

Occurrence of *Hysterothylacium* and *Anisakis* nematodes (Ascaridida: Ascaridoidea) in the tanaka's snailfish *Liparis tanakae* (Gilbert & Burke) (Scorpaeniformes: Liparidae)

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Abstract The tanaka's snailfish *Liparis tanakae* (Gilbert & Burke) (Scorpaeniformes: Liparidae) is an economically important marine fish species in China. However, the helminth parasites of this fish are still poorly known. During a helminthological survey of Chinese marine fishes from 2011 to 2012, we revealed that *L. tanakae* was heavily infected with third-stage larvae and adults of ascaridoid nematodes (total prevalence 100 % and mean intensity 82.3 nematodes per fish). Four species of third-stage larvae *Hysterothylacium liparis* Li, Xu & Zhang, 2007, *H. aduncum* (Rudolphi, 1802), *Hysterothylacium fabri* (Rudolphi, 1819), and *Anisakis pegreffii* (Campana-Rouget & Biocca, 1955) and a single species of adults *H. liparis* were differentiated and identified by morphological and molecular methods. The detailed morphology of the four species of third-stage larvae was also studied using light microscopy and scanning electron microscopy. The morphological and molecular characterization of the third-stage larvae of *H. liparis* was reported. *Liparis tanakae* represents a new host record for *A. pegreffii* and *H. fabri*. In addition, a new name, *Hysterothylacium zhoushanense* nom. nov. was also given to *Hysterothylacium zhoushanensis* Li, Liu & Zhang, 2012 to make the latinized specific epithet agree with this neuter generic name.

Introduction

The tanaka's snailfish *Liparis tanakae* (Gilbert & Burke) (Scorpaeniformes: Liparidae) is endemic to Northwest Pacific and only distributes in the Yellow Sea, East China Sea, Sea of Japan, Sea of Okhotsk and Pacific coast of Japan to northern Kuril Islands (Yamada et al. 1995). During a helminthological survey of Chinese marine fishes by two of the authors of the present study (Li and Zhang) from 2011 to 2012, large numbers of third-stage larvae and adults of ascaridoid nematodes were collected from *L. tanakae* in the Yellow Sea and East China Sea. The specific identification of any developmental stage and sex of ascaridoid nematodes is central to exploring and understanding their life cycles, epidemiology, ecology, population genetics and control (Gasser 2006; Zhang et al. 2007). However, it is often problematic to achieve the objective purely on the basis of morphological features. In recent years, many studies have proved that integrating DNA data and morphological features can overcome the limitations of traditional approaches and attain maximum efficiency for species separation and identification of ascaridoid nematodes (Zhu et al. 2007; Mattiucci et al. 2008; Shamsi et al. 2009, 2012, 2013; Mašová et al. 2010; Garbin et al. 2011; Testini et al. 2011; Xu et al. 2012; Li et al. 2012a, b, c, d; Liu et al. 2013; Zhang et al. 2013; Silva et al. 2013). Consequently, these third-stage larvae and adults of ascaridoid nematodes collected from *L. tanakae* were exactly identified utilizing morphological examination and molecular methods by sequencing and analyzing the internal transcribed spacer (ITS) of nuclear ribosomal DNA (rDNA).

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Materials and methods

Light and scanning electron microscopy

Fishes caught by commercial trawlers from the Yellow Sea (off Shidao, Shandong Province) and East China Sea (off Zhou-shan Island, Zhejiang Province), respectively, were examined for parasites. Nematodes recovered from the abdominal cavity, subcutaneous layer of the skin, muscle, and digestive tract of *L. tanakae* (Fig. 1a–d) were washed in physiological saline and then fixed and stored in 80 % ethanol until studied. Light and scanning electron microscopical studies were prepared following the methods used by Li et al. (2012a). Drawings were made with the aid of Nikon microscope drawing attachment. Measurements (the range, followed by the mean in parentheses) are given in micrometres unless otherwise stated. Voucher specimens are deposited in College of Life Science, Hebei Normal University, Hebei Province, China.

Molecular procedures

Adult nematodes were identified as *Hysterothylacium liparis* on the basis of diagnostic characters including the body size, the morphology of the lips, the length of esophagus, intestinal caecum, ventricular appendix, spicules, the number and arrangement of caudal papillae, and the morphology of the tail tip. Larval specimens were identified to different morphotypes or generic level based on the morphological characters including the position of the excretory pore, the absence or presence of the intestinal caecum, and ventricular appendix, the shape of the tail, the length and ratio of the intestinal caecum, and ventricular appendix; and then some adults and larval individuals of each morphotype were randomly selected for molecular

analysis (for details, see Table 1). Genomic DNA from individual worms was extracted using a Column Genomic DNA Isolation Kit (Shanghai Sangon, China) according to the manufacturer's instructions. DNA was eluted in elution buffer and kept at -20 °C until use. The ITS region was amplified by PCR using the primers and cycling conditions described previously (Li et al. 2012a). PCR products were checked on GoldView-stained 1.5 % agarose gel and purified by the Column PCR Product Purification Kit (Shanghai Sangon, China). Sequencing was carried out using a DyeDeoxyTerminator Cycle Sequencing Kit (v.2, Applied Biosystems, California, USA) and an automated sequencer (ABI-PRISM 377). Sequencing for each sample was carried out for both strands. Sequences were aligned using ClustalW2 (Thompson et al. 1994) and adjusted manually. The ITS sequences determined were compared (using the algorithm BLASTn) with those available in the National Center for Biotechnology Information (NCBI) database (<http://www.ncbi.nlm.nih.gov>).

Results

Third-stage larvae of *Hysterothylacium liparis* Li, Xu and Zhang, 2007

Diagnosis (n=10)

Body is small, whitish, with finely transversely striated cuticle, 5.6–11.3 (9.2) mm long; maximum width is 97–223 (183). It's cephalic end rounded, with small ventral cuticular tooth (Figs. 2a, b and 3a, b). Oral aperture is triangular, surrounded by weakly developed anlagen of lips (Fig. 3a, b). Esophagus is almost cylindrical, muscular, 796–1,505 (1,182)

Fig. 1 *Liparis tanakae* heavily infected with ascariidoid nematodes from the Yellow Sea, China: **a** anterior part of body, dorsal view; **b** nematode under the skin of the fish (arrowed); **c** nematode penetrates through the muscle of fish; **d** large numbers of nematodes occur in the digestive tract of fish

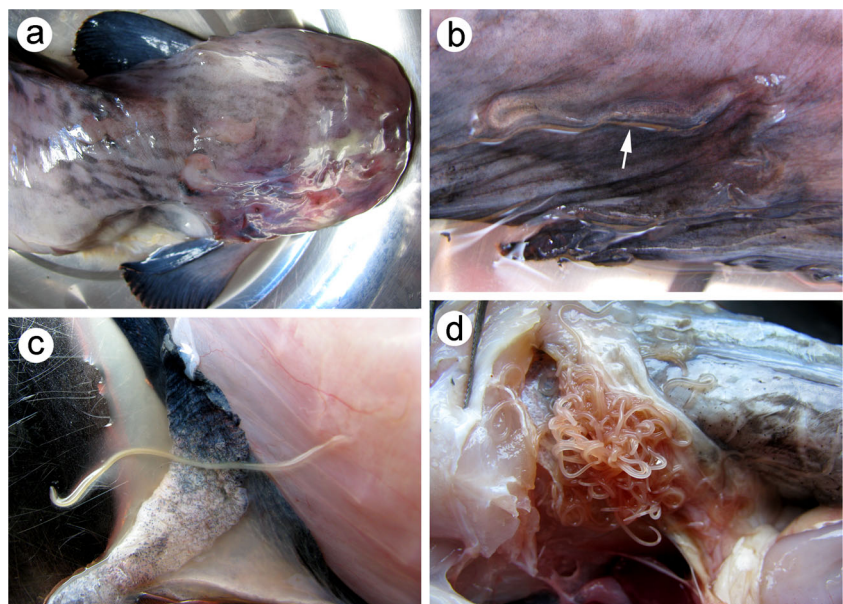


Table 1 Specimens of *Hysterothylacium* and *Anisakis* nematodes collected from *Liparis tanakae* in the Yellow Sea and East China Sea selected for molecular analysis

Species	Nematode individuals randomly selected for molecular analysis	Host	Locality
<i>Anisakis pegreffii</i>	3 third-stage larvae	<i>L. tanakae</i>	East China Sea
<i>Hysterothylacium liparis</i>	1 male, 2 females (paratypes)	<i>L. tanakae</i>	Yellow Sea, China
<i>H. liparis</i>	1 male, 2 females	<i>L. tanakae</i>	East China Sea
<i>H. liparis</i>	3 third-stage larvae	<i>L. tanakae</i>	Yellow Sea, China
<i>Hysterothylacium fabri</i>	9 third-stage larvae	<i>L. tanakae</i>	East China Sea
<i>H. fabri</i>	1 male, 1 female	<i>Uranoscopus japonicus</i>	East China Sea
<i>Hysterothylacium aduncum</i>	1 male, 1 female	<i>Lophius litulon</i>	Yellow Sea, China
<i>H. aduncum</i>	3 third-stage larvae	<i>L. tanakae</i>	Yellow Sea, China

long, and 49–97 (76) in maximum width, representing 11.4–15.6 (13.0)% of body length (Fig. 2b). Nerve ring is 252–388 (320) and excretory pore is 291–437 (375) from anterior extremity, respectively. Ventriculus oval to oblong, almost as wide as posterior region of esophagus, is 49–127 (89) long, and 44–98 (67) wide. Ventricular appendix is slightly shorter

than intestinal caecum, 441–578 (460) long, 39–74 (53) in maximum width. Intestinal caecum is 583–971 (781) long and 49–78 (62) wide, representing 57.1–75.0 % esophageal length. Ratio of intestinal caecum to ventricular appendix 1.3–1.9:1 (1.7:1) (Fig. 2b). Tail is 87–146 (102) long; tip of tail is rounded, without any ornament (Figs. 2c, d and 3c).

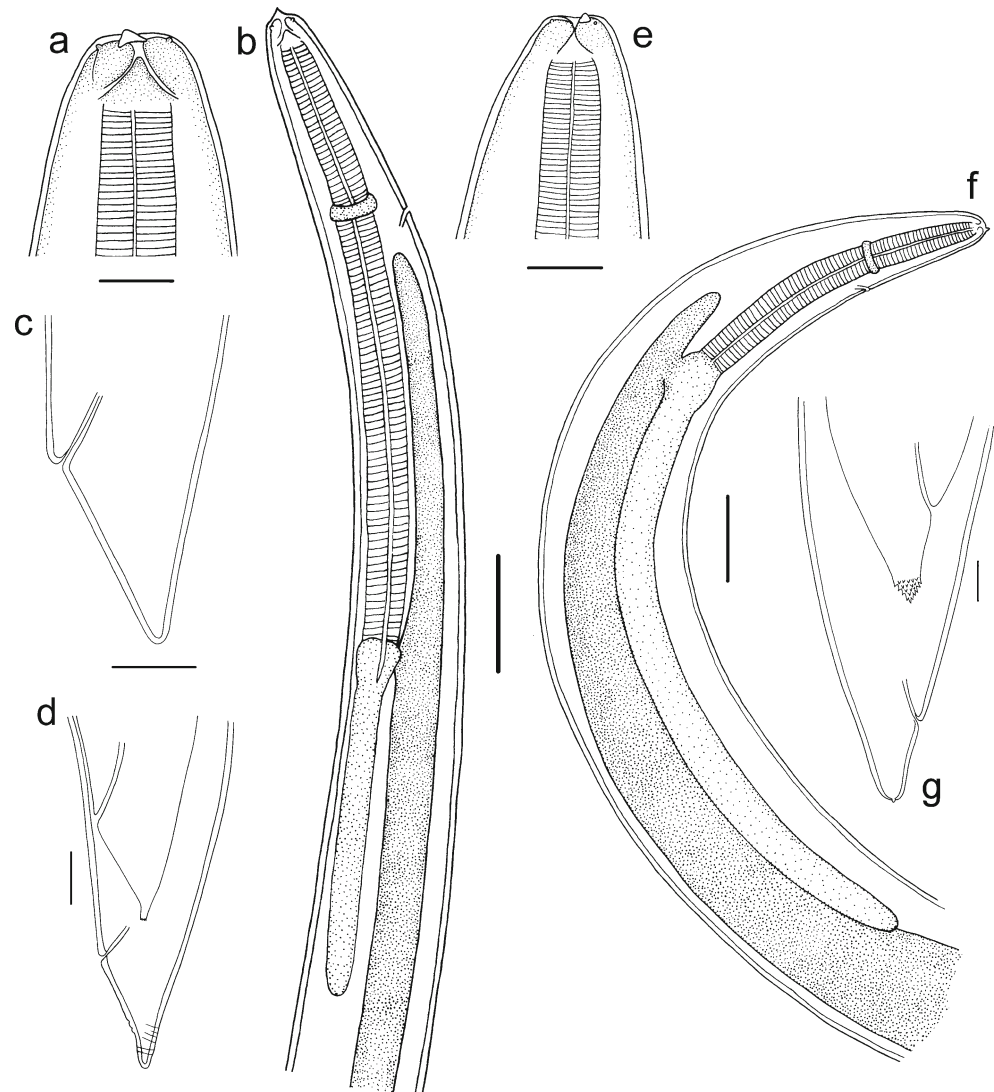
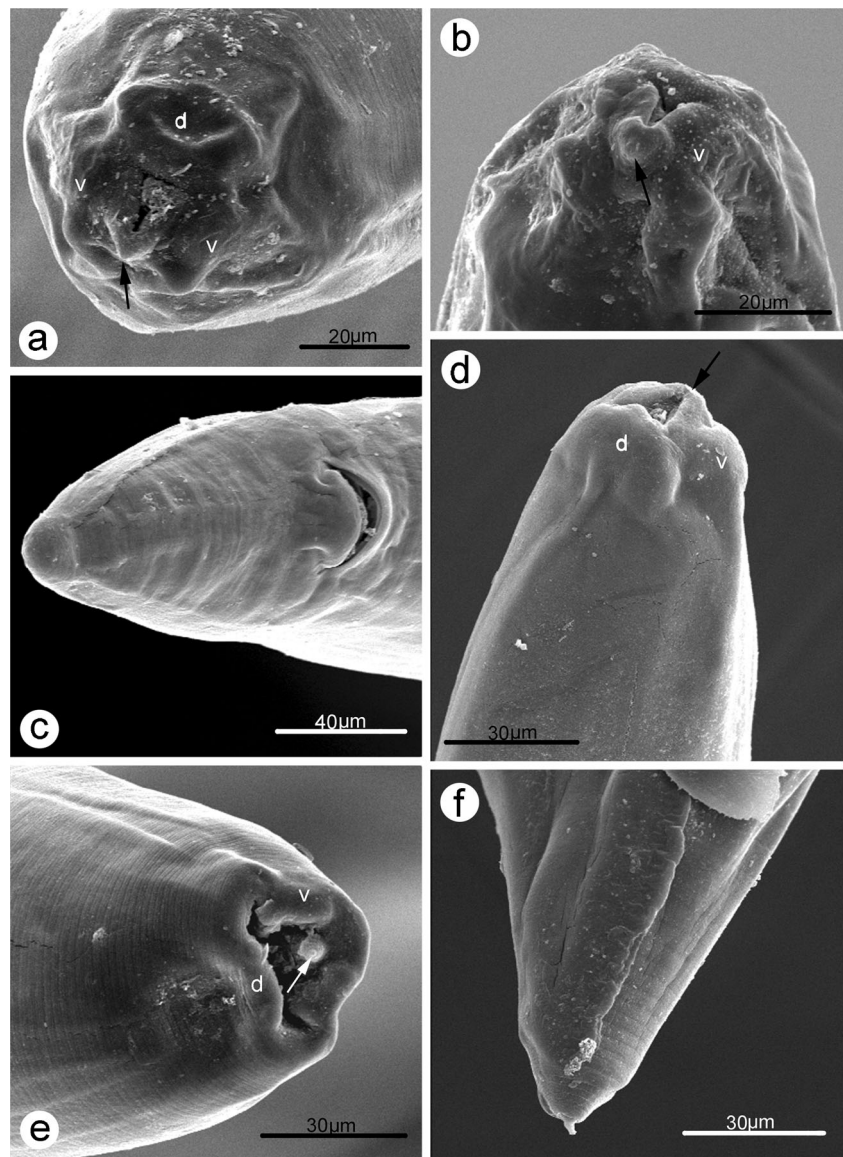
Fig. 2 Third-stage larva of *Hysterothylacium liparis* (a–d) and *Hysterothylacium fabri* (e–g) from *Liparis tanakae*. a, e cephalic end, lateral view; b, f anterior part of body, lateral view; c, d, g posterior end of body, lateral view. Scale bars: a, c–e, g 50 μ m; b, f 200 μ m

Fig. 3 Scanning electron micrographs of third-stage larva from *Liparis tanakae*.

Hysterothylacium liparis (a–c): a cephalic end, apical view (cuticular tooth arrowed); b cephalic end, subapical view (cuticular tooth arrowed); c posterior end of body, ventral view. *Hysterothylacium fabri* (d–f): d cephalic end, lateral view (cuticular tooth arrowed); e cephalic end, apical view; f tip of tail, lateral view



Host and locality of third-stage larvae

Host is Tanaka's snailfish *L. tanakae* (Gilbert & Burke) (Scorpaeniformes: Liparidae) found at Yellow Sea (off Shidao, Shandong Province) and East China Sea (off Zhoushan Island, Zhejiang Province), China.

Site of infection

Abdominal cavity, subcutaneous layer of the skin, muscle, and intestine are the sites of infection.

Prevalence and intensity of infection

Six out of ten fishes were infected with intensity of 1–118 (mean=24.5) specimens.

Voucher specimens

There were 140 specimens (HBNU-F13043L).

Adults of *Hysterothylacium liparis* Li, Xu & Zhang, 2007

Diagnosis

The diagnostic characters of the adults—see Li et al. (2007).

Host and locality of adults

Host is Tanaka's snailfish *L. tanakae* (Gilbert & Burke) (Scorpaeniformes: Liparidae) found at Yellow Sea (off Shidao, Shandong Province) and East China Sea (off Zhoushan Island, Zhejiang Province), China.

Site of infection

Intestine and stomach are the sites of infection

Prevalence and intensity of infection

Ten out of ten fishes were infected with intensity of 1–46 (mean=20.1) specimens.

Voucher specimens

There were 201 specimens (HBNU-F13044L).

DNA characterization

There were six different sequence types obtained for the ITS region of the partial rDNA based on the present material examined herein and the length are all 1,007 bp. The different sequence types showed very low level of nucleotide variability (0.1–0.2 % nucleotide difference, details see Table 2). There are 16 species of *Hysterothylacium* ITS sequences registered in GenBank and pairwise comparison between *H. liparis* and the other species of *Hysterothylacium* (except *Hysterothylacium* sp. HS1) registered in GenBank displayed 7.5 % (*H. thalassini*) to 22.3 % (*Hysterothylacium zhoushanense*) nucleotide difference. Thus, we could consider the low level of nucleotide difference among the six different genotypes obtained herein as intraspecific nucleotide difference because of the level far lower than the interspecific nucleotide difference. In addition, there was only 0.2 % of nucleotide difference between *H. liparis* and *Hysterothylacium* sp. HS1 (AM706344) in the ITS region. We considered the species *Hysterothylacium* sp. HS1 collected from *Conger myriaster* (Brevoort) (Anguilliformes: Congridae) also from the Yellow sea, China (Off Weihai, Shangdong Province) should belong to *H. liparis*. The ITS sequence of *H. liparis* are deposited in the GenBank database (<http://www.ncbi.nlm.nih.gov>) under accession numbers (KF601895–KF601900).

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Remarks

Li et al. (2007) described *Hysterothylacium liparis* and its fourth-stage larvae from the digestive tract of *L. tanakae* in the Yellow Sea, China. However, the third-stage larvae have not been found until now. The accurate identification of the third-stage larvae of *Hysterothylacium* purely based on morphological features is almost impossible because of the third-stage larvae of *Hysterothylacium* usually with very limited morphological features of taxonomic significance and their morphology commonly evidently different from the adults (Li et al. 2012a, b). The morphology and measurements of adults agree very well with the type material of *H. liparis* (deposited in College of Life Science, Hebei Normal University, Hebei Province, China), and the present material was also from the type host, *L. tanakae*, in the same or very neighboring region of the Northwest Pacific. Consequently, we considered our present specimens to be conspecific with *L. tanakae*. The morphology of the cephalic end and the tail of the present third-stage larvae is distinctly different from the adults of *H. liparis* and the relative oesophageal length of the third-stage larvae is much longer than the adults (representing 11.4–15.6 % of body length in the third-stage larvae vs. 7.1–10.9 % of body length in adults of *H. liparis*). However, the ratio of intestinal caecum and ventricular appendix of the present third-stage larvae is very similar to the adults, and they are collected from the same host *L. tanakae*. We therefore speculated that the present third-stage larvae might be congeneric with *H. liparis*. For further clarifying the taxonomic status of the present material, the ITS region of the paratypes of *H. liparis* and the present adults and putative third-stage larvae was sequenced and analyzed. The result of molecular analysis confirmed that the present adult and larval nematodes and the paratypes of *H. liparis* are homogeneous genetically and all belong to the same species.

Table 2 The sequence polymorphisms revealed at alignment positions among the different six sequence types of *Hysterothylacium liparis* in the ITS region obtained in the present study

GenBank nos.	Sequence polymorphisms revealed at alignment positions					
	216	272	395	555	736	892
KF601895	T	G	G	A	C	G
KF601896	T	A	G	A	T	G
KF601897	T	G	A	A	T	G
KF601898	T	G	G	A	T	G
KF601899	C	G	G	A	T	G
KF601900	T	G	G	G	T	A

Third-stage larvae of *Hysterothylacium fabri* (Rudolphi, 1819)

Diagnosis (n= 10)

Body is very variable in length, whitish, with finely transversely striated cuticle, 6.0–15.7 (10.8) mm long; maximum width is 243–437 (322). It's cephalic end rounded, with very small ventral cuticular tooth (Figs. 2e, f and 3d, e). Oral aperture is triangular, surrounded by weakly developed anlagen of lips (Fig. 3d,e). Esophagus is almost cylindrical, muscular, 631–1068 (866) long, 68–107 (83.0) in maximum width, representing 6.5–10.6 (8.3)% of body length (Fig. 2f). Nerve ring is 223–350 (288) and excretory pore is 262–417 (341) from anterior extremity, respectively. Ventriculus is oval

to oblong, slightly wider than posterior region of esophagus, 88–172 (115) long, 64–123 (85.0) wide. Ventricular appendix is much longer than intestinal caecum, 757–1233 (1015) long, and 49–136 (87.0) in maximum width. Intestinal caecum is very short, 181–368 (267) long, and 44–78 (64.0) wide, representing 1.9–4.0 (3.1)% esophageal length. Ratio of intestinal caecum to ventricular appendix is 0.2–0.4:1 (0.3:1) (Fig. 2f). Tail is 107–175 (125) long; tip of tail is with a very small cuticular spike (Figs. 2g and 3f).

Host and locality of third-stage larvae

Host is Tanaka's snailfish *L. tanakae* (Gilbert & Burke) (Scorpaeniformes: Liparidae) found at Yellow Sea (off Shidao, Shandong Province) and East China Sea, (off Zhou-shan Island, Zhejiang Province), China.

Site of infection

Abdominal cavity is the site of infection.

Prevalence and intensity of infection

Three out of ten fishes were infected with intensity of 1–15 (mean=8.0) specimens.

Voucher specimens

There were 19 specimens (HBNU-F13045L).

DNA characterization

There were six different sequence types obtained for the ITS region of the partial rDNA based on the present material examined herein and the length are from 1,001 to 1,002 bp. The different sequence types showed 0.1–0.4 % nucleotide variability (details see Table 3). There are three ITS sequences of *H. fabri* registered in GenBank (nos. KC852206, JQ520158, JX974558) and pairwise comparison between the present data and the three ITS sequences of *H. fabri* registered in GenBank displayed very low level of nucleotide variability

(0–0.4 % nucleotide difference). Thus, we considered the present material to be conspecific with *H. fabri*. The ITS sequences of *H. fabri* are deposited in the GenBank database (<http://www.ncbi.nlm.nih.gov>) under accession numbers (KF736939–KF736944).

Remarks

H. fabri is a common parasite found in various Mediterranean fishes (Petter et al. 1984; Petter and Radujkovic 1986; Petter and Maillard 1987; Martín-Sánchez et al. 2003). Li et al. (2008a) reported it from three marine fishes in the Chinese waters. However, *H. fabri* is still a poorly known species, especially about its taxonomic status. Martín-Sánchez et al. (2003) studied its population genetic diversity and revealed that *H. fabri* collected from the Mediterranean fishes should be a complex comprising at least three sibling species. Our unpublished molecular data also proved that there were some significant genetic variations detected in the ITS region of *H. fabri* based on the adults collected from the Uranoscopidae fish in the Chinese waters and considered at least two sibling species to be in existence within the *H. fabri* complex (Li et al., unpublished). Nevertheless, the present molecular data supported that the third-stage larvae of *H. fabri* parasitizing *L. tanakae* maybe represent a single species. Moravec (1994) described the third-stage larvae of *H. fabri* based on the specimens collected from *Salmo trutta fario* Linnaeus (Salmoniformes: Salmonidae), but his material is distinctly smaller than ours in body size (1.8–2.2 mm of Moravec's material vs. 6.0–15.7 mm of ours) and the morphology of the tail tip of his material is also different from ours (tail tip without the small spine in Moravec's material). Moravec (1994, 1998) considered that a species identification of *Hysterothylacium* larvae purely on the basis of their morphology is often problematic. Therefore, the taxonomic status of the third-stage larvae identified as *H. fabri* by Moravec (1994) remains unclear. In the present study, the adult nematodes identified morphologically as *H. fabri* collected from *Uranoscopus japonicus* Houttuyn (Perciformes: Uranoscopidae) was firstly characterized using molecular methods by sequencing and analyzing the ITS region of

Table 3 The sequence polymorphisms revealed at alignment positions between the six different sequence types of *Hysterothylacium fabri* in the ITS region obtained in the present study

GenBank nos.	Sequence polymorphisms revealed at alignment positions									
	126	260	321	447	483	523	527	870	981	998
KF736944	–	T	T	A	A	T	A	T	G	A
KF736943	T	T	T	A	A	C	A	T	A	C
KF736942	–	T	C	A	A	T	A	T	G	A
KF736941	–	T	T	A	A	T	A	C	G	A
KF736940	–	C	T	G	G	T	A	T	G	A
KF736939	–	T	T	A	A	T	G	T	G	A

rDNA, and then the ITS sequences of the randomly selected, identified morphologically as putative third-stage larvae of *H. fabri* were sequenced and compared with the sequence of adults. The results confirmed these third-stage larvae to be conspecific with the adults and all belong to *H. fabri*. *L. tanakae* represents a new host record for *H. fabri*.

Third-stage larvae of *Hysterothylacium aduncum* (Rudolphi, 1802)

Diagnosis (n=10)

Body is relatively large, very variable in length, whitish, with finely transversely striated cuticle, 11.5–28.6 (17.2) mm long; maximum width is 233–437 (350). It is cephalic end rounded, with small ventral cuticular tooth, sharply pointed. (Figs. 4a, b and 5a, b). Oral aperture is triangular, surrounded by weakly developed anlagen of lips (Fig. 5a,b). Esophagus is almost cylindrical, muscular, 1,260–2,720 (1,760) long, and 68–146 (115) in maximum width, representing 9.3–11.3 (10.4)% of body length (Fig. 4a). Nerve ring is 340–534 (433) and excretory pore is 427–612 (499) from anterior extremity, respectively. Ventriculus is oval to oblong, slightly wider than posterior

region of esophagus, 68–175 (124) long, and 68–146 (102) wide. Ventricular appendix is almost as long as intestinal caecum, 680–1,136 (870) long, and 78–107 (96.0) in maximum width. Intestinal caecum is 583–1,505 (915) long and 58–126 (92) wide, representing 46.2–57.6 % esophageal length. Ratio of intestinal caecum to ventricular appendix is 0.8–1.3:1 (1.0:1) (Fig. 4a). Tail is 146–291 (198) long, and tip of tail is with a long cuticular spike (Figs. 4c, d and 5c).

Host and locality of third-stage larvae

Host is Tanaka's snailfish *L. tanakae* (Gilbert & Burke) (Scorpaeniformes: Liparidae) found at East China Sea, off Zhoushan Island, Zhejiang Province, China.

Site of infection

Abdominal cavity is the site of infection.

Prevalence and intensity of infection

Ten out of ten fishes were infected with intensity of 1–83 (mean=26.7) specimens.

Fig. 4 Third-stage larva of *Hysterothylacium aduncum* (a–d) and *Anisakis pegreffii* (e–g) from *Liparis tanakae*. **a, e** anterior part of body, lateral view; **b, f** cephalic end, lateral view; **c, d, g** posterior end of body, lateral view. Scale bars: **a, e** 200 μ m; **b–d, f, g** 50 μ m

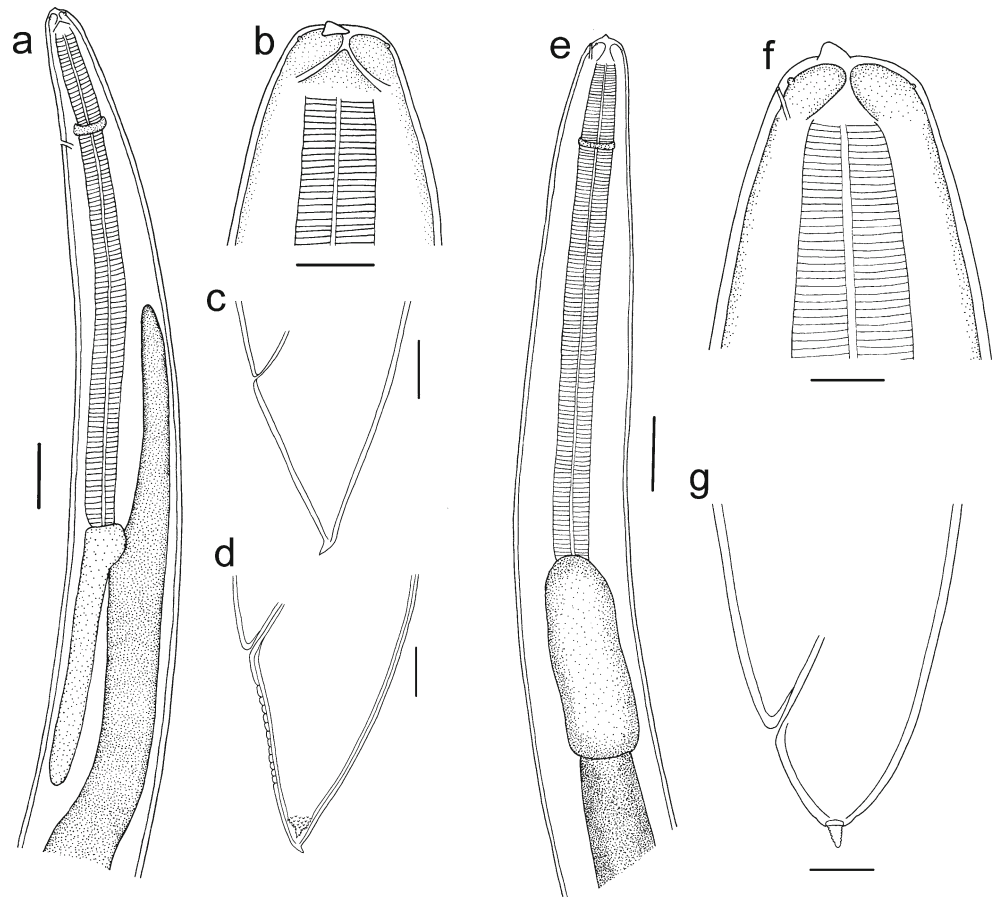
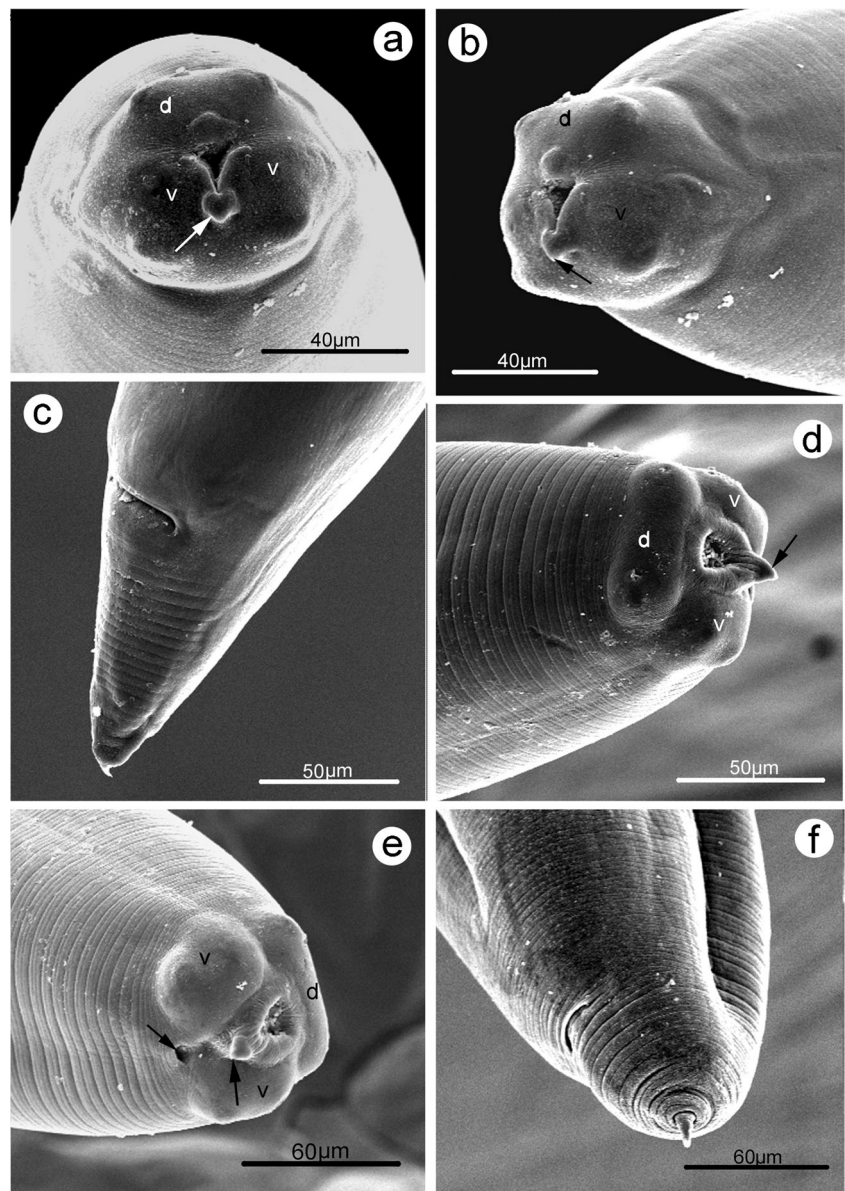


Fig. 5 Scanning electron micrographs of third-stage larva from *Liparis tanakae*. *Hysterothylacium aduncum* (a–c): **a** Cephalic end, apical view (cuticular tooth arrowed); **b** cephalic end, lateral view (cuticular tooth arrowed); **c** posterior end of body, subventral view. *Anisakis pegreffii* (d–f): **d** cephalic end, apical view (cuticular tooth arrowed); **e** cephalic end, apical view (excretory pore and cuticular tooth arrowed); **f** tip of tail, lateral view. *d* Dorsal lip; *v* Ventrolateral lip



Voucher specimens

There were 270 specimens (HBNU-F13046L).

DNA characterization

There were three different sequence types obtained for the ITS region of the partial rDNA based on the present material examined herein and the length are all 987 bp. The different sequence types showed 0.1–0.2 % nucleotide variability (for details, see Table 4). There are 35 ITS sequences of *H. aduncum* registered in GenBank and pairwise comparison between the present data and the sequences of *H. aduncum* registered in GenBank displayed very low level of nucleotide variability (0–0.5 % nucleotide difference). Thus, we

considered the present material to be conspecific with *H. aduncum*. The ITS sequences of *H. aduncum* are deposited

Table 4 The sequence polymorphisms revealed at alignment positions among the different three different sequence types of *Hysterothylacium aduncum* in the ITS region obtained in the present study

GenBank nos.	Sequence polymorphisms revealed at alignment positions		
	431	612	638
KF736938	G	T	G
KF736938	A	C	A
KF736937	A	T	A

in the GenBank database (<http://www.ncbi.nlm.nih.gov>) under accession numbers (KF736936–KF736938).

Remarks

Hysterothylacium aduncum is one of the most thoroughly studied ascaridoid nematodes parasitic in fish with respect to taxonomy (Hartwich 1975; Moravec et al. 1985; Moravec and Nagasawa 2000; Petter and Maillard 1987; Li et al. 2013), morphology (Soleim and Berland 1981), life cycles (Yoshinaga et al. 1987; Koie 1993; González 1998; Klimpel and Rückert 2005), ecology (Andersen 1993; Kalay et al. 2009), development (Balbuena et al. 1998, 2000; Iglesias et al. 2002), and population genetic diversity (Klimpel et al. 2007; Amor et al. 2011; Pekmezci et al. 2013). Recent molecular studies supported that *H. aduncum* parasitizing different marine teleosts worldwide only represents a single species, despite the existence of considerable intraspecific morphological variability and a low level of intraspecific genetic difference in some cases (Klimpel et al. 2007; Amor et al. 2011; Li et al. 2013; Pekmezci et al. 2013). The morphology of the larval forms of *H. aduncum* has been previously studied by Moravec et al. (1985), Shih and Jeng (2002), and Iglesias et al. (2002). The morphology of the present larval specimens agrees well with the descriptions by Moravec et al. (1985) and Iglesias et al. (2002), especially the relative length and ratio of the intestinal caecum, ventricular appendix and esophagus, and the morphology of the tail tip. However, the accuracy of morphological identification of larval stages of *Hysterothylacium* spp. is often incredible. For example, the specimens identified morphologically as *H. aduncum* by Shih and Jeng (2002), collected from the rabbitfish *Siganus fuscescens* (Houttuyn) (Perciformes: Siganidae) from off the Taiwan Strait, is not the real *H. aduncum*. On the basis of the morphology, measurements (especially the length and ratio of the intestinal caecum and ventricular appendix) and the host information, we considered that the material collected from *S. fuscescens* by Shih and Jeng (2002) should be *H. longilabrum* Li, Liu & Zhang, 2012. Consequently, the ITS region of rDNA were sequenced and analyzed for the accurate identification of the present larval nematodes. Comparison of the ITS sequences obtained herein with those available in GenBank confirmed that the present third-stage larval specimens from *L. tanakae* should belong to *H. aduncum*.

Third-stage larvae of *Anisakis pegreffii* Campana-Rouget & Biocca, 1955

Diagnosis (n= 3)

Body is large, whitish, with remarkably transversely striated cuticle, 15.2–20.1 (18.3) mm long; maximum width is 340–

495 (440). It is cephalic end rounded, with very large ventral cuticular tooth, sharply pointed (Figs. 4e, f and 5d, e). Oral aperture triangular, surrounded by weakly developed anlagen of lips (Fig. 5d, e). Excretory pore just at base of subventral lips (Figs. 4f and 5e). Esophagus is almost cylindrical, muscular, 1,379–1,748 (1570) long, and 97–136 (123) in maximum width, representing 8.1–9.1 (8.6)% of body length (Fig. 4e). Nerve ring is 291–311 (298) from anterior extremity. Ventriculus more or less cylindrical, much wider than esophagus, 534–728 (621) long, and 194–204 (197) wide (Fig. 4e). Intestinal caecum and ventricular appendix are absent. Tail is 97–117 (107) long, with a finger-like tip (Figs. 4g and 5f).

Host and locality of third-stage larvae

Host is Tanaka's snailfish *L. tanakae* (Gilbert & Burke) (Scorpaeniformes: Liparidae) found at East China Sea, off Zhoushan Island, Zhejiang Province, China.

Site of infection

Abdominal cavity is the site of infection.

Prevalence and intensity of infection

One out of ten fishes was infected with intensity of three specimens.

Voucher specimens

There were three specimens (HBNU-F13047L).

DNA characterization

There was only one sequence type obtained for the ITS region of the partial rDNA based on the present material examined herein and the length is 918 bp. There are 67 ITS sequences of *A. pegreffii* registered in GenBank and pairwise comparison between the present data and the ITS sequences of *A. pegreffii* registered in GenBank showed very low level of nucleotide variability (0–0.4 % nucleotide difference). Thus, we considered the present material to be conspecific with *A. pegreffii*. The ITS sequence of *A. pegreffii* is deposited in the GenBank database (<http://www.ncbi.nlm.nih.gov>) under accession number (KF736935).

Remarks

Due to the importance as disease agent in humans and marine organisms, *A. pegreffii* has received much attention. The third-stage larvae of *A. pegreffii* have been reported from various fishes and cephalopods worldwide (Abe et al. 2005; Mattiucci and Nascetti 2006; Umehara et al. 2006, 2010; Du et al. 2010;

Shamsi et al. 2011; Kuhn et al. 2011; Quiazon et al. 2011; Smrzlic et al. 2012; Zhang et al. 2007, 2013; Setyobudi et al. 2013), but there has been no report of *A. pegreffii* from the fish host *L. tanakae*. In the Chinese waters, Zhang et al. (2007, 2013), Du et al. (2010) reported this species from various fishes. The morphology of the third-stage larvae of *A. pegreffii* has been studied by Zhang et al. (2013). We compared our present material with the larval specimens of Zhang et al. (2013) deposited in College of Life Science, Hebei Normal University, Hebei Province, China. The measurements and morphology of the material of Zhang et al. (2013) agree very well with ours. The molecular characterization of ITS region of *A. pegreffii* has been studied by Abe et al. (2005), Kuhn et al. (2011), Quiazon et al. (2011), Smrzlic et al. (2012), Setyobudi et al. (2013) and Zhang et al. (2013). Pairwise comparison between the present data and the ITS sequences of *A. pegreffii* registered in GenBank further confirmed the present larval material collected from *L. tanakae* belong to *A. pegreffii*.

Hysterothylacium zhoushanense nom. nov., synonym
Hysterothylacium zhoushanensis Li, Liu & Zhang, 2012

Remarks

H. zhoushanensis was described as a new species from the flatfish *Pseudorhombus oligodon* (Bleeker) (Pleuronectiformes: Paralichthyidae) in the East China Sea (Li et al. 2012b). They proposed the latinized specific epithet *zhoushanensis*, which referred to the name of the type locality, Zhoushan Island. However, the generic names *Hysterothylacium* is in the neuter gender. Li et al. (2012b) were unaware of changing the ending of *zhoushan-ensis* to *zhoushan-ense* to make it agree with this neuter generic name. The correct ending is given now.

Discussion

The tanaka's snailfish *L. tanakae* (Gilbert & Burke) (Scorpaeniformes: Liparidae) is an economically important fish species and the annual harvests have been increasing in the east coast of China (Zhang et al. 2011; Zhou et al. 2012). However, the helminth parasites of this fish are still poorly known. To our knowledge, only *H. liparis* and *H. aduncum* has previously been reported parasitic in *L. tanakae* (Li et al. 2008b). In the present study, the high levels of infection and helminth diversity in *L. tanakae* were revealed for the first time. The prevalence and mean intensity of infection of third-stage larvae of *H. aduncum* were 100 % and 26.7 individuals per fish, respectively, which are much higher than the other three species *H. liparis*, *H. fabri*, *A. pegreffii*. Analogously, we could consider that *H. aduncum* is the dominant parasite

species in *L. tanakae* in the larval stage. In contrast, we cannot find the adults of *H. aduncum* and *H. fabri* in the alimentary tract of *L. tanakae* and only the adults of *H. liparis* occur in intestine and stomach of *L. tanakae* in this helminthological survey. Li et al. (2008b) reported the adults of *H. aduncum* occurred in the alimentary tract of *L. tanakae* in the Yellow Sea, China; however, the levels of prevalence and mean intensity of infection of *H. aduncum* adults were far lower than those of *H. liparis*. This situation possibly indicates that *L. tanakae* may not be the suitable definitive host of *H. aduncum* and *H. fabri*, but for *H. liparis*. The high levels of infection and helminth diversity in this fish can be easily explained when we considered that *L. tanakae* is a benthic, voracious predator, and large numbers of small fishes, benthic crustaceans and cephalopods are present in its diet (Xue et al. 2010; Zhang et al. 2011; Zhou et al. 2012), most of which are paratenic hosts or intermediate hosts of the species of *Hysterothylacium* and *Anisakis*. Meanwhile, the heavily infected level of third-stage larvae of *Hysterothylacium* and *Anisakis* in *L. tanakae* also reminds us that we need to be constantly alert to anisakidosis when we eat raw or undercooked meat of this marine fish.

Our present knowledge of the third-stage larvae of ascaridoid nematodes is still very limited. With the assistance of molecular techniques, for example, utilizing the ITS of nuclear rDNA, mitochondrial cytochrome oxidase subunit I (CO I) and subunit II (CO II) as genetic marker, the different larval forms of ascaridoid nematodes can be easily distinguished or separated; nevertheless, most of them hardly could be identified to the species level due to the absence of similar sequences for adult parasites. Within the genus *Hysterothylacium*, as far as we are aware, only the third-stage larvae of *H. geschei* Torres, Andrade & Silva, 1998, *H. patagonense* Moravec, Urawa & Coria, 1997, *H. cenotae* (Pearse, 1936), *H. aduncum*, *H. longilabrum*, and *H. zhoushanense* were accurately identified and described (Torres et al. 1998; Moravec et al. 1985, 1997; Iglesias et al. 2002; Li et al. 2012a, b). Most of the previous studies can only assign the larval forms of *Hysterothylacium* to different morphotypes or to generic level (Cannon 1977; Chai et al. 1986; Borges et al. 2012; Shamsi et al. 2011, 2013; Jabbar et al. 2013). The growing molecular data (i.e., ITS of rDNA, CO I and CO II of mtDNA) of the already described adults of ascaridoid nematodes will undoubtedly contribute to solve the problem.

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