parasite. They are usually confluent at the posterior end.

Ten specimens of *Tetraonchus* sp. from Dr. AWAKURA were examined. The shape of the chitinous parts is completely identical with that of our specimens. Their measurements are as follows: total length of the ventral anchors, 64–72 μm; that of the dorsal anchors, 67–73 μm; length of the bar, 40–43 μm; and length of the cirrus accessory, 65–76 μm. Since his specimens were not flattened sufficiently, the measurements were shorter than ours. Thus, we regard *T.* sp. of AWAKURA (1966) as identical with *T. awakurai* n. sp.

The specific name was dedicated to Dr. AWAKURA who described first this species.

The structure of the chitinous parts, the entire worm and the terminal genitalia are presented in Figs. 2–8.

Tetraonchus oncorhynchi n. sp.

Host: *Oncorhynchus masou*, 0+year.

Locality and date: Yamanashi Pref., Sept. 1976.

Specimens: The holotype and some paratypes are deposited in the Meguro Parasitological Museum, M. P. M. Coll. No. 19180, and the other paratypes in the authors' collection.

Description

1) *Chitinous parts* (Nos. measured: 10): Total length of the ventral and dorsal anchors are all represented by **a** in Fig. 1 (**a**≧**b**) except one instance; in one of a pair of the dorsal anchors of one specimen measured, **b** in Fig. 1 (83 μm) is longer than **a** (79 μm), because of a well extended inner root (Fig. 13, right), while in the other, **a** is almost same as **b** in length (78 μm). Total length of the ventral anchors is 78–86 μm (82 μm) long. The inner and outer basal parts are 45–56 μm (50 μm) and 48–54 μm (51 μm) long, respectively. The point is 31–38 μm (35 μm) long, more slender than that of *T. awakurai*. The maximum width ranges 48–58 μm. Total length of the dorsal anchors is 75–83 μm (79 μm). Both the inner and outer roots are much longer than those of the ventral anchors. The outer root is somewhat thicker than the inner one. The inner and outer basal parts are 48–62 μm (54 μm) and 44–51 μm (49 μm) long, respectively. The maximum width is 43–53 μm. The ventral bar is straight to V-shaped, and 52–65 μm long by 9–17 μm wide. Two "fan-shaped" bars are situated at intervals to each other, their sizes being 19–24 μm long by 24–28 μm wide. Eight pairs of marginal hooks are present. They are same in shape and size, each being 14–16 μm long.

The cirrus which is 54–58 μm (56 μm) in length and 3 μm in maximum width, is a much narrower tube than that of *T. awakurai* and strongly curved at the base. There is no supplementary supporting process. The accessory somewhat resembles that of *T. awakurai* in shape, but is a little shorter than the latter in length, 60–69 μm (64 μm).

2) *General structure* (based on eight stained specimens): The structure of *T. oncorhynchi* n. sp. is basically identical with that of *T. awakurai* n. sp.

The body is slender, 705–1530 μm long by 237–296 μm wide. The transversely wide opisthaptor, 91–126 μm long by 161–239 μm wide, is well differentiated from the body proper.

The pharynx is 55–86 μm long by 64–95 μm wide. The intestine is almost a simple tube and narrower than that of *T. awakurai*. The cement glands, in most cases, originates in the opisthaptor.

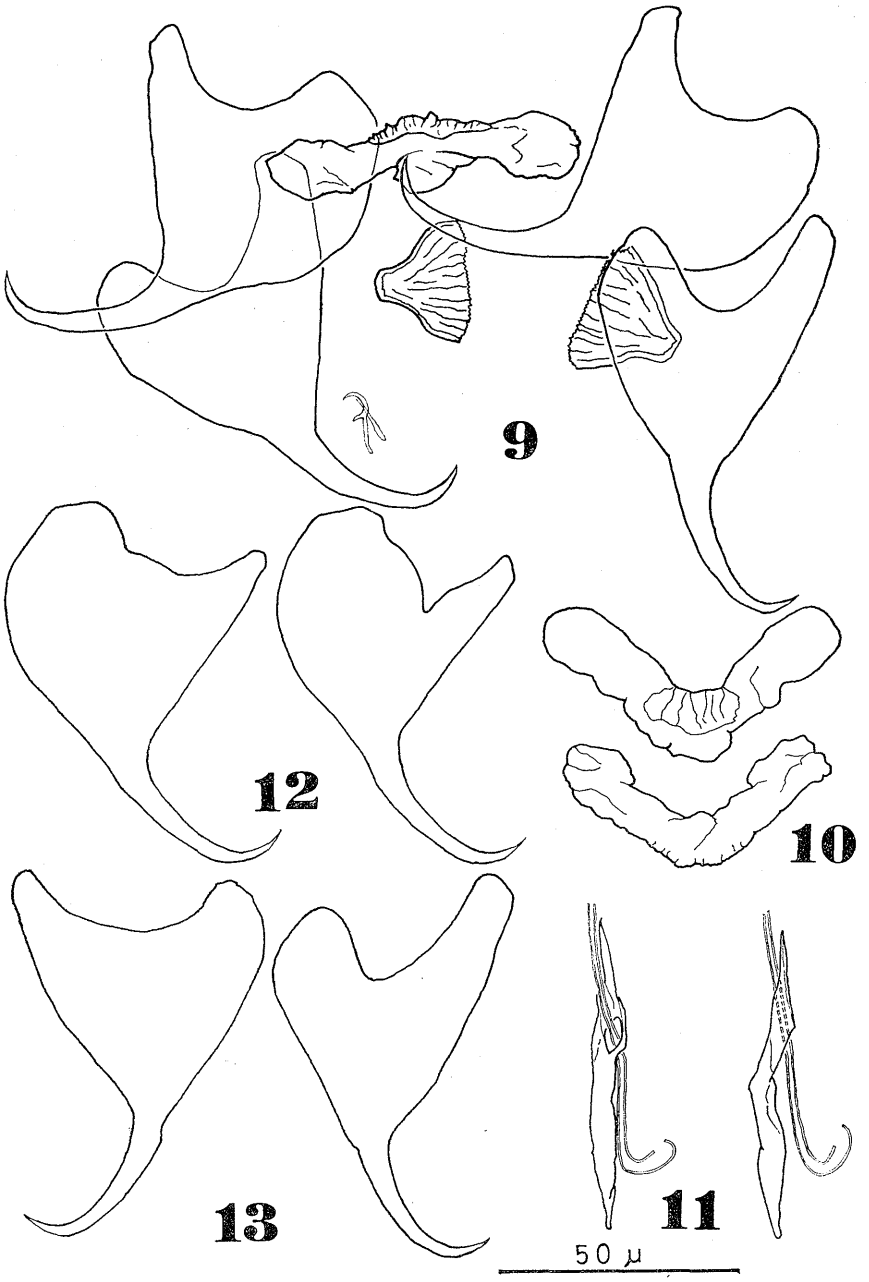
The testis is elongated in shape and enormously large, 167–521 μm (350 μm) long by 138–227 μm wide, occupying a large part of the posterior region of the body proper. The vesicula seminalis is 55–82 μm long by 23–27 μm wide. The prostatic reservoir on the right is 18–27 μm long by 10–13 μm wide. Very often the left one empties and becomes shrunk. It measures 19 μm×7 μm and when empty, 23–26 μm×6–7 μm.

The ovary is elongated and almost straight in shape. It is located at the right side of the body, 129–186 μm long by 52–91 μm wide. The vagina is absent. The receptaculum seminis is smaller than that of *T. awakurai*, and its width ranges 8–25 μm.

The chitinous parts and the entire worm are presented in Figs. 9–14.

Discussion

SPASSKY and ROYTMAN (1958)[9] asserted that the genus *Tetraonchus* should be divided into two independent genera on the basis of their morphological and ecological characters. They proposed a new genus *Salmonchus* for parasites of Salmonidae, while species parasitic on Esocidae and Thymallidae were left in *Tetraonchus*. STRELKOV (1963)[10] reduced these genera into subgenera of *Tetraonchus*, taking up the presence of the "fan-shaped"



Figs. 9–13. Chitinous parts of *T. oncorhynchi* n.sp., all paratypes. 9: An anchor complex and a marginal hook, ventral view. 10: Ventral bars. 11: Copulatory organs. 12: Ventral anchors of unusual shape. 13: Dorsal anchors of unusual shape.

bars as a main diagnostic character. ERGENS (1971)[11] found the "fan-shaped" bars not only in the members of *T.* (*Salmonchus*) but also in *T.* (*Tetraonchus*). According to STRELKOV's observation, the "fan-shaped" bars was absent in the members of *T.* (*Tetraonchus*). Since ERGENS' findings indicate that there is no ground to divide *Tetraonchus* into two subgenera, it may be most appropriate at present to follow his opinion that the genus *Tetraonchus* has a uniform character systematically and setting the subgenera up under the genus is unnecessary.

Tetraonchus awakurai n. sp. resembles *T.* sp. 1 of ERGENS (1971) and *T. gvosdevi* (SPASSKY et

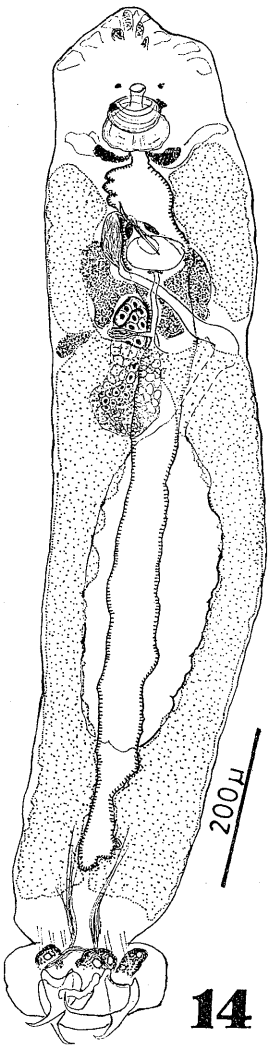


Fig. 14. The entire worm of *T. oncorhynchi* n.sp. of holotype, ventral view.

ROYTMAN, 1960),[12] but it differs from the latter two species in the shape of the cirrus. In *T. awakurai* the cirrus is gradually widened proximally and becomes narrowed and slightly curved at the base, while in *T.* sp. 1 of ERGENS the cirrus base is not narrowed and almost straight, and in *T. gvosdevi* the cirrus is very long and strongly curved at the base. *T. oncorhynchi* n. sp. differs from all other species by having the short and narrow cirrus tube without the supplementary supporting process. *T. awakurai* n. sp. was obtained both from the masu salmon and the rainbow trout. The size of the chitinous parts was almost same between the two groups regardless of host species. However, the body itself of the tetraonchids obtained from the rainbow trout was considerably small and poorly matured, suggesting that the fish may possibly be an occasional host.

In our observation on the two new species, not only the bar but also the anchors are variable in shape, whereas the shape of the copulatory organ is usually consistent. The anchors sometimes vary in shape to such a degree that **b** is about 4 μm longer than **a** in Fig. 1.

The marginal hooks of the members of *Tetraonchus* have been known to exist in eight pairs. Although *T. oncorhynchi* n. sp. has eight pairs of them, in *T. awakurai* n. sp. they are usually absent, as mentioned above. Examinations of adult specimens of the present two species and of eight species of *Tetraonchus* from Mongolia supplied by Dr. ERGENS revealed that *Tetraonchus* species could be divided into three groups according to the number of the marginal hooks. In the first group where *T. awakurai, T. gvosdevi* and *T.* sp. 1 of ERGENS (1971) are included, the marginal hooks are absent. In the second group where *T. oncorhynchi, T. skrjabini, T. lenoki, T. roytmani* and *T. rogersi* are included, they are present in eight pairs. In *T. spasskyi*, only one hook (probably one of the pair I*) remains, possibly included in the first group. In *T. huchonis*, two pairs (pair I* situated in the center of the opisthaptor and pair II*, the posteriormost one) are lost during its development, and the remaining six pairs are found. This species belongs to the third group. In adult *T. awakurai* the marginal hooks are usually absent, but their domus and sometimes the remnants of the hooks are preserved at their initial locations. Meanwhile, juvenile *T. awakurai* often has their remnants and sometimes even the full marginal hooks. All these facts are explained as follows. The marginal hooks originally exist in eight pairs. In *T. awakurai*, when the marginal hooks become lost their ability to attach to the gill tissues of fish as the parasite develops, they are gradually absorbed into the body, but are not thrown off, and at last, only their domus remain at the original sites. Thus, the disappearance of the marginal hooks is considered to be a phenomenon accompanied by the development of the parasite. The systematical and ecological significance of the structure should be investigated in more detail in future.

Internal morphology of *Tetraonchus* has been little elucidated. Morphologically, the present species (possibly all the species of *Tetraonchus*) are

* The mode of numbering the pairs of the marginal hooks are due to LLEWELLYN (1963)[18].

characterized by having a single intestine and two prostatic reservoirs and by absence of the vagina. Only the members of the superfamilies Tetraonchoidea and Udonelloidea are known to have a single intestine. This is apparently a primitive character as BYCHOWSKY (1957)[14] mentioned. The granules in each prostatic reservoir are coarse and not stained with carmine unlike the case of *Dactylogyrus minutus* in which the granules in one of the reservoirs are stained with carmine.[15] Each content of two reservoirs of the present parasites may be same with each other and derived from a single mass of the prostatic cells. The oviduct, after forming the receptaculum seminis, is connected with the common vitelline duct. According to the figure of *T. monenteron*, the type species of the genus, described by BYCHOWSKY (1957),[14] the vagina opens on the right margin and is connected with the oviduct. According to MIZELLEE and WEBB (1953),[5] the vagina is absent in *T. monenteron*, but a lightly chitinized vagina is present in *T. rauschi* (a synonym of *T. borealis*) and is continuous with a relatively large vaginal tube which is chitinized to the same degree. To regard the absence of the vagina as a key for identifying the genera, it should be re-examined if the vagina is absent or not in all the members of *Tetraonchus*, especially in *T. monenteron*. The morphological characteristics as the existence of a single intestine and the lack of the vagina mean that *T. awakurai* n. sp., *T. oncorhynchi* n. sp. and possibly all the members of *Tetraonchus* may be very primitive monogeneans and also may occupy a particular systematical position.

Acknowledgements

The authors would like to thank Dr. R. ERGENS, Institute of Parasitology, Czechoslovak Academy of Sciences, Czechoslovakia for having donated us the microscopic preparations of eight species of *Tetraonchus* from Mongolia including *T.* sp. 1 of ERGENS (1971) for comparative study, and Dr. T. AWAKURA, Hokkaido Salmon Hatchery, Japan, for having donated us the microscopic preparations of *T.* sp. of AWAKURA (1966). Thanks are also due to Dr. Sh. KAMEGAI, Meguro Parasitological Museum, Japan, for his helpful guidance, and Mr. K. INOUE, Okutama Branch, Tokyo Metropolitan Fisheries Experimental Station for collecting the fish.

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