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REVIEW

The biology of *Contracaecum osculatum* sensu lato and *C. osculatum* A (Nematoda: Anisakidae) in Japanese waters: a review

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Abstract The paper reviews the biology of *Contracaecum osculatum* sensu lato and *C. osculatum* A, a member of the *C. osculatum* complex, parasitic in marine finfishes as larvae and in pinnipeds as adults in Japanese waters. Before *C. osculatum* A was discovered in 1998, larval nematodes identifiable as *C. osculatum* s. l. had been reported as various types of *Contracaecum* or *C. osculatum*. To date, larval and adult *C. osculatum* s. l. have been recorded from 13 species of teleosts and six species of pinnipeds, respectively, and there is a record of larval *C. osculatum* s. l. from euphausids. Larval and adult *C. osculatum* A are known from Alaska pollock *Theragra chalcogramma* and bearded seals *Erignathus barbatus*, respectively. Larval *C. osculatum* s. l. are commonly found in commercially important gadid fishes, especially in Alaska pollock. Two cases of human infection with *C. osculatum* s. l. have been reported in Hokkaido. Since larval *C. osculatum* s. l. can invade the intestinal mucosa of experimentally infected puppies, the larvae have the potential to penetrate the wall of the human digestive canal.

Key words: Anisakidae, *Contracaecum osculatum* A, *Contracaecum osculatum* sensu lato, Nematoda, parasite, pinnipeds, *Theragra chalcogramma*

INTRODUCTION

The *Contracaecum osculatum* complex is a sibling species group in the nematode family Anisakidae (Mattiucci *et al.*, 2007; Mattiucci and Nascetti, 2008). This species complex consists of five members: three Arctic species (*C. osculatum* A, *C. osculatum* B, and *C. osculatum* sensu stricto) and two Antarctic species (*C. osculatum* D and *C. osculatum* E). These members are differentiated genetically using allozyme markers. Nematodes of this species complex are found in marine finfishes as larvae and in pinnipeds as adults (Mattiucci *et al.*, 2007; Mattiucci and Nascetti, 2008). *Contracaecum osculatum* A occurs in Japanese waters (Mattiucci *et al.*, 1998; Paggi *et al.*, 1998), but before this species was discovered, larval nematodes identifiable with *C. osculatum* sensu lato had been reported as *C. osculatum*, *Contracaecum* (Type A), *Contracaecum* (Type E) or others in Japan. Larvae of these nematodes and *C. osculatum* A parasitize commercially important gadid fishes in Japanese waters, and two cases of human infection with *C. osculatum* s. l. have been reported in Japan. Therefore, both *C. osculatum* s. l. and *C. osculatum* A are important parasites in this country from the viewpoints of fisheries and human parasitology. Based on the information published between 1951 and 2012, this paper

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reviews various aspects of the biology of *C. osculatum* s. l. and *C. osculatum* A in Japanese waters. In this review, the scientific and common names of fishes follow those recommended by Froese and Pauly (2012).

IDENTIFICATION

In Japan, larval *C. osculatum* s. l. were reported using various names of types during the 1960s and 1970s. The larvae from Alaska pollock *Theragra chalcogramma* were described as "*Contracaecum* (Type B)" by Koyama *et al.* (1969), but this type was later renamed "*Contracaecum* sp. (Type A)" by Koyama (1974). Also, the larvae from Alaska pollock and Pacific cod *Gadus macrocephalus* were reported as "*Contracaecum* sp. (type E)" by Kikuchi *et al.* (1970). Shiraki (1974) further described "*Contracaecum*-type larva (A)"from the two gadid species and several other teleosts, and suggested that this larva is identical to *C. osculatum* s. l. Since the mid-1980s, such names have not been used in Japan, but *C. osculatum* s. l. instead has been adopted in the publications (Moravec *et al.*, 1985; Urawa, 1986; Miyamoto, 1990; Kato *et al.*, 1992; Nagasawa, 1993; Konishi and Sakurai, 2002; Abe and Yagi, 2005). Oshima (1974) reviewed various aspects of larval *Contracaecum* reported in Japan mainly during the 1960s and 1970s.

On the other hand, Fagerholm (1988) examined adult nematodes from a ribbon seal *Phoca fasciata* (as *Histriophoca fasciata*) from Hokkaido and identified them as "*C. osculatum*," which has been currently regarded as *C. osculatum* s. l. Before this work, "*Contracaecum callotariae*" was described by Yamaguti (1951) from a northern fur seal *Callorhinus ursinus* (as *Callotaria ursina*) from the Pacific Ocean near Japan. However, Margolis (1956) treated this nematode as a junior synonym of "*C. osculatum*". Yamaguti's paper has never been cited in the current literature.

Recently, based on genetic and morphological studies, both third-stage larvae and adults of *C. osculatum* s. l. taken from Alaska pollock and bearded seals *Erignathus barbatus*, respectively, were identified as *C. osculatum* A (Mattiucci *et al.*, 1998; Paggi *et al.*, 1998).

MORPHOLOGY OF LARVAE

A description of larval *C. osculatum* s. l. was described by Koyama *et al.* (1969) and Kikuchi *et al.* (1970) using specimens from Japanese gadid fishes. Shiraki (1974) and Koyama (1974) also reported the morphology of larval *C. osculatum* s. l. According to Koyama *et al.* (1969) and Kikuchi *et al.* (1970), the larvae possess a relatively small boring tooth and a tapering tail without mucron (Fig. 1). The ventriculus is short, and the venticular appendix is longer than the intestinal caecum. The renette cell is large. The excretory pore opens between subventral lips. The reproductive system is not developed at all. The body is 10.3-27.3 (mean 19.3) mm long and 0.29-0.74 (0.51) mm (Koyama *et al.*, 1969). Also, the morphology of the larvae reported by these authors is morphologically identical to that of larval *Contracaecum* sp. described by Berland (1961) from Norwegian marine fishes.

The external morphology of larval *C. osculatum* s. l. (as "*Contracaecum* type B") from Pacific cod was reported by Weerasooriya *et al.* (1986) based on a scanning electron microscopic (SEM) study. Compared with larvae of three other anisakid genera (*Anisakis*, *Pseudoterranova* and *Hysterothylacium*), larval *C. osculatum* s. l. are characterized by a well-differentiated cephalic structure and a slit-like transverse mouth opening. The cuticle has somewhat regularly spaced, continuous, transverse grooves, which are rather broad and possess a double banded appearance. Parallel, irregularly spaced, longitudinal ridges are seen between the grooves. The tail end is conical. Subsequently, using the same SEM

photographs shown by Weerasooriya *et al*. (1986), the external morphology of the larvae (as "*Contrcaecum osculatum*, third stage") was demonstrated by Ishii *et al.* (1991).

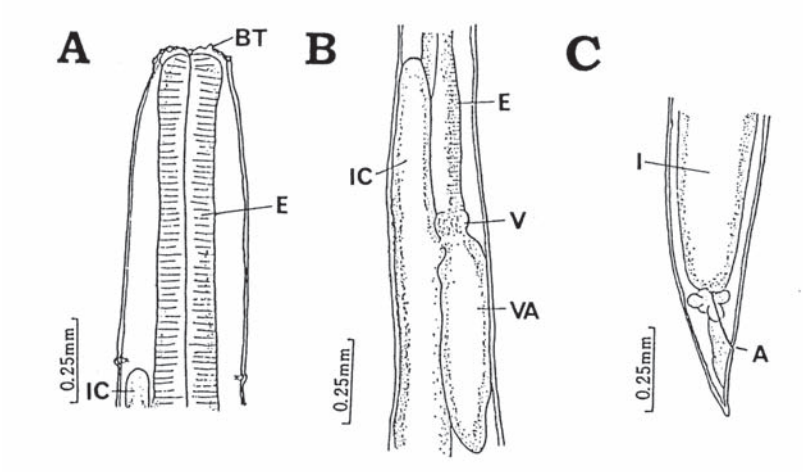

Fig. 1. Anterior (A), middle (B) and posterior (C) parts of a third-stage larva of *Contracaecum osculatum* sensu lato (reported as Type E) (modified from Kikuchi *et al.*, 1970). A: anus, BT: boring tooth, E: esophagus, I: intestine, IC: intestinal caecum, VT: ventriculus, VA: ventricular appendix.

LIFE CYCLE IN JAPANESE WATERS

Based on a work in Europe by Køie and Fagerholm (1995), *C. osculatum* sensu stricto has three hosts in its life cycle: crustacean paratenic hosts, fish intermediate hosts and seal final hosts. In Japan, euphausiids are known to harbor larval *C. osculatum* s. l.: Shimazu and Oshima (1972) found a larva of "*Contracaecum* larva (Type B)" in *Euphausia pacifica* collected in the western North Pacific off northern Honshu and described it. This is the only record of larval *C. osculatum* s. l. from marine invertebrates in Japanese waters.

Marine finfishes serve as intermediate hosts for *C. osculatum* s. l. Third-stage larvae of this nematode have been reported to date from the following 13 species of teleosts belonging to 7 orders and 10 families (fishes are arranged based on their systematics): Alaska pollock *Theragra chalcogramma* (Koyama *et al.*, 1969; Kikuchi *et al.*, 1970, 1975; Sasaki, 1973; Oishi *et al.*, 1974; Suzuki and Oishi, 1974; Suzuki and Matsufuji, 1975, 1976, 1982; Suzuki, 1976, 1979; Mattiucci *et al.*, 1998; Paggi *et al.*, 1998; Konishi and Sakurai, 2002; see also Nagasawa, 1993), Pacific cod *Gadus macrocephalus* (Shiraki, 1969, 1974; Kikuchi *et al.*, 1970, 1975; Oishi *et al.*, 1974; Suzuki and Oishi, 1974; Weerasooriya *et al.*, 1986; Abe and Yagi, 2005; see also Nagasawa, 1993) (Gadiformes: Gadidae); Pacific herring *Clupea pallasii pallasii* (as *C. pallasi*) (Shiraki, 1974) (Clupeiformes: Clupeidae), Japanese anchovy *Engraulis japonicus* (as *Engraulis japonica*) (Kato *et al.*, 1992) (Clupeiformes: Engraulidae); Arctic rainbow smelt *Osmerus mordax dentex* (as *O. mordax*) (Shiraki, 1969, 1974) (Salmoniformes: Osmeridae), chum salmon *Oncorhynchus keta* (Urawa, 1986), masu salmon *Oncorhynchus masou masou* (Kagei *et al.*, 1970a, 1970b) (Salmoniformes: Salmonidae); three-spined stickleback *Gasterosteus aculeatus aculeatus* (as *G. aculeatus*) (Moravec *et al.*, 1985) (Gasterosteiformes: Gasterosteidae); great sculpin *Myoxocephalus polyacanthocephalus* (as *Ainocottus ensiger*) (Suzuki and Matsufuji, 1975; Miyamoto, 1990) (Scorpaeniformes: Cottidae), smooth lumpsucker *Aptocyclus ventricosus* (Machida, 1985)

(Scorpaeniformes: Cyclopteridae); chub mackerel *Scomber japonicu*s (Kosugi, 1972) (Perciformes: Scombridae); flathead flounder *Hippoglossoides dubiu*s (Shiraki, 1974) and Pacific halibut *Hippoglossus stenolepis* (Miyamoto, 1990) (Pleuronectiformes: Pleuronectidae). This host list shows that *C. osculatum* s. l. is not host-specific to finfishes but has a wide host range. Since the two gadid fishes are commercially important, they, especially Alaska pollock, have been frequently examined for the nematode. Moravec *et al.* (1985) found *C. osculatum* s. l. in three-spined stickleback from a freshwater lake, but the infected fish presumably returned from the sea because this fish species is anadromous.

The final hosts of *C. osculatum* s. l. reported in Japanese waters are six species of pinnipeds in two families (cf. Nagasawa, 1999): northern fur seals *Callorhinus ursinus* (Yamaguti, 1951; Machida, 1969b, 1969c, 1971; Takahashi *et al.*, 1998), Steller sea lions *Eumetopias jubatus* (as *E. jubata*) (Orihara, 1963; Ishikura *et al.*, 1996; Takahashi *et al.*, 1998) (Carnivora: Otariidae); spotted seals *Phoca largha* (Nakamura *et al.*, 1965; Machida, 1969a; Miyamato *et al.*, 1979), ribbon seals *Phoca fasciata* (Miyamato *et al.*, 1979; Fagerholm, 1988), harbor seals *Phoca vitulina* (Ito *et al.*, 1998; Takahashi *et al.*, 1998) and bearded seals *Erignathus barbatus* (Ishikura *et al.*, 1996; Mattiucci *et al.*, 1998; Paggi *et al.*, 1998; Takahashi *et al.*, 1998) (Carnivora: Phocidae). Of these pinnipeds, ribbon seals are a preferred final host for *C. osculatum* s. l., because worms can mature into adulthood in these hosts (Konishi and Sakurai, 2002). On the other hand, northern fur seals appear to be a non-preferred final host, because almost of all worms remain as larvae in the hosts (Machida, 1971).

OCCURRENCE OF LARVAE IN ALASKA POLLOCK

As indicated above, there are many records of larval *C. osculatum* s. l. in Alaska pollock in Japanese waters. For this fish species occurring in Hokkaido waters, several stocks have been proposed, and infection levels of larval *C. osculatum* s. l. differ between stocks (Konishi and Sakurai, 2002). Fish of the Nemuro Strait stock were most heavily infected (mean abundance of worms per fish was 7.7 in fish of 450-474 mm long), followed by those of the Pacific coast stock and the Sea of Japan stock (mean abundances were 2.5-4.8 and 1.2, respectively, in the same length class). This regional difference in infection of larval *C. osculatum* s. l. appears to be related with the distribution patterns of final hosts, especially those of ribbon seals, a preferred host of the nematode (Konishi and Sakurai, 2002). Ribbon seals migrate to the Nemuro Strait for wintering and feed abundantly on Alaska pollock (Deguchi *et al.*, 2004). Larval *C. osculatum* s. l. are thus likely to be transmitted from Alaska pollock to ribbon seals in this strait by the prey-predator relationship.

Infection level of larval *C. osculatum* s. l. increases with fish age (Konishi and Sakurai, 2002). The highest number of worms recorded from a single Alaska pollock is 227: this heavily infected fish was caught in the Nemuro Strait (Suzuki and Oishi, 1974).

Larval *C. osculatum* s. l. are usually found on the pyloric caeca of fish, but some are present on other organs in the body cavity (Suzuki and Oishi, 1974). There was no muscular infection.

INFECTIVITY OF LARVAE TO HUMANS AND OTHER MAMMALS

Two cases of human infection with *C. osculatum* s. l. were found in Hokkaido between 1980 and 1996 (Ishikura *et al.*, 1996). Compared with human cases caused by *Anisakis* (26,661 cases) and *Pseudoterranova* (789 cases), the number of cases caused by *C. osculatum* s. l. is quite small, but the disease due to this parasite does exist in Hokkaido. Of the 1,085 worms recovered from the patients of anisakidosis in this region, *C. osculatum* s. l. constitutes only 0.2% (2 worms).

Shiraki (1969), who experimentally administered 50 live larvae of *C. osculatum* s. l. collected from Alaska pollock to a rabbit, found that all the worms lost their motility by three hours postinfection (PI) in the host's stomach, small intestine and caecum. However, different results were obtained by Kikuchi *et al.* (1975) who also made an experimental infection of larval *C. osculatum* (*s. l.*) from Pacific cod, using 3-4 month-old puppies. A total of 230 live larvae were orally administered to nine puppies (20, 25 or 30 worms per puppy), which were dissected at 3-70 hours PI. Worms moved to the small intestine by 5-8 hours PI and then to the large intestine, where they were most abundant at 24-30 hours PI. Worms invading the mucosa of the small intestine were first observed at 5-8 hours PI, and pathological changes were induced at the attachment sites. Of the worms administered, 134 (58%) were found invading the mucosa, and the remaining 61 (27%) and 35 (15%) were free in the lumen and were expelled, respectively. Based on this observation, Kikuchi *et al.* (1975) suggested the possibility that larval *C. osculatum* s. l. penetrate into the wall of the human digestive canal and induce pathological lesions.

Kitayama *et al*. (1967) found a parasitic granuloma in the stomach of a dog from Nemuro, Hokkaido, caused by concurrent infections with larvae of *Contracaecum* sp. and *Pseudoterranova* (as *Terranova*) sp. The authors did not identify these larval nematodes at species level, but it is likely that both are identical to *C. osculatum* s. l. and *P. decipiens* s. l., respectively, because these species are found in marine finfishes collected off Hokkaido (Shiraki, 1974).

FUTURE STUDY

Third-stage larvae and adults of *C. osculatum* s. l., respectively, from Alaska pollock and bearded seals caught in Japanese waters have been identified as *C. osculatum* A (Mattiucci *et al.*, 1998; Paggi *et al.*, 1998). It is thus likely that the nematodes reported before 1998 from Japan as "*C. osculatum*" and various types of larval *Contracaecum* sp. are also identical to *C. osculatum* A. This is probably true for the nematodes collected in subarctic waters off northern Japan, because their final hosts (pinnipeds) migrate to such cold waters. However, when larval and adult *C. osculatum* A were found in Japan (Mattiucci *et al.*, 1998; Paggi *et al.*, 1998), the worms were reported simply as "*C. osculatum* (s. l.)" without describing their morphological characters. As indicated above, *C. osculatum* s. l. have been reported from 13 fishes of finfishes and six species of pinnipeds in Japan. Therefore, it is desirable to collect specimens from these finfish and pinniped hosts and conduct a morphological and genetic study, using such specimens, in order to clarify whether they are identical to *C. osculatum* A.

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日本周辺海域におけるアニサキス科線虫 *Contracaecum osculatum* sensu lato と *C. osculatum* A の生物学：総説

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要　旨　わが国周辺海域の魚類や鰭脚類にそれぞれ幼虫や成虫が寄生するアニサキス科線虫の *Contracaecum osculatum* sensu lato と *C. osculatum* A の生物学的知見を纏めた。わが国では1998年に *C. osculatum* A が見出されるまでは，近年 *C. osculatum* s. l. とされる線虫が型を付した名前や単に *C. osculatum* として報告されてきた。これまでに *C. osculatum* s. l. はオキアミ類1種，海産魚類13種，鰭脚類6種から記録され，*C. osculatum* A はスケトウダラとアゴヒゲアザラシに見出されている。*C. osculatum* s. l. の幼虫はスケトウダラとマダラ，特に前者によく寄生している。*C. osculatum* s. l. の幼虫を実験的に子犬に感染させると腸粘膜に穿入するため，人間の消化管壁にも穿入する可能性があり，実際にわが国で人体寄生例が2例知られる。

キーワード：アニサキス科線虫，寄生虫，スケトウダラ，鰭脚類，*Contracaecum osculatum* A, *Contracaecum osculatum* sensu lato