Zoological Studies **41**(2): 208-215 (2002)

Zoological Studies

Hysterothylacium aduncum (Nematoda: Anisakidae) Infecting a Herbivorous Fish, *Siganus fuscescens*, off the Taiwanese Coast of the Northwest Pacific

Hsiu-Hui Shih[1,*] and Ming-Shiou Jeng[2]

[1]*Department of Zoology, National Taiwan University, Taipei, Taiwan 106, R.O.C.*

[2]*Institute of Zoology, Academia Sinica, Taipei, Taiwan 115, R.O.C.*

(Accepted February 19, 2002)

Hsiu-Hui Shih and Ming-Shiou Jeng (2002) *Hysterothylacium aduncum* (Nematoda: Anisakidae) infecting a herbivorous fish, *Siganus fuscescens,* off the Taiwanese coast of the Northwest Pacific. *Zoological Studies* **41**(2): 207-214. *Hysterothylacium aduncum* (Rudolphi, 1802) infecting a herbivorous fish, *Siganus fuscescens*, and 1 invertebrate zooplankton, a chaetognath, is reported off the Taiwanese coast of the Northwest Pacific. Third-stage larvae were found in the body coelom of the chaetognath, *Sagitta* sp. Fourth-stage larvae and adults were recovered from the stomach and intestines of *S. fuscescens*. Nematodes are described, measured, and illustrated. The prevalence of parasitic infection, mean intensity, and abundance were calculated from samples collected from 2 different Taiwanese coastal locations. The life cycle of *H. aduncum*, involving the host species considered, is also postulated.http://www.sinica.edu.tw/zool/zoolstud/41.2/208.pdf

Key words:*Hysterothylacium aduncum*, *Siganus fuscescens*, Rabbitfish, *Sagitta* sp., Chaetognath.

Hysterothylacium aduncum (Rudolphi, 1802) Deardorff and Overstreet, 1981, is one of the anisakid nematodes which may cause anisakidosis. It lives as sexually mature adults in the digestive tracts of marine teleosts. Its larvae are known to occur in marine invertebrates and in fish (Køie 1993a). The 3rd-stage larvae have been found encapsulated in the mesentery and viscera of a wide range of fish that act as transport hosts (Berland 1961, Køie 1993a). This anisakid has a cosmopolitan and circumpolar distribution. It is found mainly in marine teleosts in temperate and cold waters (Berland 1991), although a few freshwater fishes have been recorded as hosts (Moravec et al. 1985). In the Northern Hemisphere, *H. aduncum* has been reported in fishes collected in the Northeastern Atlantic and the seas north of Europe (Køie 1993b), the Mediterranean Sea (Petter and Maillard 1987), and the Adriatic Sea (Petter and Radujkovic 1989). This nematode is also common in the Pacific and Atlantic waters of North America (Margolis and Arthus 1979, Marcogliese 1996). Comparatively, there is little available information on this nematode recorded from the Northwest Pacific and adjacent waters. Adults of *H. aduncum* collected in the Pacific Ocean off the Kushiro and Tsugaru Straits, Hokkaido, Japan, were first described by Moravec and Nagasawa (1985). Larvae were first recorded from marine fish in the Gulf of Tong King (Sun et al. 1992) and from the Bohai Sea, both off mainland China (Ma et al. 1997).

The rabbitfish, *Siganus fuscescens* Houttuyn, 1782, is herbivorous and distributed in inshore shallow waters. It is an economically important fish species and is cultured in marine cage farms in Taiwan. Since mid-September 2000, the obstruction of this fish's gastrointestinal tract by nematodes has been noted along all seashores of Taiwan.

The aim of this work is to examine the parasitism of *S. fuscescens* captured at 2 locations in the Northwest Pacific off Taiwan, Yehliu (26°N) and Hualien (24°N), and to identify that the infecting

*To whom correspondence and reprint requests should be addressed. Tel: 886-2-23630231 ext. 3818. Fax: 886-2-23636233. E-mail: shihhh@ccms.ntu.edu.tw

nematode is *H. aduncum*. According to the published life history of *H. aduncum*, zooplankton can be the 1st or even 2nd intermediate host (Køie 1993a). Therefore, zooplankton samples were collected between these 2 zones and investigated as possible intermediate hosts for this nematode from the Northwest Pacific.

MATERIALS AND METHODS

In total, 61 adult *S. fuscescens* specimens were collected between September and October 2000 from 2 locations on the eastern and northeastern coasts of Taiwan (Fig. 1). Samples were collected from commercial catches and individual fishermen.

Fish were measured for total length and weight. As each fish was dissected, examinations were made of the heart, liver, spleen, stomach, intestine, gall bladder, swim bladder, and gonads. Recovered parasites were washed in physiological saline, fixed in 5% formalin, and cleared in glycerine for examination. After examination, specimens were preserved in 70% ethanol. Nematodes were counted to obtain the prevalence and abundance (terminology of Margolis et al. 1982). They were then measured and identified using a compound microscope (Olympus) equipped with a calibrated ocular micrometer. All measurements are given in millimeters.

For scanning electron microscopy, adult worms were re-fixed with 1% glutaraldehyde for 12 h and post-fixed with OsO_4 for 1 h. They were then dehydrated, critically dried, and ion sputter-coated. Coated worms were observed under a scanning electron microscope (Hitachi-S2500, Japan) at 15 kV.

Zooplankton samples were taken 4 times in October 2000 from a location in northern Taiwan near Yehliu (Fig. 1). The invertebrates were fixed and preserved in 5% glycerine in 70% ethanol, sorted and identified using a stereomicroscope. All chaetognaths containing nematodes were sorted and photographed using a Zeiss microscope (Axioskop) with an attached Olympus camera (C-2000Z). Larval nematodes dissected from chaetognaths were examined and measured using the same procedure as with the adults.

RESULTS

In the endoparasitic examination, nematodes were recorded only from the stomach and intestine of the samples examined. All of the nematodes were *Hysterothylacium aduncum*. Table 1 shows that the prevalence, mean intensity, and abundance of this nematode were very similar between samples collected from the 2 locations. An extraordinarily high prevalence (70% from Yehliu, 86% from Hualien) and mean intensity (23.8 from Yehliu samples and 27.3 from Hualien samples) were found during this survey. The heavy obstruction of the gastrointestinal tract (Fig. 2), a common phenomenon observed within this study, demonstrated the high mean intensity.

Of approximately 10,000 zooplankton specimens examined, the chaetognath, *Sagitta* sp., was the only species that harbored the parasite. Infected specimens harbored only 1 nematode, *H. aduncum*, found in the body coelom (Fig. 3). The infection rate was 0.05% in these chaetognaths. None of the crus-

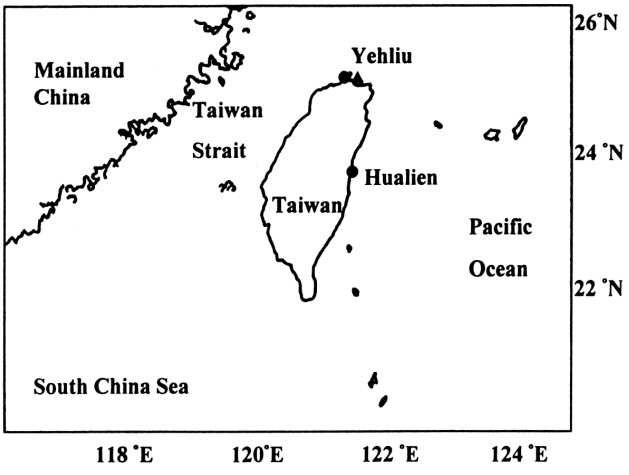

Fig. 1. Map of Taiwan with rabbitfish (●) and plankton (▲) sampling sites.

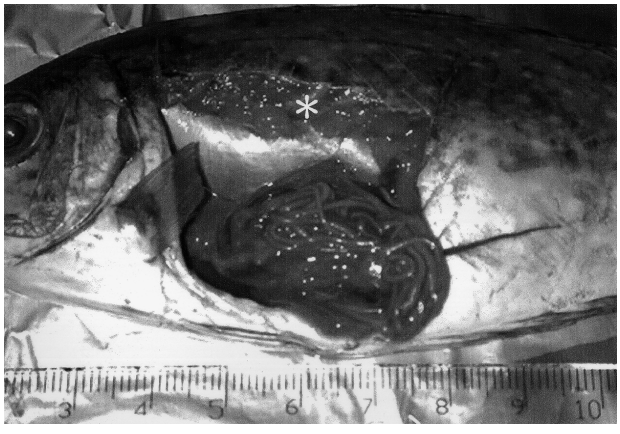

Fig. 2. *Hysterothylacium aduncum* adults in rabbitfish, *Siganus fuscescens*, intestine. Part of the fish's abdominal muscle is cut away (✱).

taceans examined, including amphipods, euphausiids, stomatopods, zoeas, and copepods were infected by this nematode.

Third-stage larvae harvested from the coelom of the chaetognath were divisible into 2 size groups: < 3.5 mm and > 10 mm (Fig. 3). All of them possessed a ventral boring tooth, an intestinal caecum, a short ventriculus, a ventricular appendix, an excretory pore situated just behind the nerve ring, and a conical tail terminating in a spike. Within the tail a "cactus-tail" of the 4th-stage larva can be seen (Fig. 4). The larvae averaged 6.27 (3.34-11.56) in length

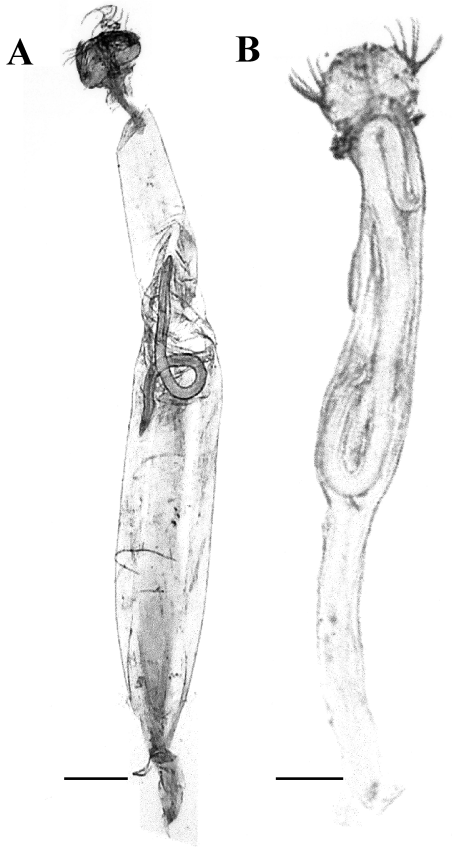

Fig. 3. Naturally infected chaetognaths, *Sagitta* sp., harboring only a single 3rd-stage *Hysterothylacium aduncum* larva within its body coelom. Two larvae sizes are shown: (A) < 3.5 mm and (B) > 10 mm. Scale bar: 2 mm in (A), 1 mm in (B).

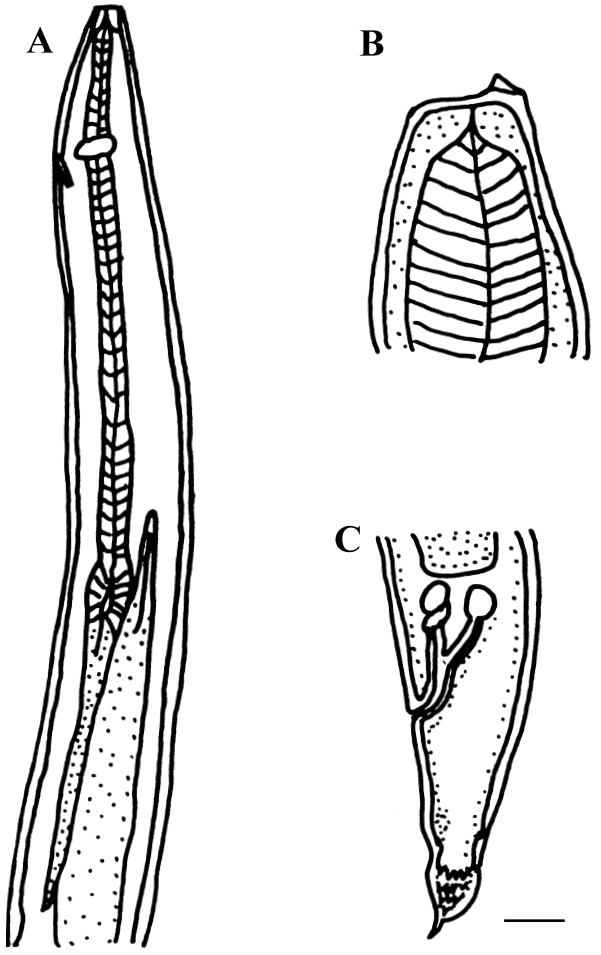

Fig. 4. Third-stage *Hysterothylacium aduncum* larva taken from naturally infected chaetognath. (A) Anterior part, (B) anterior extremity, (C) posterior extremity. Scale bar: 0.1 mm in (A), 0.05 mm in (B, C).

Table 1. Prevalence (P), mean intensity (MI), and abundance (A) of *Hysterothylacium aduncum* infecting free-living rabbitfish, *Siganus fuscescens,* in the Northwest Pacific off the east and northeast coasts of Taiwan

Location	Date	Sample size	Mean weight[1] (g)	Mean length[1] (cm)	P (%)	MI	A
Yehliu	Sept.2000	30	49.9 ± 5.3	14.6 ± 0.9	70.0	23.8	16.66
Hualien	Oct.2000	31	52.3 ± 6.4	16.0 ± 0.6	86.0	27.3	23.48

[1] Presented as the Mean ± S.D.

and 0.098 (0.040-0.110) in width at the nerve ring. The mean length of the esophagus was 0.78 (0.57-1.92), the intestinal caecum was 0.150 (0.095-0.242), and the ventricular appendix was 0.430 (0.350-0.560). The ratio of cecal to ventricular appendix lengths was 1: 2.87-3.75. The nerve ring averaged 0.204 (0.108-0.288) from the anterior extremity. Rectal glands were present and surrounded the rectum.

Fourth-stage larvae collected from rabbitfish digestive tracts had 3 distinct lips with short interlabia and a spinous tail, a feature sometimes referred to as a "cactus tail" (Fig. 5). The larvae averaged 15.42 (10.71-20.64) in length and 0.22 (0.16-0.32) in width at the nerve ring. The mean length of the esophagus was 1.37 (0.92-1.69), the intestinal caecum 0.23 (0.12-0.29), and the ventricular appendix 0.482 (0.43-0.76) . The ratio of caecal to ventricular appendix lengths was 1: 2.12-4.15. The nerve ring averaged 0.42 (0.38-0.52) from the anterior extremity.

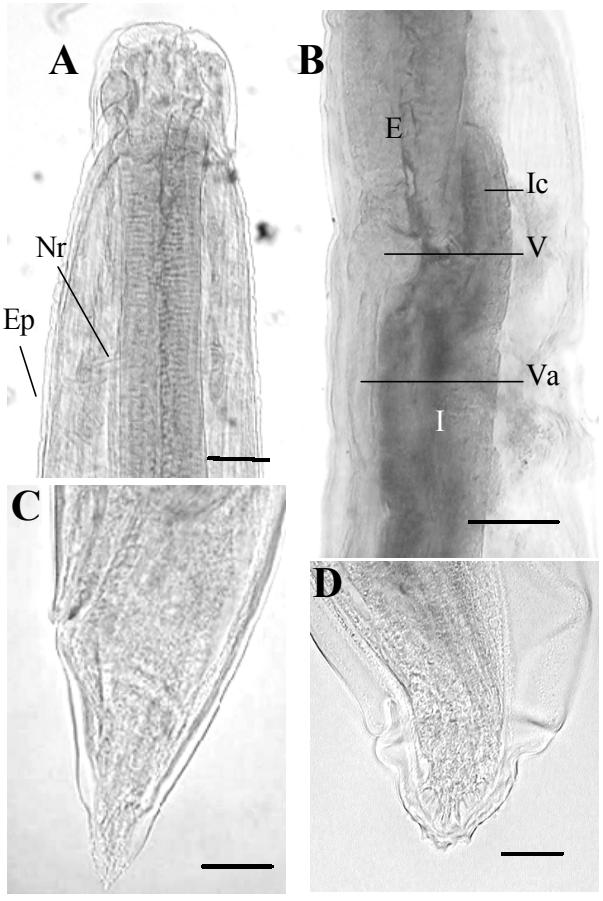

Fig. 5. Fourth-stage *Hysterothylacium aduncum* larva harvested from the intestine of a rabbitfish. (A) Anterior part, excretory pore just behind the nerve ring, (B) body at the intestinal-ventricular junction level, (C) posterior part, (D) posterior extremity. E, esophagus; Ep, excretory pore; I, intestine; Ic, intestinal caecum; Nr, nerve ring; V, entriculus; Va, ventricular appendix. Scale bar: 0.1 mm, except 0.025 mm in (D).

Adult worms harvested from rabbitfish digestive tracts had 1 distinct dorsal lip, 2 ventrolateral lips with short interlabia, and a "cactus tail" (Fig. 6). These structures were very evident on scanning electron micrographs (Fig. 7). At the separate margins of the 3 large lips, an interlabium (Fig. 7A) which was shorter than the lips was observed. Caudal papillae were present in males (Fig. 7B), and the caudal spike of the cactus tail was longer in females (Fig. 7C).

Females averaged 22.1 (10.3-43.2) in length and 0.33 (0.19-0.42) in width. The mean length of the esophagus was 2.31 (1.29-3.37), the intestinal caecum 0.46 (0.22-0.59), and the ventricular appendix 0.89 (0.64-1.09). The ratio of caecal to ventricular appendix lengths was 1: 1.45-3.20. The nerve ring averaged 0.68 (0.64-0.72) from the anterior extremity. A vulva without salient lips opened in the first 1/3 of the body length. Uterine eggs had smooth thin shells. Males averaged 18.7 (8.9-24.8) in length and 0.26 (0.12-0.36) in width. The mean length of the esophagus was 1.83 (1.26-2.96), the intestinal caecum 0.38 (0.32-0.44), and the ventricular appendix 0.82 (0.64-0.96). The ratio of cecal to ventricular appendix lengths was 1: 1.49-3.25. The nerve ring averaged 0.55 (0.41-0.68) from the anterior extremity. The spicules are ventrally alate (Fig. 6E) and averaged 0.78. About 23 pairs of precloacal papillae are arranged in 2 subventral rows; the 10 posterior pairs are closer together (Fig. 7B). Nematode samples described were stored at the Department of Zoology, National Taiwan University.

DISCUSSION

Hysterothylacium has been found to be a valid genus which now includes those species previously considered as members of the junior synonym *Thynnascaris*. These species were considered members of *Contracaecum* in the past (Deardorff and Overstreet 1981). *Hysterothylacium* and *Contracaecum* are closely related genera in the family Anisakidae. They have now been differentiated morphologically by the location of the excretory pore (Deardorff and Overstreet 1980). The excretory pore of *Hysterothylacium* sp. is located at or near the level of the nerve ring, whereas in *Contracaecum* sp., it occurs at the anterior end near the base of the lips. In addition, the definitive hosts of *Hysterothylacium*

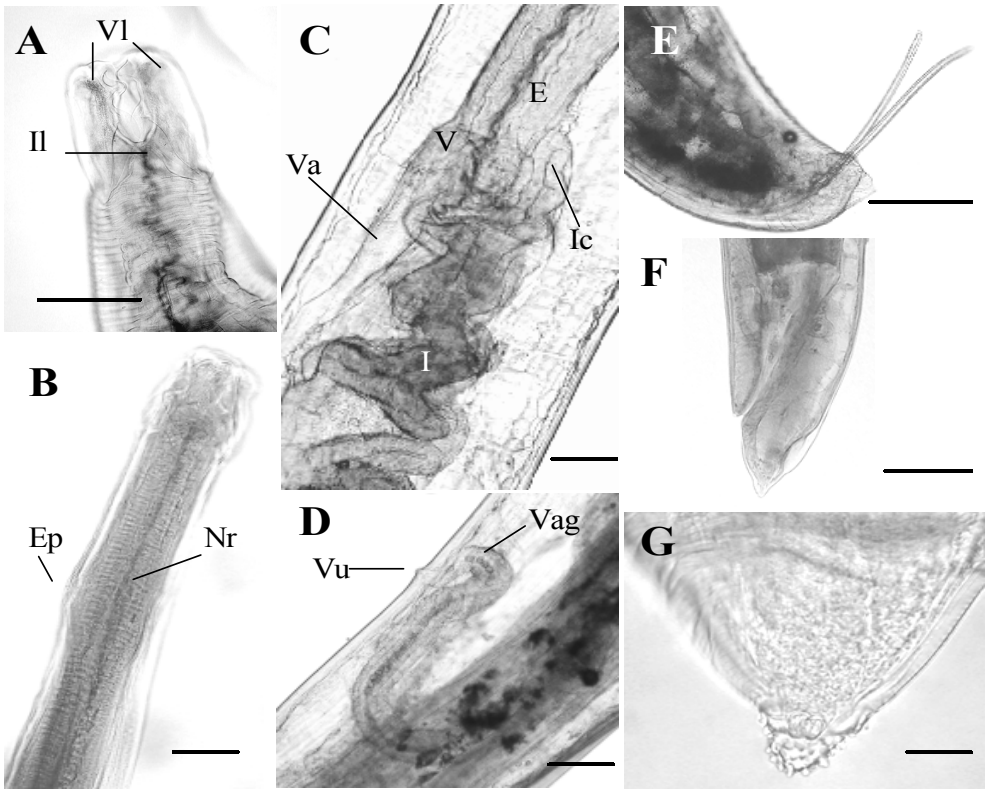

Fig. 6. Adult *Hysterothylacium aduncum* harvested from rabbitfish intestine. (A) Anterior extremity, ventral view; an interlabium is present between the 2 ventrolateral lips, (B) anterior part, excretory pore located just behind the nerve ring, (C) body at the intestinal-ventricular junction level, (D) the vagina opened outside the cuticle, and a vulva is formed, (E) posterior part of a male; 2 spicules are present, (F) posterior part of a female, (G) posterior extremity of a female; a cactus tail is shown. E, esophagus; Ep, excretory pore; I, intestine; Ic, intestinal caecum; Il, interlabium; Nr, nerve ring; Va, ventricular appendix; Vag, vagina; Vl, ventrolateral lips; Vu, vulva. Scale bar: 0.2 mm, except 0.1 mm in (C, D).

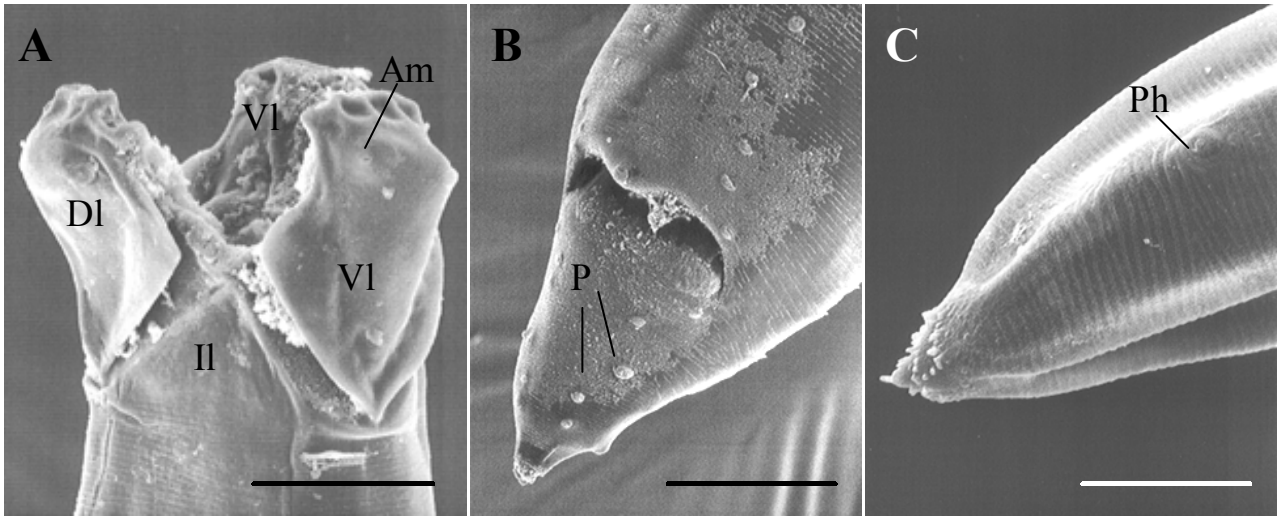

Fig. 7. SEM of adult *Hysterothylacium aduncum* recovered from intestines of infected rabbitfish. (A) Anterior extremity, (B) posterior extremity of a male, (C) posterior extremity of a female. Am, amphid; Dl, dorsal lip; Il, interlabium; P, papilla; Ph, phasmid; Vl, ventolateral lip. Scale bar: 50 μm in (A), 75 μm in (B), and 30 μm in (C).

are piscivorous fishes, not birds and mammals as for *Contracaecum* (Deardorff and Overstreet 1980). The geographic distributions of the various definitive hosts of these 2 genera differ. *Contracaecum* is found in inshore shallow water fishes, while *Hysterothylacium* has an intermediate distribution between inshore and open-water fishes (Moser and Hsieh 1992).

All of the nematodes examined at the larval and adult stages collected from the chaetognath and rabbitfish hosts in this study are considered to belong to the species *H. aduncum* (Rudolphi 1802) Deardorff and Overstreet, 1981. The 3rd-stage and 4th-stage larvae as well as adult worms examined were characterized by the arrangement of the digestive organs, the position of the excretory pore just behind the nerve ring, and the typical "cactus tail" (Yoshinaga et al. 1987a b, Navone et al. 1998). In addition, the morphometric measurements show agreement with those of *H. aduncum* in that the intestinal caecum is usually shorter than the ventricular appendix (Deardorff and Overstreet 1981, Vidal-Martínez et al. 1994).

Until recently, no *Hysterothylacium* species had been described from North and West Pacific fish. Unidentified *Hysterothylacium* 3rd-stage larvae were reported from 4 species of marine fishes from the Bohai Sea, China (Ma et al. 1997). Female *Hysterothylacium* worms were recorded from *Cleisthenes pinetorum herzensteini* caught in the Northwest Pacific off Kushiro, Hokkaido, Japan (Moravec and Nagasawa 1985, Yagi et al. 1996). *Hysterothylacium* sp. larvae were also reported from *Muraenesox cinereus* and *Trichiurus haumela* from China (Sun et al. 1992). There are 2 important contributions from this study. First, this is the 1st record in the entire world of a herbivorous, algae-eating fish, *Siganus fuscescens*, as a definitive host for *H. aduncum*. Second, although the chaetognath, *Sagitta* sp., is a common intermediate host for *Hysterothylacium* nematodes in the Atlantic and adjacent waters (Øresland 1986, Svendsen 1990), this is the 1st case in the Northwest Pacific in which *Sagitta* sp. has been recorded as an intermediate host for *H. aduncum*. In addition to larger piscivorous teleost fish, which eat smaller fish and harbor the adult worms in its gut (Køie 1993a), a quite different definitive or transfer host, the relatively smaller herbivorous rabbitfish, is now confirmed to be involved in the life cycle of *H. aduncum*.

Based on the above observations and information concerning the trophic webs, a postulated life cycle of *H. aduncum* in the studied area is suggested (Fig. 8). Rabbitfish possibly eat infected chaetognaths and harbor 4th-stage larvae and adult worms in its gut, thus playing the role of a definitive or transport host. But it is not the intermediate host, since no 3rd-stage larvae were found encysted in the viscera or free in its body cavities in this study. The chaetognaths, *Sagitta* sp., are unlikely to be the 1st intermediate host, because they are carnivorous and

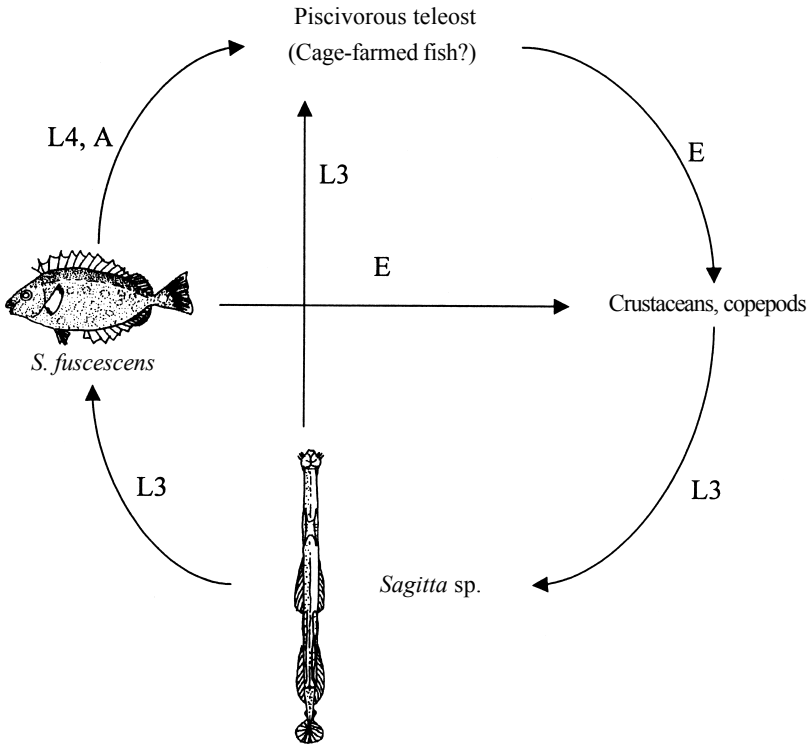

Fig. 8. Postulated life cycle for *Hysterothylacium aduncum*. E, eggs; L3, 3rd-stage larvae; L4, 4th-stage larvae; A, adults.

predatory on zooplankton (Svendsen 1990). Infected crustaceans and copepods, which may play the role of 1st intermediate hosts (Køie 1993a), were not found in this study. Chaetognaths can be infected by eating copepods or crustacean larvae and become the 2nd intermediate or transport hosts of *H. aduncum* (Øresland 1986, Svendsen 1990).

The possibility of *H. aduncum* larvae being transported through the zooplankton community into cage farms and infecting economically important fish in Taiwanese waters is indicated in figure 8. The presence of *H. aduncum* larvae and adults was reported in several native fishes or marine cage farm fish along the South Atlantic Ocean such as in Chilean waters (González 1998). The survey of this nematode in the fauna associated with cage-reared salmonids in Chile showed that the maximum values for the prevalence and mean intensity (79% and 4.9, respectively) of this nematode occurred in the warm springtime. These values are similar to those found in the wild fish, *Merluccius australis* that constitutes their natural definitive host (González 1998). In addition, the highest prevalence and mean intensity (54.8% and 1.40, respectively) for this nematode in whitings, *Merlangius merlangus euxinus*, was also in the warm months of July/August from the Turkish coast of the Black Sea (Ismen and Bingel 1999). In comparison, a similar prevalence value, but a much higher mean intensity (23.8 and 27.3 at 2 locations) for *H. aduncum* was observed in wild rabbitfish in this study. Heavy gastrointestinal tract obstruction was a common feature of infected fish. Whether this implies a dormant threat to the rising cage farming industry in Taiwanese waters or simply an opportunistic infection in rabbitfish is being further investigated.

Acknowledgments: This work was supported by the Council of Agriculture, Fisheries Adminstration under grant 90AS-1.1.4-FA-F1(7). We would like to extend our sincere thanks to Dr. K.T. Shao, Director of the Institute of Zoology, Academia Sinica, for help with fish and zooplankton sample collection.

REFERENCES

Berland B. 1961. Nematodes from some Norwegian marine fishes. Sarsia **2:** 1- 50.

Berland B. 1991. *Hysterothylacium aduncum* (Nematoda) in fish. ICES Identification leaflets for diseases and parasites of fish and shellfish, Leaflet No. 44, 4 p.

Deardorff TL, RM Overstreet. 1980. Review of *Hysterothylacium* and *Iheringascaris* (both previously = *Thynnascaris*) (Nematoda: Anisakidae) from the northern Gulf of Mexico. Proc. Biol. Soc. Wash. **93:** 1035-1079.

Deardorff TL, RM Overstreet. 1981. Larval *Hysterothylacium* (=*Thynnascaris*) (Nematoda: Anisakidae) from fishes and invertebrates in the Gulf of Mexico. Proc. Helminthol. Soc. Wash. **48:** 113-126.

González L. 1998. The life cycle of *Hysterothylacium aduncum* (Nematoda: Anisakidae) in Chilean marine farms. Aquaculture **162:** 173-186.

Ismen A, F Bingel. 1999. Nematode infection in the whiting *Merlangius merlangus euxinus* off Turkish Coast of the Black Sea. Fish. Res. **42:** 183-189.

Køie M. 1993a. Aspects of the life cycle and morphology of *Hysterothylacium aduncum* (Rudolphi, 1802) (Nematoda, Ascaridoidea, Anisakidae). Can. J. Zool. **71:** 1289-1296.

Køie M. 1993b. Nematode parasites in teleosts from 0 to 1540 m depth off the Faroe Islands (the North Atlantic). Ophelia **38:** 217-243.

Ma HW, TJ Jiang, FS Quan, XG Chen, HD Wang, YS Zhang, MS Cui, WY Zhi, DC Jiang. 1997. The infection status of anisakid larvae in marine fish and cephalopods from the Bohai Sea, China and their taxonomical consideration. Korean J. Parasitol. **35:** 19-24.

Marcogliese DJ. 1996. Larval parasitic nematodes infecting marine crustaceans in eastern Canada. 3 *Hysterothylacium aduncum*. J. Helminthol. Soc. Wash. **63:** 12-18.

Margolis L, JR Arthus. 1979. Synopsis of the parasites of fishes of Canada. Bull. Fish. Res. Board Can. **199:** 269.

Margolis L, GW Esch, JC Holmes, AM Kuris, GA Schad. 1982. The use of ecological terms in parasitology (report of an ad hoc committee of the American Society of Parasitologists). J. Parasitol. **68:** 131-133.

Moravec F, K Nagasawa. 1985. *Ichthyofilaria japonica* sp.n. (Philometridae) and some other nematodes from Hokkaido, Japan. Vest. cs. Spolec. Zool. **49:** 211-223.

Moravec F, K Nagasawa. S Urawa. 1985. Some fish nematodes from fresh waters in Hokkaido, Japan. Praha **32:** 305-316.

Moser M, J Hsieh. 1992. Biological tags for stock separation in Pacific herring *Clupea harengus pallasi* in California. J. Parasitol. **78:** 54-60.

Navone GT, NH Sardella, JT Timi. 1998. Larvae and adults of *Hysterothylacium aduncum* (Rudolphi, 1802) (Nematoda: Anisakidae) in fishes and crustaceans in the South West Atlantic. Parasite **5:** 127-136.

Øresland V. 1986. Parasites of the chaetognath *Sagitta setosa* in the western English Channel. Mar. Biol. **92:** 87-91.

Petter AJ, C Maillard. 1987. Ascarides de poissons de Méditerranée occidentale. Bull. Mus. Natl. d'Histoire Nat., Paris, 4e série, sec. A, **9:** 773-798.

Petter AJ, J Radujkovic. 1989. Parasites des Poissons marins du Montenegro: Nematodes. Acta Adriatica **30:** 195-236.

Sun SZ, T Koyama, N Kegei. 1992. Morphological and taxonomical studies on Anisakidae larvae found in marine fishes of China II. Gulf of Tong King. Chin. J. Parasitol. Parasit. **10:** 108-112. (in Chinese with English abstract)

Svendsen YS. 1990. Hosts of third stage larvae of *Hysterothylacium* sp. (Nematoda, Anisakidae) in zooplankton from outer Oslofjord, Norway. Sarsia **75:** 161-167.

Vidal-Martínez VM, D Osorio-Sarabia, RM Overstreet. 1994. Experimental infection of *Contracaecum multipapilatum* (Nematoda: Anisakinae) from Mexico in the domestic cat. J. Parasitol. **80:** 576-579.

Yagi K, K Nagasawa, H Ishikura, A Nakagawa, N Sato, K Kikuchi, H Ishikura. 1996. Female worm *Hysterothylacium*

aduncum excreted from human: a case report. Jpn. J. Parasitol. **45:** 12-23.

Yoshinaga T, K Ogawa, H Wakabayashi. 1987a. Experimental life cycle of *Hysterothylacium aduncum* (Nematoda: Anisakidae) in fresh water. Fish Pathol. **22:** 243-251.

Yoshinaga T, K Ogawa, H Wakabayashi. 1987b. New record of third-stage larvae of *Hysterothylacium aduncum* (Nematoda: Anisakidae) from *Neomysis intermedia* (Crustacea: Mysidae) in a freshwater lake in Hokkaido, Japan. Nippon Suisan Gakkai. **53:** 63-65.

太平洋西北臺灣沿岸之象魚發生有鉤宮脂線蟲感染

施秀惠[1] 鄭明修[2]

本研究中報告位於太平洋西北海域臺灣沿岸之水族發生有鉤宮脂線蟲感染，受感染者為嗜食藻類的褐籃子魚（象魚）和浮游動物中的箭蟲。由箭蟲體腔內分離出此線蟲之第三期幼蟲，而在象魚腸道及胃中分離出第四期幼蟲和成蟲。本文中詳細描述第三、第四期及成蟲之特徵，測量並圖示其各種構造。由臺灣兩處海岸收集象魚標本，分別統計此線蟲之盛行率、平均密度和豐富度。最後根據調查所知可能之寄主，推測此線蟲在臺灣海域可能的生活史。

關鍵詞：宮脂線蟲，褐籃子魚，象魚，箭蟲屬，箭蟲。

[1] 國立臺灣大學動物學系

[2] 中央研究院動物研究所