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▣ MINI-REVIEW

Current Status and Perspectives of Cysticercosis and Taeniasis in Japan

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Abstract: This mini-review describes recent epidemiological trends in cysticercosis and taeniasis in Japan. Some of the topics discussed herein were presented at the first symposium on "Current perspectives of *Taenia asiatica* researches", that was held in Osong in Chungbuk Province, South Korea, in October 2011 and organized by Prof. K. S. Eom, Chungbuk National University School of Medicine. To better understand the trends in the occurrence of cysticercosis and taeniasis in Japan, clinical cases reported in 2005 have been updated. In addition, the current status of *Taenia asiatica* infections successively occurring in Japan since 2010 is also discussed.

Key words: *Taenia solium*, *Taenia asiatica*, *Taenia saginata*, taeniasis, cysticercosis, Japan

INTRODUCTION

Cysticercosis, a parasitic disease caused by *Taenia solium* cysticercus, is one of the important parasitic diseases. Neurocysticercosis (NCC) is accepted to refer to cysts in the central nerve system, including the parenchyma and ventricles of the brain and the spinal cord. Subcutaneous cysticercosis (SCC) is used for the cysticercosis presenting the form of firm, mobile nodules, mainly in the soft tissues and muscles of on the trunk and extremities. NCC is clinically more serious than SCC because of the severity of the neurologic symptoms, such as epileptic seizures and paralysis that can result from infection. The disease constitutes a major public health problem in many parts of the world, including China, Southeast Asia, India, sub-Saharan Africa, and Latin America [1]. Cysticercosis has also become an important parasitic disease in developed countries, such as the United States, particularly in California and other states with a large immigrant population [2]. In Japan, although *T. solium* cysticercosis/taeniasis was endemic to the Okinawa region in southern Japan 50-60 years ago [3,4], the disease is no longer endemic in the area. Nonetheless, sporadic cases of cysticercosis have been reported in Japan, primarily among Japanese returning from abroad and foreigners coming to Japan (Table 1) [5].

Conversely, taeniasis, which is caused by infection with the adult tapeworm of *T. solium* or *Taenia saginata*, occurs worldwide, except in countries where people do not eat pork and beef for religious reasons [1]. Taeniasis caused by *Taenia asiatica* is restricted to countries in Asia, including South Korea, China, Taiwan, the Philippines, Vietnam, Thailand, Indonesia, and Japan [6]. In Japan, sporadic cases of taeniasis have been reported and most of them were caused by infection with *T. saginata* and were imported cases until *T. asiatica* infections were confirmed in 2010 (Table 2). Compared to cysticercosis, taeniasis is innocuous or asymptomatic, with most patients presenting with slight intestinal illness and mental discomfort due to persistent expulsion of the proglottids.

In Japan, the "Ordinance for Enforcement of the Food Sanitation Act" based on the Food Sanitation Law stipulates that food-borne parasitic diseases such as cysticercosis and taeniasis be treated as cases of food poisoning and that authorities be notified of their occurrence immediately. However, because parasitic diseases have never reported based on the law, it is not possible to accurately estimate the incidence of cysticercosis/taeniasis in Japan. Therefore, the author previously examined the epidemiological trends in cysticercosis and taeniasis based on clinical cases in Japan published in scientific journals [5]. Since then, new cases of cysticercosis and taeniasis have been reported and several cases of cysticercosis have been newly diagnosed in our department. The Department of Parasitol-

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Table 1. Demographic and clinical data for cysticercosis cases reported in Japan (1990-2011)

Case No.	Year	Patient (Nationality/Age/Sex)	Type of cysticercosis	Diagnostic criteria	Presumed locality of infection	References
1	1990	Japanese/40/F	NCC (multiple)	CT/Pathology	Japan (Tokunoshima, Kagoshima)	55
2	1991	Korean/73/M	NCC (multiple)	CT/MRI/Pathology	Korea	86
3	1991	Japanese/33 /M	NCC (solitary)	CT/MRI/Serology	Honduras	87
4	1991	Japanese/29/F	SCC (solitary)	Pathology/Serology	Thailand	41
5	1991	Japanese/48/M	Intramedullary spinal (solitary)	CT/MRI/Pathology	Thailand	44
6	1992	Chinese/20/M	NCC (multiple)	CT/MRI/Pathology	China (Heilongjiang province)	88
7	1992	Japanese/41/F	NCC (multiple)	CT/MRI/Serology/Pathology	Hong Kong, Korea or Japan	89
8	1992	Japanese/30/M	NCC (multiple, racemose-type)	CT/MRI/Pathology	Japan (Ginowan, Okinawa)	50, 51
9	1992	Korean/42/M	NCC (multiple)	CT/MRI/Serology	Korea	90
10	1993	Japanese/44/F	NCC (solitary)	CT/Pathology	Japan	56
11	1993	Japanese/46/M	Ocular (solitary)	Funduscope/Pathology	Vietnam or Cambodia	45
12	1993	Japanese/41/F	NCC (solitary)	CT/MRI/Pathology	Japan	57
13	1993	Brazilian/26/F	NCC (multiple)	CT/Pathology	Brazil	91
14	1993	Japanese/49/F	NCC (multiple)	CT/MRI/Pathology	China	92
15	1993	Japanese/53/M	NCC (multiple, racemose-type ?)	CT/MRI/Pathology	Taiwan	55
16	1994	Korean/48/M	NCC (multiple) and SCC (systemic)	CT/MRI/X ray/Pathology	Korea	42
17	1994	Korean/43/F	NCC (multiple) and SCC (systemic)	CT/MRI/X ray/Pathology	Korea	42
18	1994	Japanese/72/F	NCC (racemose-type)	CT/MRI/Serology/Pathology	China	53
19	1994	Chinese/24/M	NCC (racemose-type)	CT/MRI/Pathology	China	54
20	1994	Japanese/44/M	NCC (solitary)	CT/MRI/Pathology	Japan	54
21	1994	Japanese/52/F	NCC (solitary)	CT/MRI/Serology	Japan	58
22	1995	Japanese/21/F	NCC (multiple)	MRI/PET	Japan	59
23	1996	Japanese/39/M	NCC (solitary)	MRI/Pathology	Japan	60
24	1996	Japanese/39/M	SCC (solitary)	Pathology	China	43
25	1996	Korean/70/F	NCC (multiple)	CT/MRI/Serology/Pathology	Korea	93
26	1997	Chinese/68/M	NCC (multiple) and SCC (multiple)	CT/MRI/X ray/Serology	China (Heilongjiang province)	7
27	1998	Chinese/48/M	NCC (multiple)	CT/MRI	China	8
28	1998	Japanese/37/M	SCC (solitary)	Pathology	Japan	9
29	1998	Japanese/34/M	NCC (multiple), SCC (multiple) and taeniasis	CT/MRI/Serology	China (Jiujiang, Jiangxi Province)	10
30	1998	Japanese/59/M	NCC (multiple)	CT/Pathology	China	11
31	1999	Japanese/19/F	NCC (solitary)	MRI/Pathology	India	12
32	1999	Chinese/55/M	NCC (multiple)	CT/MRI/Endoscopy/Pathology	China	13
33	1999	Japanese/46/M	NCC (solitary)	CT/MRI/Pathology	Indonesia, Nigeria, or Nepal	14, 15
34	2000	Japanese/45/F	SCC (multiple)	CT/Pathology	Thailand	16
35	2000	Cambodian/29/M	NCC (multiple) and SCC (multiple)	CT/MRI/X ray/Pathology	Cambodia	17
36	2001	Japanese/53/F	Ocular (solitary)	Funduscope/US	No information	18
37	2001	Japanese/43/F	NCC (solitary)	CT/MRI/Pathology	Thailand	19
38	2001	Unknown/73/M	NCC (multiple)	CT/MRI	No information	20
39	2001	Japanese/70/M	NCC (racemose type)	CT/MRI/Serology	Philippines	21
40	2002	Japanese/26/M	NCC (solitary)	CT/MRI/Pathology/Serology	Japan	22
41	2003	Japanese/22/F	NCC (solitary)	CT/MRI// US/Pathology	India	23
42	2004	Japanese/53/F	NCC (solitary)	CT/MRI/Pathology/DNA	India, Vietnam, Thailand or Myanmar	24, 25
43	2004	Chinese/50/M	Ocular (solitary) and NCC (solitary)	Funduscope/CT/Serology/Pathology	China (Heilongjiang province)	26
44	2004	Japanese/83/M	SCC (systemic)	CT/X ray/Pathology/DNA	China	27, 28
45	2005	Chinese/44/F	NCC (multiple) and SCC (multiple)	CT/MRI/X ray	China	29
46	2005	Chinese/21/F	NCC (solitary)	CT/MRI/PET/Serology	China (Harbin, Heilongjiang province)	30

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Table 1. (*Continued from the previous page*) Demographic and clinical data for cysticercosis cases reported in Japan (1990-2011)

Case No.	Year	Patient (Nationality/Age/Sex)	Type of cysticercosis	Diagnostic criteria	Presumed locality of infection	References
47	2005	Filipino/9/F	NCC (solitary)	CT/MRI/Pathology/DNA	Philippines	31
48	2006	Japanese/24/F	NCC (solitary)	MRI/Pathology/DNA	Indonesia or Korea	32
49	2006	Indian/28/F	NCC (multiple)	CT/MRI/DNA	India	33
50	2006	Brazilian/42/F	NCC (racemose-type)	CT/MRI/Pathology/DNA	Brazil	34
51	2007	Japanese/38/F	NCC (solitary)	CT/MRI/Pathology/DNA	Nepal	35
52	2007	Japanese/84/M	SCC (systemic)	CT/X ray/DNA	Japan (Okinawa)	36
53	2007	Japanese/51/F	NCC (multiple)	CT/MRI	Japan (Okinawa)	This study
54	2007	Japanese/31/F	NCC (multiple)	CT/MRI	India	This study
55	2008	Indian/44 /F	NCC (multiple)	MRI/Serology/DNA	India	37
56	2008	Chinese/30/M	NCC (multiple) and SCC (multiple)	CT/MRI/X ray/Serology	China	This study
57	2008	Japanese/39/F	NCC (multiple)	CT/MRI/PET/Serology	Asian or African countries	This study
58	2009	Japanese/24/M	Ocular and taeniasis	Funduscope/US/Serology	Malawi	38
59	2009	Korean/38/M	NCC (multiple)	CT/MRI/SEM/Pathology	Korea	39
60	2009	Japanese/20/F	NCC (multiple) and taeniasis	CT/MRI/Serology/Capsule endoscopy/DNA	India	This study
61	2009	Japanese/61/M	NCC (multiple, racemose-type)	CT/MRI/US/Serology/Pathology/DNA	India, Thailand, China or Vietnam	This study
62	2010	Japanese/53/M	NCC (racemose-type)	CT/MRI/Pathology/Serology/DNA	Japan (Uruma, Okinawa)	40
63	2010	Japanese/58/F	SCC (multiple)	CT/MRI/X ray/DNA	Japan (Akita or Okinawa)	This study
64	2010	Chinese/46/F	NCC (multiple) and SCC (multiple)	CT/MRI/US/Serology	China (Harbin, Heilongjiang province)	This study
65	2010	Japanese/31/M	NCC (multiple), SCC(multiple) and taeniasis	CT/MRI/Serology/DNA	India	This study
66	2011	Nepalese/35/M	SCC (solitary)	CT/US/Serology/Pathology/DNA	Nepal	This study

ogy at the National Institute of Infectious Diseases, Tokyo routinely performs diagnostic tests requested for parasitic diseases from domestic and foreign medical institutions, and cysticercosis and taeniasis also are acceptable for diagnosis.

The purpose of this article is to overview the current status of cysticercosis/taeniasis in Japan and to update the data that was reported in 2005 [5] based on the cases cited in PubMed (National Library of Medicine) and Japana Centra Revuo Medicina as well as cases diagnosed in our department over the last 5 years (2007-2011).

CLINICAL CASES

Cysticercosis

According to Nishiyama and Araki [4], as many as 389 cases of cysticercosis were reported in Japan from 1908 to 1997. However, 24 cases reported between 1943 and 1979 were not included in the study. Furthermore, 41 cases, including 10 cases diagnosed by our department, have been newly confirmed between 1997 and 2011 (cases 26-66 in Table 1) [7-40]. Taken together, this gives a total of 454 cysticercosis cases that have been reported in Japan between 1908 and 2011. Table 1 shows 66 of the cysticercosis cases that have been reported over the last 22 years (1990-2011) along with cases confirmed by our department between 2007 and 2011.

Of these 66 cases, 54 (66.7%) were NCC; NCC with multiple cysts (28/54, 51.9%; Fig. 1E) was more frequent than NCC with a solitary cyst (13/54, 33.5%; Fig. 1A, B and Fig. 2A, B, E). Between 1990 and 2011, total 17 cases of SCC were reported as cases 4 [41], 16-17 [42], 24 [43], 26 [7], 28 [9], 29 [10], 34 [16], 35 [17], 44 [27,28], 45 [29], 52 [36], 56, 63, 64, 65, and 66. Two of them were systemic intramuscular cysticercosis with numerous calcified cysts; cases 44 [27,28] and 52 [36] (Fig. 1F, G; Fig. 2D, F). Very rarely, intramedullary cysticercosis in case 5 [44] and ocular cysticercosis in cases 11 [45], 36 [18], 43 [26], and 58 [38] have also been reported. Ten cases of NCC with either SCC or ocular cysticercosis were reported in cases 16-17 [42], 26 [7], 29 [10], 35 [17], 43 [26], 45 [29], 56 , 64, and 65 (Table 1). More interestingly, dual infection of cysticercosis and taeniasis was observed in 4 cases; 29 [17], 58 [51], 60, and 65 (Table 1). Furthermore, the adult tapeworm in case 41 was observed in the small intestine using capsule endoscopy to confirm the presence of the adult worm (Table 2).

Cysticercosis diagnosis is generally performed by imaging,

Table 2. Demographic and clinical data for taeniasis reported in Japan (1990-2011)

Case No.	Year	Patient (Nationality /Age/Sex)	Etiologic agent (diagnostic criteria)	Presumed locality of infection	References
1	1990	Japanese/72/M	*T. saginata* (/Morphology)	?	63
2	1990	Korean/52/M	*T. saginata* (Serology/Morphology)	?	64
3	1990	Japanese/34/M	*T. saginata* (Morphology)	Ethiopia	65
4	1990	Japanese/32/M	*T. saginata* (Morphology)	Japan	65
5	1990	Japanese/26/M	*T. saginata* (Morphology)	Ethiopia or Somalia	65
6	1992	Japanese/10/F	*T. saginata* (Morphology)	Japan	66
7	1994	Japanese	*T. saginata* (Morphology)	Iran	53
8	1994	Japanese	*T. saginata* (Morphology)	?	53
9	1994	Japanese	*T. saginata* (Morphology)	?	53
10	1994	Japanese	*T. saginata* (Morphology)	France or Germany	53
11	1994	Japanese	*T. saginata* (Morphology)	Germany	53
12	1996	Japanese/53/F	*T. saginata* (Morphology)	?	67
13	1996	Japanese/26/M	*T. saginata* (Morphology)	Bolivia	68
14	1996	Japanese/47/M	*T. saginata* (Morphology)	Cote D'Ivoire	69
15	1997	Japanese/23/F	*T. saginata* (Morphology)	Europe	70
16	1998	Brazilian/45/M	*T. saginata* (Colonoscope/Morphology)	Brazil	71
17	1998	Japanese/34/M	Probably *T. solium* with NCC	China (Jiujiang, Jiangxi Province)	10
18	2001	Filipino/32/F	*T. saginata* (Morphology)	Philippines	72
19	2001	Japanese/26/M	*T. saginata* (Morphology)	Japan or India	73
20	2001	Japanese/47/M	*T. saginata* (Morphology)	Indonesia	73
21	2001	Japanese/30/M	*T. saginata* (Morphology)	Ethiopia	73
22	2001	Japanese/60/M	*T. saginata* (Morphology)	Japan	73
23	2002	Japanese/30/M	*T. saginata* (Morphology)	Ethiopia	74
24	2002	Japanese/51/M	*T. saginata* (Morphology)	Thailand	74
25	2002	Japanese/46/M	*T. saginata* (Morphology)	Africa	75
26	2003	Japanese/24/F	*T. saginata* (Morphology)	Vietnam	77
27	2007	Japanese/45/M	*T. saginata* (DNA)	Thailand or Indonesia	76
28	2007	Cambodian/16/M	*T. saginata* (DNA)	Cambodia	94
29	2007	Japanese/58/M	*T. saginata* (DNA)	Korea	94
30	2007	Japanese/32/M	*T. saginata* (DNA)	Ethiopia	94
31	2007	Japanese/33/M	*T. saginata* (DNA)	Cambodia or Ethiopia	94
32	2007	Japanese/40/F	*T. saginata* (DNA)	China, Kenya, Monaco or Croatia	This study
33	2007	Japanese/25/M	*T. saginata* (Endoscope/Morphology)	Laos	78
34	2008	Japanese/26/F	*T. saginata* (DNA)	Nicaragua, Laos or Indonesia	This study
35	2008	Japanese/26/M	*T. saginata* (DNA)	Indonesia	This study
36	2008	Japanese/45/M	*T. saginata* (DNA)	Vietnam or China	This study
37	2009	Japanese/24/M	*Taenia* sp. (Morphology) with ocular type	Malawi	38
38	2009	Japanese/63/M	*T. saginata* (DNA)	Thailand	This study
39	2009	Japanese/57/M	*T. saginata* (DNA)	Thailand	This study
40	2009	Japanese/49/M	*T. saginata* (DNA)	Thailand	This study
41	2009	Japanese/20/F	*T. solium* (Capsule endoscope/DNA) with NCC	India	This study
42	2010	Japanese/58/M	*T. asiatica* (DNA)	Japan	81, 84
43	2010	Japanese/41/F	*T. asiatica* (DNA)	Japan	81, 84
44	2010	Japanese/55/M	*T. asiatica* (DNA)	Japan	81, 84
45	2010	Japanese/40/M	*T. asiatica* (DNA)	Japan	81, 84
46	2010	Japanese/31/M	*T. asiatica* (DNA)	Japan	82, 84
47	2010	Japanese/41/M	*T. asiatica* (DNA)	Japan	83
48	2010	Japanese/28/M	*T. asiatica* (DNA)	Japan	83
49	2010	Japanese/30/M	*T. asiatica* (DNA)	Japan	83, 84
50	2010	Japanese/60/M	*T. asiatica* (DNA)	Japan	83

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Table 2. (*Continued from the previous page*) Demographic and clinical data for taeniasis reported in Japan (1990-2011)

Case No.	Year	Patient (Nationality /Age/Sex)	Etiologic agent (diagnostic criteria)	Presumed locality of infection	References
51	2010	Japanese/39/F	*T. asiatica* (DNA)	Japan	83, 84
52	2010	Japanese/24/F	*T. asiatica* (DNA)	Japan	83
53	2010	Japanese/31/M	*T. solium* (endoscopy/DNA) with NCC	India	This study
54	2010	Japanese/39/M	*T. asiatica* (DNA)	Japan	84
55	2010	Japanese/56/M	*T. saginata* (DNA)	Thailand	This study
56	2010	Japanese/26/F	*T. asiatica* (DNA)	Japan	84
57	2010	Japanese/43/F	*T. asiatica* (DNA)	Japan	84
58	2010	Filipino/31/F	*T. asiatica* (DNA)	Philippines	This study
59	2011	Japanese/46/M	*T. saginata* (DNA)	Malaysia	This study
60	2011	Japanese/35/M	*T. saginata* (DNA)	Mali	This study
61	2011	Japanese/52/M	*T. saginata* (DNA)	Thailand	This study
62	2011	Japanese/24/F	*T. saginata* (DNA)	Indonesia (Bali)	This study
63	2011	Japanese/41/M	*T. saginata* (DNA)	Senegal	This study
64	2011	Thai/21/M	*T. solium* (DNA)	Thailand	This study
65	2011	Japanese/33/M	*T. saginata* (DNA)	Sudan	This study
66	2011	Japanese/54/M	*T. asiatica* (DNA)	Japan	This study
67	2011	Japanese/38/M	*T. asiatica* (DNA)	Japan	This study
68	2011	Ethiopian/24/F	*T. saginata* (DNA)	Ethiopia	This study
69	2011	Japanese/12/M	*T. asiatica* (DNA)	Japan	This study
70	2011	Japanese/54/M	*T. asiatica* (DNA)	Japan	This study
71	2011	Japanese/42/F	*T. saginata* (DNA)	France	This study
72	2011	Ethiopian/26/F	*T. saginata* (DNA)	Ethiopia	This study
73	2011	Japanese/41/F	*T. asiatica* (DNA)	Japan	This study

serologic, and histopathologic examinations. In our department, molecular identification of the etiologic agents is routinely performed, if surgically removed materials are available [46-48]. Indeed, the usefulness of molecular methods for diagnosing the causative agents has successfully been demonstrated by the identification of 2 genotypes of *T. solium* cysticercus as well as confirmation of the agents in paraffin-embedded sections [24,25,28,31,33-35,37,40]. In addition, the localities where the patients were infected can also be inferred based on the DNA sequences of the causative agents [32,49].

In SCC, X-ray examinations have revealed the presence of rod-like, scattered, calcified lesions in the soft tissues of the extremities (Fig. 1F, G; Fig. 2D, F). These calcified cysts have histopathologically been confirmed to be *T. solium* in cases 16-17 [42], 26 [7], 52 [36], and 44 [27,28] (Fig. 2A, C, E).

Two types of *T. solium* cysticercus, cellulose- and racemose-types, are known to exist. The cellulose-type cysticercus is characterized by a single bladder measuring 3 to 18 mm in diameter with an invaginated scolex and primarily found in the cerebral parenchyma and musculature. The racemose-type presents as large multilobulated cystic lesions lacking a scolex and appears to prefer the cisternal and ventricular systems or subarachnoid space [2]. Indeed, the racemose-type cysticercus is frequently found in the subarachnoidal spaces as multilobulated lesions (Fig. 1C, D). Although cysticercosis due to racemose-type *T. solium* cysticercus is relatively rare, 8 cases have been documented in Japan in cases 8 [50,51], 15 [52], 18 [53], 19 [54], 39 [21], 50 [34], 61, and 62 [40] (Table 1; Fig. 1C, D; Fig. 2C). Of these, mitochondrial DNA analysis using histopathologic sections revealed that etiologic *T. solium* was the Asian genotype in 3 cases, 50 [32], 61, and 62 [40], and American/African genotype in case 50 [34] (Table 1). The racemose-type cysticercus is considered to be an aberrant, multilobular, non-viable *T. solium* cysticercus, possibly the degenerated form of a cysticercus in the basal subarachnoid space. Molecular analysis using formalin-fixed and paraffin-embedded histopathologic specimens has proved that the racemose-type cysticercus is *T. solium* in cases 50 [34], 61, and 62 [40].

Most of the cysticercosis cases in Japan are imported cases, meaning that the patients either lived in or visited countries where cysticercosis and taeniasis are still endemic, and where they are presumed to have been exposed to *T. solium* eggs. However, 13 cases have suggested that infection occurred within Japan (cases 1 [55], 8 [50,51], 10 [56], 12 [57], 20 [54], 21 [58], 22 [59], 23 [60], 28 [9], 40 [22], 52 [36], 53, and 62 [40]. NCC was diagnosed by imaging findings (Fig. 1), serology, histopa-

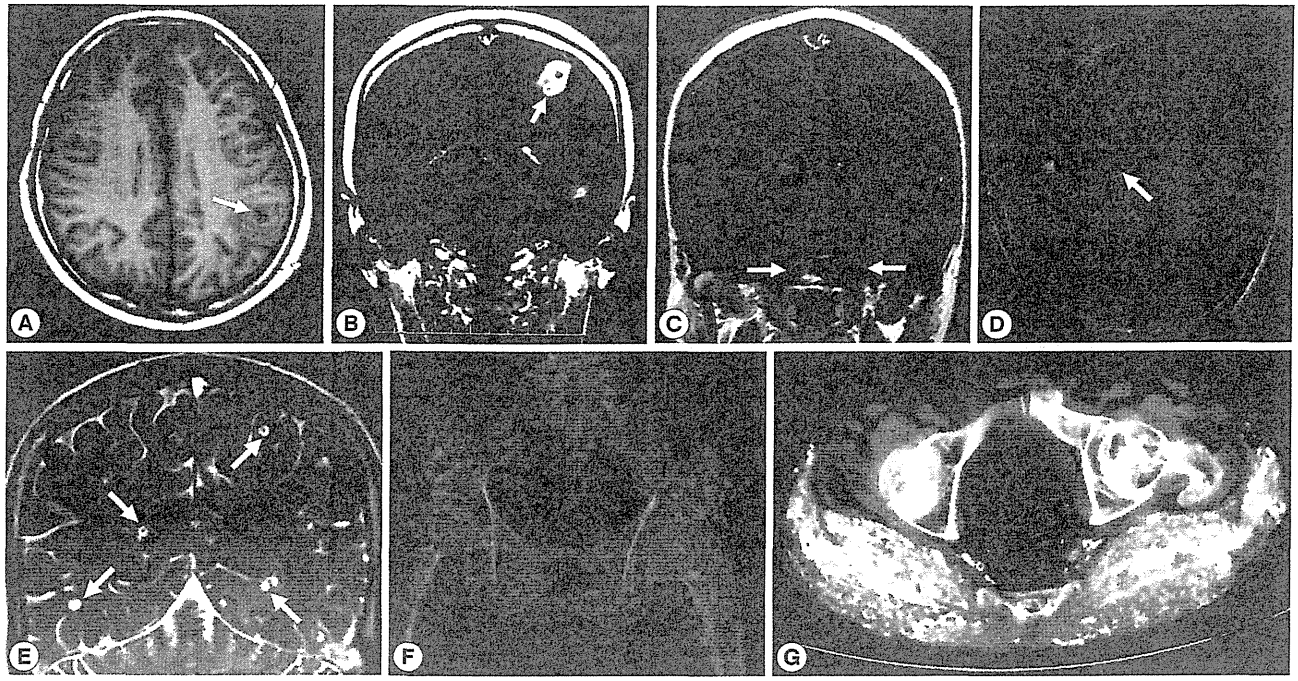

Fig. 1. Imaging findings of selected cysticercosis cases. (A) plain CT image showing a solitary lesion at the left occipitoparietal area (case 48 [32], courtesy of Prof. H. Matsuoka). (B) MRI showing one of multiple cystic lesions in the left frontal and temporal lobes (case 49 [33]). (C) MRI showing a rasemose-type lesion at the basal cistern (case 50 [34], courtesy of Dr. T. Oda). (D) MRI FLAIR findings showing a giant and multilobulated mass in the subarachnoidal spaces of the right frontal lobe (case 62 [40], courtesy of Dr. S. Shiiki). (E) Cisterography showing multiple cysts in the brain (case 60, courtesy of Prof. A. Chiba). (F) X-ray findings showing typical rice grain calcifications in the muscles of buttocks and lower extremity (case 44 [27, 28], courtesy of Dr. T. Nagase). (G) CT findings showing numerous calcified cysts in muscles of the of the buttocks (case 52 [36], courtesy of Dr. M. Tsuda).

thology (Fig. 2A, C, D, E), and molecular analysis.

Taeniasis

Table 2 shows 73 clinical taeniasis case reports that have been published in journals between 1990 and 2011 and diagnosed by our department between 2007 and 2011. In addition to these, 26 cases have been reported [61,62]. The most commonly encountered taeniasis cases were *T. saginata* infections and 48 cases (65.8%) have been confirmed to date (Table 2). Of these 48 cases, 45 were imported cases [63-78]. Although the route of infection is unknown, the possibility also exists that 4 of these cases may be attributable to domestic infections; cases 4 [65], 6 [66], 19 [73], and 22 [73]. *T. solium* taeniasis is extremely rare in Japan and only 1 case was reported in Okinawa in 1988 [79]. However, taeniasis solium cases with either NCC, SCC, or ocular cysticercosis have been confirmed, and all these were imported in cases 29 [10], 58 [38], 60, and 65 (Table 1) and cases 17 [10], 41, 53, and 64 (Table 2). Taeniasis caused by *T. asiatica* has been also recently successively confirmed in Japan and this will be discussed in the following chapter.

Taeniasis is usually diagnosed based on proglottid morphology. However, since *T. saginata*, *T. solium*, and *T. asiatica* are all morphologically similar, it is not always possible to accurately differentiate them. As a result, more reliable molecular diagnoses are currently employed to differentiate between taeniasis infections in our department [46-48]. Most recently, *T. solium* tapeworms have been observed in the small intestine using capsule endoscopy in cases 41 [23] and 53.

CURRENT STATUS OF *T. ASIATICA* INFECTION IN JAPAN

Although *T. asiatica* was not previously considered to occur in Japan [5], retrospective molecular analyses of proglottids revealed that 2 *T. asiatica* infections occurred in Tottori Prefecture on Honshu Island, Japan, in 1968 and 1996 [6]. Unfortunately, it is unknown whether the 2 Japanese cases were domestic infections or imported cases. As the number of Japanese travelers visiting Asian countries has increased, so too has the ber of people from other Asian countries visiting Japan

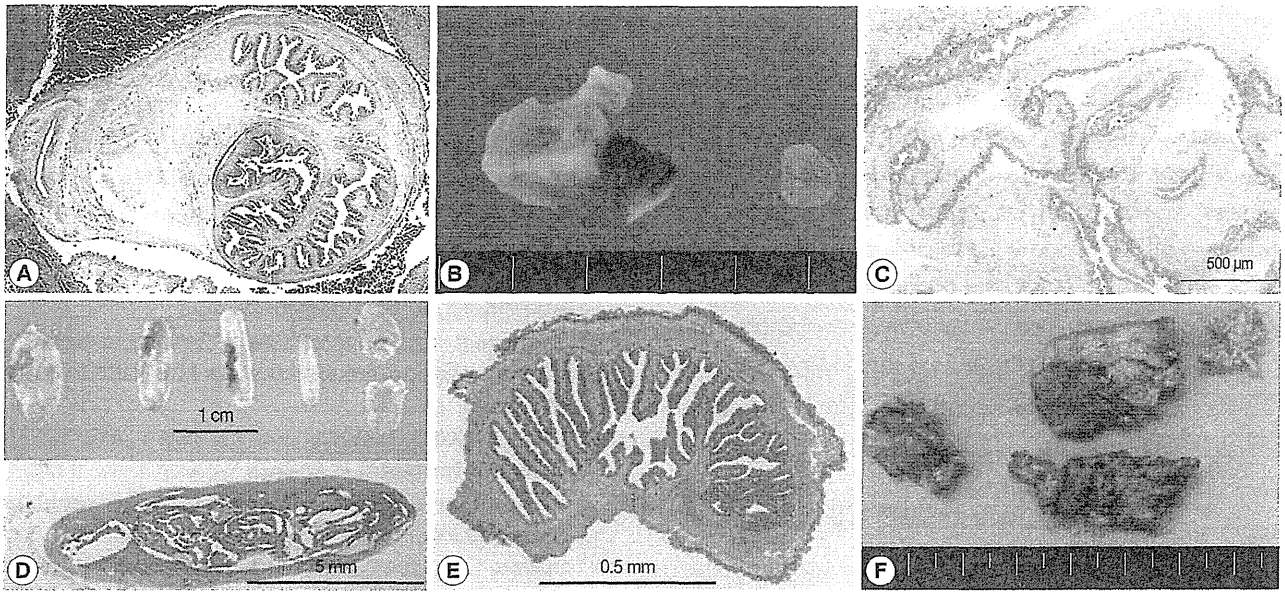

Fig. 2. Histopathologic findings of cystic lesions from cysticercosis patients. (A) A cellulose-type cysticercus characterized by rabyrinth-like structure (case 40 [22], courtesy of Dr. S. Matsunaga). (B) and (E) A resected lesion and a cellulose-type cysticercus (case 48 [32], courtesy of Prof. H. Matsuoka). (C) Racemose-type cysticercus characterized by complicated cystic walls (case 62 [40], courtesy of Dr. S. Shiiki). (D) SCC showing typical rice grain calcifications in the muscles of buttocks and lower extremity and the section of the calcified lesion (case 44 [27, 28], courtesy of Dr. T. Nagase). (F) Surgically removed calcified lesions (case 52 [36], courtesy of Dr. Tsuda). Sections (A, C , D, and E) were stained with hematoxylin and eosin.

may mean that the likelihood of encountering cases of imported *T. asiatica* is increasing. Surprisingly, from June 2010 to December 2011, an increasing number of human cases with taeniasis have been diagnosed in the Kanto region, including Tokyo and the neighboring 5 prefectures (Gumma, Tochigi, Saitama, Chiba, and Kanagawa) in central Honshu [80-84]. Of 31 taeniasis cases, 20 were attributed to *T. asiatica*. *Taenia asiatica* tapeworms were identified based on nucleotide sequence analysis of the mitochondrial cytochrome *c* oxidase subunit 1 gene [25] and allelic analysis of the 2 nuclear genes for elongation factor 1-α and ezrin-radixin-moesin-like protein genes [85].

Nineteen out of 20 patients infected with *T. asiatica* were Japanese nationals residing in the Kanto area and 1 was a Filipino woman living in same area (Tochigi). Fifteen patients stated that they frequently ate raw pig liver (*sashimi*). Sixteen had never been overseas or, if they had undertaken any international travel, they traveled to countries where *T. asiatica* is not endemic. The infection in the Filipino woman who has returned to the Philippines several times was also considered to have been occurred in Japan.

The occurrence of taeniasis due to *T. asiatica* infection is thus considered to have occurred within Japan by the following reasons: i) most of the patients had never been overseas or traveled to areas where *T. asiatica* is not endemic, ii) most patients had histories of eating raw pig liver, iii) based on interviews with patients and meat inspectors, pigs that had been produced and slaughtered in the Kanto region were strongly suspected to be possible sources of infection, iv) although Japan imports pork from Canada, Mexico, and Europe, no raw pig liver is imported from these countries. At present, the reasons why *T. asiatica* infections successively occurred in the Kanto region, a region within which the disease was not reported previously, have not yet been satisfactorily clarified. Considering that patients have occurred now, it is possible that the workers and pigs on farms in the Kanto region currently constitute the *T. asiatica* reservoirs responsible for these infections. We have been investigating the prevalence of *T. asiatica* metacestodes in pigs from these farms in collaboration with local meat inspection centers. In addition, we have also disseminated information describing precautions against *T. asiatica* infections in Infectious Agents Surveillance Reports (http://idsc.nih.go.jp/iasr/ 32/374/kj3741.html) published by the Infectious Diseases Information Center at the National Institute of Infectious Diseases [80-84].

CONCLUSIONS

It is expected that cysticercosis and taeniasis will primarily be detected as imported cases with the increasing numbers of Japanese travelers to foreign countries where these diseases are endemic or visitors from these areas increase. The occurrence of human infections due to *T. asiatica* is currently restricted to the Kanto region in Japan, and the origins of infection have not yet been clarified. Thus, further occurrence of the disease is likely to occur, medical practitioners should be aware of the importance accurately identifying the causative agent responsible for infection.

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VALIDITY OF THE BEAR TAPEWORM *DIPHYLLOBOTHRIUM URSI* (CESTODA: DIPHYLLOBOTHRIIDAE) BASED ON MORPHOLOGICAL AND MOLECULAR MARKERS

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ABSTRACT: The bear tapeworm *Diphyllobothrium ursi* is described based upon the morphology of adult tapeworms recovered from the brown bear (*Ursus arctos middendorffi*) and larval plerocercoids found in sockeye salmon (*Oncorhynchus nerka*) from Kodiak Island in Alaska in 1952. However, in 1987 *D. ursi* was synonymized with *Diphyllobothrium dendriticum*, and the taxonomic relationship between both species has not subsequently been revised. In this study mitochondrial cytochrome *c* oxidase subunit 1 gene (*cox1*) sequences of holotype and paratype *D. ursi* specimens that had been preserved in a formalin-acetic acid-alcohol solution since the time the species was initially described approximately 60 yr ago were analyzed. Molecular and phylogenetic analysis of the *cox1* sequences revealed that *D. ursi* is more closely related to *D. dendriticum* than it is to *Diphyllobothrium nihonkaiense* and *Diphyllobothrium latum*. In addition to molecular evidence, differences in the life cycle and ecology of the larval plerocercoids between *D. ursi* and *D. dendriticum* also suggest that *D. ursi* is a distinct species, separate from *D. dendriticum* and *D. nihonkaiense*, and also possibly from *D. latum*.

Although *Diphyllobothrium* Cobbold, 1858, contains at least 38 species (Kamo, 1999; Rausch, 2005), the taxonomic status of several of these species is currently unclear and in need of revision. *Diphyllobothrium ursi* Rausch, 1954 (Diphyllobothriidea; Diphyllobothriidae) was described in 1952 based on adult tapeworms collected from brown bear (*Ursus arctos middendorffi*) and larval plerocercoids obtained from sockeye salmon (*Oncorhynchus nerka*) from Kodiak Island in Alaska (Rausch, 1954). Adult *D. ursi* tapeworms were then found in black bears (*Ursus americanus*) from Alaska (Rausch and Hilliard, 1970) and Quebec, Canada (Frechette, 1978; Juniper, 1978), as well as in humans from British Columbia, Canada (Margolis et al., 1973; Ching, 1984). Subsequently *D. ursi* was synonymized with *Diphyllobothrium dendriticum* (Nitzsch, 1824) (Andersen et al., 1987), but no studies have attempted to resolve the taxonomic and phylogenetic relationships between these species at the DNA level since then.

Congeneric species of *Diphyllobothrium* in North America and the countries bordering the northern Pacific Ocean include *Diphyllobothrium alascense* Rausch et Williamson, 1958 (Rausch and Williamson, 1958), *Diphyllobothrium cordatum* (Leuckart, 1863) (Markowski, 1952), *Diphyllobothrium dalliae* Rausch, 1956 (Rausch, 1956), *D. dendriticum* (Andersen et al., 1987), *Diphyllobothrium ditremum* (Creplin, 1825) (Andersen et al., 1987), *Diphyllobothrium klebanovskii* Muratov and Posokhov, 1988 (Muratov and Posokhov, 1988), *Diphyllobothrium lanceolatum* (Krabbe, 1865) (Markowski, 1952), *Diphyllobothrium latum* (Linnaeus, 1758) (Andersen et al., 1987), *Diphyllobothrium nihonkaiense* Yamane et al., 1986 (Yamane et al., 1986; Wicht et al., 2008), and *D. ursi* (Rausch, 1954). Although *D. klebanovskii* has recently been synonymized with *D. nihonkaiense* based on molecular evidence (Arizono et al., 2009), the phylogenetic relationships among *Diphyllobothrium* species, including *D. alascense*, *D. dalliae*, and *D. ursi*, have not yet been clarified.

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The purpose of the present study was to determine whether *D. ursi* is a valid species or not based on morphological characters and phylogenetic analysis of mitochondrial DNA sequence data from *D. ursi* type specimens. The morphological characteristics of *D. ursi* were also redescribed because micrographs of the proglottids had not been published since the initial description of the species.

MATERIALS AND METHODS

Diphyllobothrium ursi type specimens examined in this study

The holotype and paratype specimens of *D. ursi* that were used in this study were collected from a brown bear in 1952. These specimens had been preserved in formalin-acetic acid-alcohol solution (FAA) over 60 yr, since the time the species was initially described and deposited at the Helminthological Collection of the U.S. National Museum (USNM; currently the U.S. National Parasite Collection, USNPC, slide no. 49355). Parts of holotype and paratype specimens preserved in FAA were sent to Department of Medical Zoology, Kyoto Prefectural University for morphological characterization and molecular analysis (Fig. 1).

Molecular analysis

For molecular analysis, 2 type specimens of *D. ursi* preserved in FAA were embedded in paraffin and sectioned to facilitate extraction of the DNA from the fixed proglottids (Yamasaki et al., 2007). Genomic DNA was then extracted from the 10-μm thick, unstained paraffin-embedded sections using a DEXPAT kit (Takara Bio Inc., Otsu, Japan) according to the manufacturer's instructions. Compared to *Ex Taq*–based DNA polymerase, preliminary results revealed that KOD-FX DNA polymerase (Toyobo, Osaka, Japan) showed higher fidelity, amplification efficiency, and elongation capability. The target DNA fragments were, therefore, amplified using KOD FX DNA polymerase in a reaction volume of 25 μl.

Because DNA was considered to have been degraded by long-term fixation, short overlapping DNA fragments (< 300 bp) of the mitochondrial cytochrome *c* oxidase subunit 1 gene (*cox1*) were amplified using primer pairs designed based on the *cox1* genes of *D. nihonkaiense* (AB268585) and *D. latum* (AB269325). The PCR protocol consisted of an initial denaturation step of 94 C for 15 min, followed by 35 cycles of 94 C for 30 sec, 58 C for 30 sec, 72 C for 1 min, and a final extension step of 72 C for 5 min. The short fragments amplified by PCR were then tentatively sequenced. The overlapping fragments of *cox1* were re-amplified using 27 primer pairs designed based on the tentative *cox1* sequences of *D. ursi* (Table I). Twenty-seven overlapping fragments were successfully amplified (data not shown), and all of the sequences were then subsequently joined. Samples for DNA sequencing were prepared using an ABI PRISM BigDye Terminator Cycle Sequencing Reaction kit (ver. 3.1, Applied Biosystems Inc., Foster City, California), and the resultant sequence ladders were read with a 3100 Genetic Analyzer and/or 3730 xl DNA Analyzer (Applied Biosystems Inc.).

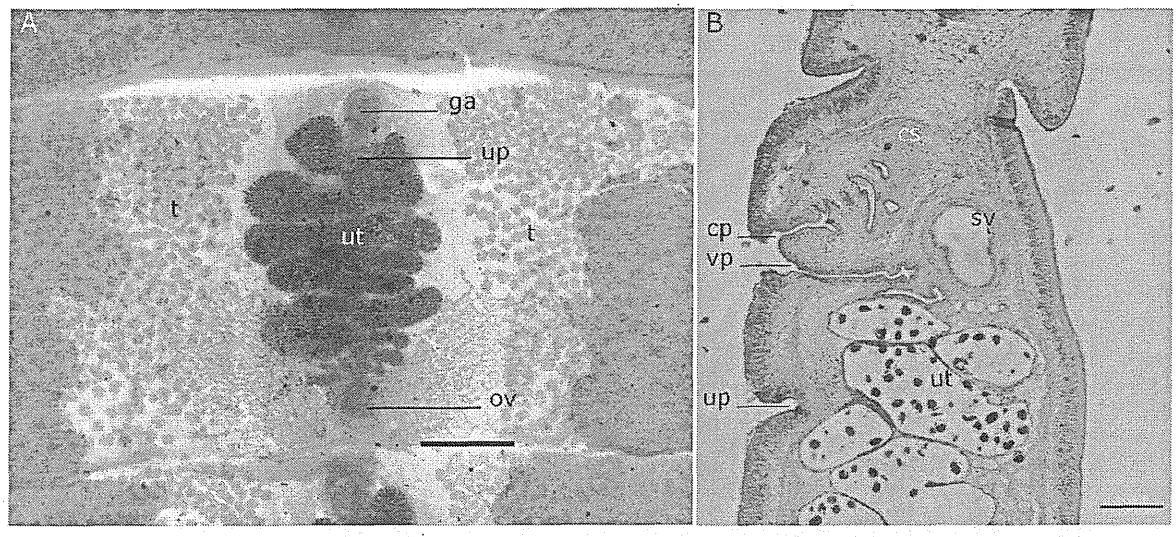

FIGURE 1 The holotype specimen of *Diphyllobothrium ursi* used for morphological characterization. (A) Gravid proglottid stained with acetic acid-carmine. ga, genital atrium; ov, ovary; t, testis; up, uterine pore; ut, uterus. (B) Medial sagittal section of a segment stained with hematoxylin and eosin. Cirrus sac is positioned obliquely to the anterior-posterior axis, and a seminal vesicle is located immediately posterior to the cirrus sac. cp, cirrus pore; cs, cirrus sac; sv, seminal vesicle; up, uterine pore; ut, uteri containing eggs; vp, vaginal pore. Scale bars = 1 mm (A) and 200 μm (B).

Phylogenetic analysis

Phylogenetic analyses were performed using Bayesian inference (BI) and the neighbor-joining (NJ) methods. Phylogenetic trees were constructed using the complete *cox1* sequences (1,566 bp) of 7 taxa of Diphyllobothriidae: *D. ursi* (AB605762, AB605763), *D. dendriticum* (AM412738, AB573182), *D. nihonkaiense* (AB364645, AB268585, AM412599, AM412560, AB544064, AB684623), *D. latum* (AB269325, AB504899, FM209181), *D. ditremum* (FM209182), *Diplogonoporus balaenopterae* (AB355623, AB355628), and *Spirometra erinaceieuropaei* (AB369249). The DNA sequence data for the BI and NJ analyses were converted into NEXUS and FASTA formats using the MEGA program (ver. 5.05). The BI analysis was performed using MrBayes (ver. 3.1.2), and the General Time Reversible model was selected (Ronquist and Huelsenbeck, 2003). Kimura's 2-parameter model (K2P) was selected for the NJ analysis (Kimura, 1980). Phylogenetic trees were rooted with *S. erinaceieuropaei* as an out-group. Clades were assessed by posterior probability and bootstrap resampling (1,000 replicates) of the BI and NJ trees, respectively. Pairwise genetic distances based on the complete *cox1* sequences (1,566 bp) were evaluated by the NJ method with the K2P substitution model (gamma parameter = 1), which is considered suitable for estimating genetic distances when using mitochondrial DNA (Brown et al., 1982).

REDESCRIPTION

Diphyllobothrium ursi

(Fig. 1)

Diagnosis

Genital atrium in anterior half of segment, near anterior margin; uterine pore on mid-line, immediately posterior to genital atrium; posterior portion of uterus markedly coiled, consisting of 5 to 8 loops each side of mid-line; anteriormost loops frequently extending considerably beyond genital pore. Ovary at posterior margin of segment, with 2 distinct lobes connected by narrow reticulated isthmus near center. Testes abundant in each proglottid and disposed in disconnected lateral fields or extending across mid-line, anterior to genital pore. Cirrus pore and vaginal pore in anterior portion in medial sagittal section of gravid proglottid (Fig. 1B). Cirrus pore and vaginal pore surrounded by elevated area of papilla-like protrusions. Uterine pore posterior to genital atrium. Cirrus sac pyriform and relatively large. Seminal vesicles spherical with thick wall, situated immediately posterior to distal end of cirrus sac.

Molecular-phylogenetic relationships between *D. ursi* and related *Diphyllobothrium* species

The nucleotide sequence data of *cox1* for *D. ursi* were deposited in the DDBJ/GenBank database under accession numbers AB605762 (paratype) and AB605763 (holotype). Phylogenetic trees were constructed using the complete *cox1* sequences (1,566 bp) of 7 taxa belonging to the Diphyllobothriidae produced trees with similar topologies. *Diphyllobothrium ursi* formed a monophyletic cluster and was more closely related to *D. dendriticum* than to *D. nihonkaiense* or *D. latum*, even though the posterior probability (0.76) and bootstrap value (63) were relatively low (Fig. 2). The genetic distance between the 2 *D. ursi* individuals was 0.0165, which is considered typical for intraspecific variation. Table II shows the pairwise genetic distances between *D. ursi* and related *Diphyllobothrium* species. The genetic distance between *D. ursi* and *D. dendriticum* ranged from 0.0442 to 0.0570 (mean = 0.0502). The genetic distance between *D. ursi* and *D. nihonkaiense*, and between *D. ursi* and *D. latum*, ranged from 0.0546 to 0.0617 (mean = 0.0580) and from 0.0735 to 0.0887 (mean = 0.0809), respectively.

DISCUSSION

Despite initially being described as a new species in 1954 (Rausch, 1954), *D. ursi* was synonymized with *D. dendriticum* for the following reasons (Andersen et al., 1987). The proglottids of adult *D. ursi* appear to exhibit considerable morphological variation in a variety of hosts; specimens from the brown bear deposited in the USNPC had large, broad segments and were more muscular than those of *D. dendriticum*. Even so, the specimens deposited at the USNPC by Andersen et al. (1987) conform to the description of *D. dendriticum*. However, *D. ursi* can be separated from *D. dendriticum* and *D. ditremum* by several characters, including relatively larger eggs (average 65 × 43 μm), larger number of testis (≤ 1,650), and a strobila exceeding 11 m (Rausch, 1954). As compared with adult *D. ursi*, *D. alascense*, *D. cordatum*, and *D. dalliae* that are distributed in North America

TABLE I. Oligonucleotide primer pairs used to amplify the *cox1* gene of *Diphyllobothrium ursi*.

trnW/F*:	5′-AAGGTCATTTGATGAATACAGA-3′
R185–160:	5′-GTGATTAAAAATTTATAACAGTCTGA-3′
F1–25:	5′-ATGACTAATYTTAAAGTTTTTAGTT-3′
R130–106:	5′-AATTAATCCGTATCATAACACTAAG-3′
F110–135:	5′-GTGTTATGATACGGATTAATTTTGTT-3′
R275–250:	5′-AGGCCAGACAGCAAAGGTATTAAATA-3′
F121–145:	5′-CGGATTAATTTTGTTGAGCCTTATT-3′
R265–241:	5′-ACAAAGGTATTAAATAGTTACCAAA-3′
F160–185:	5′-TCAGACTGTTATAAATTTTTAATCAC-3′
R344–320:	5′-AAAAACAAAATAGAAGGAAATAATA-3′
F310–335:	5′-AGTGCGTGATTATTATTTCCTTCTAT-3′
R475–453:	5′-CCCTAGATAAACCAGCTAAATGT-3′
F415–438:	5′-AGGGATAGTAAGGGTGTTGATTTT-3′
R640–616:	5′-TACGATCAAATAACAACATTGTAAT-3′
F575–600:	5′-CAATTCTTTTGCTTTTAACTATTCCT-3′
R745–721:	5′-GTAAAATTAAAACATATACTTCAGG-3′
F500–524:	5′-GTACCCTTTACACAGCCTTTGTTGA-3′
R640–620:	5′-TACGATCAAATAACAGCATTG-3′
F446–470:	5′-TTTCTTTACATTTAGCTGGTTTATC-3′
R640–620:	5′-TACGATCAAATAACAGCATTG-3′
F600–624:	5′-TGTATTAGCTGCTGCTATTACAATG-3′
R745–721:	5′-GTAAAATTAAGACATACACCTCAGG-3′
F634–655:	5′- GATCGTAAATTTGGTTCAGCTT-3′
R755–735:	5′-CCAAAACCAGGTAAAATTAAA-3′
F720–745:	5′-CCCTGAAGTATATGTTTTAATTTTAC-3′
R905–881:	5′-GCCGTCTTTACATCTAAACCCACTG-3′
F875–899:	5′-TGTTTACAGTGGGTTTAGATGTAAA-3′
R1180–1156:	5′-AAAACACTATTATACTTATATAAGA-3′
F880–905:	5′-ACAGTGGGTTTAGATGTAAAGACGGC-3′
R1060–1035:	5′-CACCCCCGATAGTGAATAACACAATA-3′
F900–925:	5′-GACGGCTGTTTTCTTTAGTTCAGTTA-3′
R1100–1076:	5′-ATATTATCAAGTACACAAGCAGAAA-3′
F1030–1055:	5′-TCATTTATTGTGTTATTCACTATCGG-3′
R1200–1175:	5′-AACTGGCCACCACCAAACAAAAAACA-3′
F1035–1060:	5′-TATTGTGTTATTCACTATCGGGGGTG-3′
R1205–1180:	5′-GTAATAACTGGCCACCACCAAACAAA-3′
F1040–1064:	5′-TGTTATTCACTATCGGGGGTGTTAC-3′
R1160–1135:	5′-TAAGAACCTAGTGACATTACATAATG-3′
F1050–1075:	5′-TATCGGGGGTGTTACGGGTATTATTC-3′
R1250–1225:	5′-ACTATACAATGACATTGTAGTAAATA-3′
F1095–1120:	5′-TAATATTTTGCATGATACTTGGTTTG-3′
R1325–1301:	5′-CACACACGACGRGGTAAACCGCACA-3′
F1180–1205:	5′-TTTGTTTGGTGGTGGCCAGTTATTAC-3′
R1360–1335:	5′-GAATATTAATTCAAGCATAACCTGAC-3′
F1180–1204:	5′-TTTGTTTGGTGGTGGCCAGTTATTA-3′
R1379–1354:	5′-AAGGAACCTATTGACAAAGAATATT-3′
F1220–1244:	5′-ATAAGTATTTACTACAATGCCATTG-3′
R1369–1345:	5′-TTGAGCAAAGAATATTAATTCAAGC-3′
F1305–1328:	5′-CGGTTTACCACGTCGTGTGTGTGT-3′
R1474–1449:	5′-TTGCTGAACTACCATAATAACCTAAA-3′
F1330–1354:	5′-TATGAGTCAGGTTATGCTTGAATTA-3′
R1566–1543:	5′-CTATAAAGCCAACATACTATAATC-3′
F1460–1485:	5′-ATGGTAGTTCAGCAACTTTATTAAAT-3′
trnT/R†:	5′-ATTTACAAAACCAGTATTCTAATTAAA-3′

* Based on the gene for *trnW* of *D. nihonkaiense*.
† Based on the gene for *trnT* of *D. nihonkaiense*.

are morphologically smaller and distinct from *D. ursi* (Rausch and Williamson, 1958; Markowski, 1952; Rausch, 1956).

Rausch and Hilliard (1970) reported that *D. ursi* may be a junior synonym of *Diphyllobothrium gondo* Yamaguti, 1942,

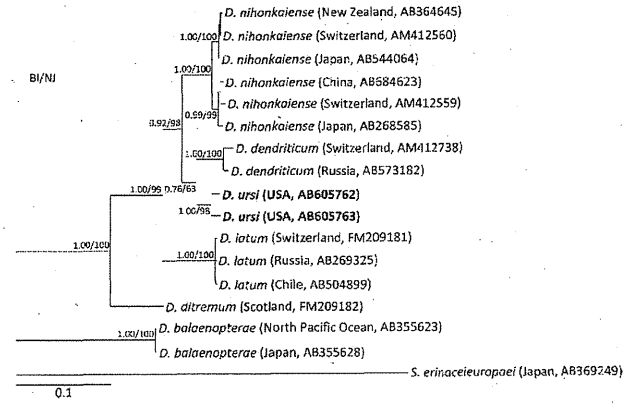

FIGURE 2. Phylogenetic tree inferred using complete *cox1* sequence data (1,566 bp) from *D. ursi* and related *Diphyllobothrium* species by Bayesian inference (BI) and neighbor-joining (NJ) methods. Numbers at nodes indicate posterior probabilities and bootstrap values (1,000 replicates) for the BI and NJ analyses, respectively. Posterior probabilities were estimated based on 1 million generations. Scale bar indicates the number of base substitutions/site. Nucleotide sequence data were obtained from the following accession numbers: *D. ursi* (AB605762, AB605763), *D. dendriticum* (AM412738, AB573182), *D. nihonkaiense* (AB364645, AB268585, AM412599, AM412560, AB544064, AB684623), *D. latum* (AB269325, AB504899, FM209181), *D. ditremum* (FM209182) and *Diplogonoporus balaenopterae* (AB355623, AB355628). *Spirometra erinaceieuropaei* (AB369249) was used as an out-group.

which was collected from the small intestine of a short-finned pilot whale *Globicephala scammoni* (currently *Globicephala macrorhynchus* Gray, 1846, Delphinidae, Cetacea) (Yamaguti, 1942). However, the scolex of adult *D. ursi* is relatively small and muscular, measuring 1.0–1.5 mm in length and 0.75 mm in width; it also has a variable shape and is always spatulate. Conversely, the scolex of adult *D. gondo* (syntype: MPM Coll. No. 22304) deposited at the Meguro Parasitological Museum (MPM) in Tokyo, Japan, is small and wrinkled or funnel-shaped, measuring 0.5–0.6 mm long by 0.3–0.45 mm wide (Yamaguti, 1942; Kamo et al., 1982). Given these marked differences in the adult morphology of *D. ursi* and *D. gondo*, the 2 species are not considered to be synonymous. Adult *D. nihonkaiense* and *D. latum* have spatulated scolices, measuring 2.4–2.8 mm long by 1.2–1.5 mm wide and 1.5–2.5 mm long by 0.8–1.6 mm wide, respectively. Collectively, these morphological features indicate that *D. ursi* is distinct from *D. nihonkaiense* and *D. latum*.

It is difficult to estimate how much genetic distance constitutes inter- or intra-specific variation, particularly since genetic distances vary depending on the target genes and the length of the nucleotide sequences examined (Nakao et al., 2007; Wicht et al., 2010). The genetic distance between *D. latum* and *D. nihonkaiense* calculated using data from 12 mitochondrial protein-coding gene sequences ranged from 0.0438 (cytochrome *b* gene, 1,070 bp) (Wicht et al., 2010) to 0.152 (ATPase subunit 6 gene, 510 bp) (Nakao et al., 2007). The genetic distance between *D. nihonkaiense* and *D. latum* measured using the *cox1* gene sequence data (1,566 bp) was 0.0804 ± 0.0035 (Table II), and the genetic distances between *D. ursi* and *D. dendriticum* was 0.0502. Arizono et al. (2009) reported that the genetic distances of *D. nihonkaiense* vs. *D. dendriticum* and *D. nihonkaiense* vs. *D. latum* estimated based on partial *cox1* sequences (711 bp) were 0.062–

TABLE II. Pairwise genetic distance between *D. ursi* and the related *Diphyllobothrium* species including *Diplogonoporus balaenopterae* and *Spirometra erinaceieuropaei*.

Species	*D. ursi*	*D. dendriticum*	*D. nihonkaiense*	*D. latum*	*D. ditremum*	*D. balaenopterae*	*S. erinaceieuropaei*
D. ursi							
D. dendriticum	0.0502 ± 0.0054						
D. nihonkaiense	0.0580 ± 0.0031	0.0749 ± 0.0036					
D. latum	0.0809 ± 0.0078	0.0767 ± 0.0006	0.0804 ± 0.0035				
D. ditremum	0.1130 ± 0.0001	0.1021 ± 0.0023	0.1118 ± 0.0016	0.1020 ± 0.0000			
D. balaenopterae	0.1718 ± 0.0062	0.1688 ± 0.0026	0.1709 ± 0.0030	0.1473 ± 0.0011	0.1557 ± 0.0013		
S. erinaceieuropaei	0.2310 ± 0.0041	0.2343 ± 0.0016	0.2333 ± 0.0032	0.2316 ± 0.0000	0.2163	0.2156 ± 0.0015	

* Genetic distance (mean ± SD) calculated by the neighbor-joining method using Kimura's 2-parameter model based on 1,566 bp-complete *cox1* sequences.

0.070 and 0.075–0.078, respectively. Although the genetic distances estimated using both partial and complete *cox1* sequences were slightly different, a genetic distance of 0.050 to 0.060 could be considered sufficient for distinguishing between *Diphyllobothrium* species. In this way, *D. ursi* can be separated from *D. dendriticum* and *D. nihonkaiense*, and probably from *D. latum*, at the molecular level. Given that the posterior probabilities and bootstrap values were relatively low, further studies are necessary to examine the relationships between *D. ursi* and *D. dendriticum* that are distributed in North and South America.

The number of *Diphyllobothrium* species that have been described since *Taenia lata* (= synonym of *D. latum*) was described has contributed toward the current confusion regarding the taxonomy, distribution, and host preference of species in this genus (Dick et al., 2001; Scholz et al., 2009). However, *Diphyllobothrium* species can be divided into 3 groups based on their life cycles: (1) freshwater (life cycle is completed in freshwater), (2) anadromous (associated with salmonids migrating from freshwater to the sea), and (3) marine (the entire life cycle occurs in the sea) (Scholz et al., 2009). Within the context of these different life cycles, several interesting differences have been observed in *D. ursi*, *D. dendriticum*, and *D. nihonkaiense*. For example, the plerocercoids of *D. ursi* form cysts on the stomach serosa of sockeye salmon, and the adult tapeworms parasitize bears (Carnivora, Ursidae) and, occasionally, humans as definitive hosts (Rausch, 1954; Hilliard, 1960; Rausch and Hilliard, 1970). Like *D. nihonkaiense*, *D. ursi* belongs to the anadromous group. Conversely, the plerocercoids of *D. dendriticum* are typically found in the viscera and on walls of the body cavity in Arctic chaar (*Salvelinus alpinus*), brook trout (*Salvelinus fontinalis*), lake trout (*Salvelinus namaycush*), Dolly varden (*Salvelinus malma*), rainbow trout (*Oncorhynchus mykiss*), and smolts and adult migrants of sockeye salmon in North America (Andersen et al., 1987). The adult tapeworms infect piscivorous birds, especially gulls and pelicans, mammals such as red foxes and bears (Andersen et al., 1987; Rausch and Hilliard, 1970), and, occasionally, humans (Wicht et al., 2007). The plerocercoids of *D. nihonkaiense* have been found in the muscle tissue of masu salmon (*Oncorhynchus masou*), chum salmon (*Oncorhynchus keta*), and pink salmon (*Oncorhynchus gorbuscha*) (Yamane et al., 1986; Ando et al., 2001), with adult tapeworms infecting brown bears and humans (Kamo, 1999).

In addition to the molecular evidence reported here, the differences in the life cycles, and the site tropism of the plerocercoids support the assertion that *D. ursi* is a valid species, which can be distinguished from *D. dendriticum*, *D. nihonkaiense*, and *D. latum* using molecular and morphological markers.

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Molecular Detection of *Diphyllobothrium nihonkaiense* in Humans, China

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The cause of diphyllobothriosis in 5 persons in Harbin and Shanghai, China, during 2008–2011, initially attributed to the tapeworm *Diphyllobothrium latum*, was confirmed as *D. nihonkaiense* by using molecular analysis of expelled proglottids. The use of morphologic characteristics alone to identify this organism was inadequate and led to misidentification of the species.

Diphyllobothriosis is a fishborne cestodiasis caused by infection with adult tapeworms belonging to the genus *Diphyllobothrium* Cobbold, 1858 (*1–5*); the most frequent etiologic agents are *D. latum* and *D. nihonkaiense*. Humans are infected by ingesting raw or undercooked fish infected with larval plerocoercoids. Adult tapeworms can grow to »2 10 m in length in the human small intestine (*1–6*). Despite the large size of the tapeworms, clinical symptoms can be absent or mild and include mild abdominal pain, watery diarrhea, and abdominal discomfort (*3–7*). *D. latum* infection can also cause vitamin B_{12}-deficiency anemia (*5*).

Diphyllobothriosis caused by *D. nihonkaiense* has been extensively reported in Japan (*3,4*), but it has also occurred autochthonously in South Korea (*8*) and the Far Eastern Federal District of Russia (originally reported as *D. klebanovskii* infection [*9*]). Imported cases have been reported in Europe (*6*), North America (*10*), and New Zealand (*7*) in recent years.

In mainland China, 15 cases of diphyllobothriosis among humans have been reported since the first report in 1927 through 2012; the etiologic species was identified as *D. latum* by morphologic characteristics (*11–13*; Table) and molecular markers (*14,15*). No cases of diphyllobothriosis had been reported in large cities such as Beijing and Shanghai during 1951–2007 (*11*). However, we confirm 4 cases of *D. nihonkaiense* infection in humans in Shanghai, previously identified as *D. latum* infection, during 2008–2011, as well as 1 case in the moderately populous city of Harbin in Heilongjiang Province.

The Study

We examined 5 recent infections of humans with *Diphyllobothrium* spp. (Table, cases 12, 16–19) that occurred in China. Each case had been originally reported as a *D. latum* infection on the basis of morphologic identification only. Case 12 was reported in Harbin City, Heilongjiang Province, in 2009 (*15*). The 4 cases reported in Shanghai were diagnosed at the National Institute for Parasitic Diseases, Shanghai, on the basis of morphologic features of passed strobila. Case-patient 16 lived in Japan, but it was suggested that he acquired the tapeworm in Shanghai where he had frequently eaten raw salmon. Case-patient 17 was a 10-year-old girl from Japan. Whether she became infected in Shanghai or Japan was unclear because of lack of information. Case-patients 18 and 19 acquired the infection in Shanghai because they had never been abroad.

Because all patients in Shanghai had eaten raw salmon, we decided to re-examine how the causative *Diphyllobothrium* spp.were identified. *D. latum* infection is associated with consumption of freshwater fish such as perch (*Perca* spp.), not Pacific salmon (*Oncorhynchus keta*, *O. masou*) and Atlantic salmon (*Salmo salar*) in the Northern Hemisphere (*1–5*). To expand diagnostic parameters and clarify the point of misidentification, we re-identified *Diphyllobothrium* spp. by examining the tapeworms' morphologic features and using a molecular marker. In a sample from case-patient 12, only proglottids stained with acetic acid–carmine were available for testing by both methods (Figure 1, panel A). Proglottids obtained from 4 case-patients in Shanghai were preserved in either 10% formalin (case-patient 16) or 70% ethanol (case-patients 17–19) after collection (Table). Parts of the proglottids were embedded in paraffin, and sagittal sections were prepared for morphologic observation.

For molecular identification of the *Diphyllobothrium* spp., genomic DNA samples were extracted from specimens by using a DNeasy Blood & Tissue Kit (QIAGEN, Hilden, Germany). In specimens from case-patients 17–19, the mitochondrial cytochrome *c* oxidase subunit 1 gene (*cox1*, 1,566 bp) was amplified by PCR by using *Ex Taq* DNA polymerase (Takara Bio, Shiga, Japan) (*7*). In formalin-fixed samples of proglottids from case-patients 12

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Table. Cases of infection with *Diphyllobothrium* species in humans reported in mainland China, 1927–2012*

Case no.	Residence/place of eating fish, if different	Proglottids expelled	Suspected source of infection	Proglottid specimen fixative	Morphologic identification	Molecular identification	GenBank accession no. for *cox1* gene	Reference
1–4	Harbin/Heilongjiang	NA	NA	NA	*D. latum*	NT	NA	(*11*)
5	Beijing	NA	NA	NA	*D. latum*	NT	NA	(*11*)
6	Shanghai	NA	NA	NA	*D. latum*	NT	NA	(*11*)
7	Beijing (returned from United States)	NA	NA	NA	*D. latum*	NT	NA	(*11*)
8	Guangzhou (returned from Argentina)	NA	NA	NA	*D. latum*	NT	NA	(*11*)
9	Harbin/Heilongjiang	NA	Raw fish	NA	*D. latum*	NT	NA	(*11*)
10	Yanji/Jilin	NA	Raw fish	NA	*D. latum*	NT	NA	(*11*)
11	Fujian (lived in Yokohama, Japan, until 1996)	2003 Jan	*Plecoglossus altivelis*	NA	*D. latum*	NT	NA	(12)
12	Harbin/Heilongjiang	2009 Jan	Raw fish	10% formalin	*D. latum*	*D. nihonkaiense*	AB684625	(13) and this study
13	Heilongjiang	NA	Salmon	NA	*D. latum*	*D. latum*	NA	(*14*)
14	Jilin	NA	Salmon	NA	*D. latum*	*D. latum*	NA	(*14*)
15	Shanghai, 2008-2011/Japan, China	2011 Dec	Raw sea and freshwater fish	NA	*D. latum*	*D. latum*	NA	(*15*)
16	Shanghai/Japan (returned from Japan in June 2008; ate raw salmon in Shanghai)	2008 Oct	Raw salmon	10% formalin	*D. latum*	*D. nihonkaiense*	AB684624	This study
17	Shanghai	2011 Sep	Raw salmon	70% ethanol	*D. latum*	*D. nihonkaiense*	AB684621	This study
18	Shanghai. Ate raw salmon in April 2011	2011 Jun	Raw salmon	70% ethanol	*D. latum*	*D. nihonkaiense*	AB684622	This study
19	Shanghai. Ate raw salmon in 2011	2011 Jul	Raw salmon	70% ethanol	*D. latum*	*D. nihonkaiense*	AB684623	This study

*Identification of *Diphyllobothrium* spp. was performed by morphologic identification alone in cases 1–11; organisms in each case were identified as *D. latum*. Cases 12, 16–19 were assessed by morphologic and molecular identification; morphologic identification of all specimens was *D. latum*. Molecular identification varied from morphologic findings in 5 of 8 tested specimens.; NA, not available; NT, not tested.

and 16, DNA degradation caused by the fixative meant that only shorter *cox1* fragments (249 bp, corresponding to sites 880–1128 of *cox1*) could be amplified successfully by PCR by using KOD FX DNA polymerase (Toyobo, Osaka, Japan). DNA sequencing of amplicons was performed with a 3100-*Advant* Genetic Analyzer or 3730 xl DNA Analyzer (Life Technologies, Foster City, CA, USA). Phylogenetic analysis was performed by the maximum likelihood method (MEGA 5.05, http://megasoftware.net/mega.php) and Bayesian inference (MrBayes ver.3.1.2, http://mrbayes.sourceforge.net/). Clades were assessed by bootstrap resampling (1,000 replicates) and a posterior probability (10^6 generations) for the maximum likelihood and Bayesian inference trees, respectively. *Diphyllobothrium* spp. isolated from case-patients 12 and 16 were identified on the basis of sequence identity (%) by performing a BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi) analysis of a DNA Data Bank of Japan (http://www.ddbj.nig.ac.jp).

Accurately identifyingthe *Diphyllobothrium* spp. isolated from case-patient 12 on the basis of morphologic features alone was difficult (Figure 1, panel A). In Figure 1, panels B–E show the sagittal sections of the proglottids from case-patients 16–19. The angle formed by the cirrus sac and the anterior–posterior axis of the proglottids was used as a criterion for differentiating *D. latum* from *D. nihonkaiense* (*1*), even though this criterion is not considered definitive: the angle is usually horizontal in *D. latum*, but oblique in *D. nihonkaiense*. Nonetheless, in this study, on the basis of morphologic criteria, tapeworms from case-patients 16, 17, and 19 were identified as *D. latum* (Figure 1, panels B, C, and E) and the tapeworm found in case-patient 18 was identified as *D. nihonkaiense* (Figure 1, panel D).

Phylogenetic trees based on the complete *cox1* nucleotide sequences showed the same topologies in maximum likelihood and Bayesian inference analyses, implying that the 3 isolates from persons in China (case-patients 17–19; GenBank [http://www.ncbi.nlm.nih.gov/genbank/] accession numbers AB684621–AB648623) are *D. nihonkaiense* (Figure 2). The 2 isolates (AB684625 and AB684624) from case-patients 12 and 16, respectively, were excluded from the analysis because they produced smaller PCR products, but they were identified as *D. nihonkaiense* on the basis of their 99%–100% sequence identity to *D. nihonkaiense*.

The 5 *Diphyllobothrium* spp. tapeworms examined in this study were previously identified as *D. latum* on the basis of morphologic characteristics, as were 3 of the 5 when we re-examined their morphologic characteristics. However, the 5 etiologic agents were confirmed as *D. nihonkaiense*

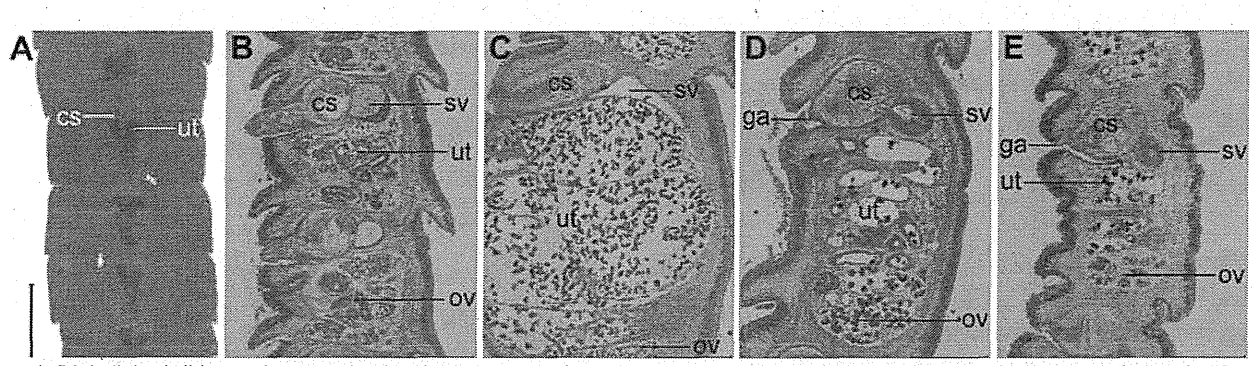

Figure 1. Diphyllobothriid samples examined in the present study, China, 2008–2012. A) Proglottids stained with acetic acid–carmine from case-patient 12. B–E) Sagittal sections of proglottids stained with hematoxylin-eosin from case-patients 16–19. cs, cirrus sac; ut, uterus; sv, seminal vesicle; ov, ovary; ga, genital atrium. Scale bar in panel A represents 2 mm; scale bars in panels B–E represent 500 μm.

by molecular analysis. This discrepancy in the identity of these agents may be attributed to the morphologic similarities between the species and the century-long confusion between the parasite *D. latum* and the parasite that caused human diphyllobothriosis associated with the consumption of Pacific salmon in Japan (*1–3*). Diphyllobothriosis caused by *D. nihonkaiense* has also been reported in South Korea (*10*) and in the Far Eastern Federal District of Russia (*11*) and is considered to be autochthonous and linked to the consumption of wild Pacific salmon in these regions. Therefore, some cases of diphyllobothriosis reported in mainland China were probably caused by infections with *D. nihonkaiense*; case-patient 12 (*15*) in this study is considered to have had such a case. However, a recent report stating that the causative species of 2 diphyllobothriosis cases in northeastern China was *D. latum* suggests that *D. latum* is also indigenous to mainland China (*8*).

Conclusions

We confirmed human diphyllobothriosis caused by *D. nihonkaiense* in mainland China by using a mitochondrial DNA marker. Reassessment of a case in Harbin revealed that some, if not all, of the autochthonous diphyllobothriosis cases were likely initially misdiagnosed as *D. latum* infection because of morphologic similarities between *D. nihonkaiense* and *D. latum* tapeworms. Consequently, molecular analysis is indispensable not only for avoiding diagnostic confusion among *Diphyllobothrium* spp., but also for facilitating the acquisition of reliable epidemiologic and epizootic information and improving clinical relevance and preventive controls for diphyllobothriosis.

Information on diphyllobothriosis and warnings of the potential risks associated with infection by its local species should be disseminated to food handlers, restaurant owners, physicians, and consumers. Because

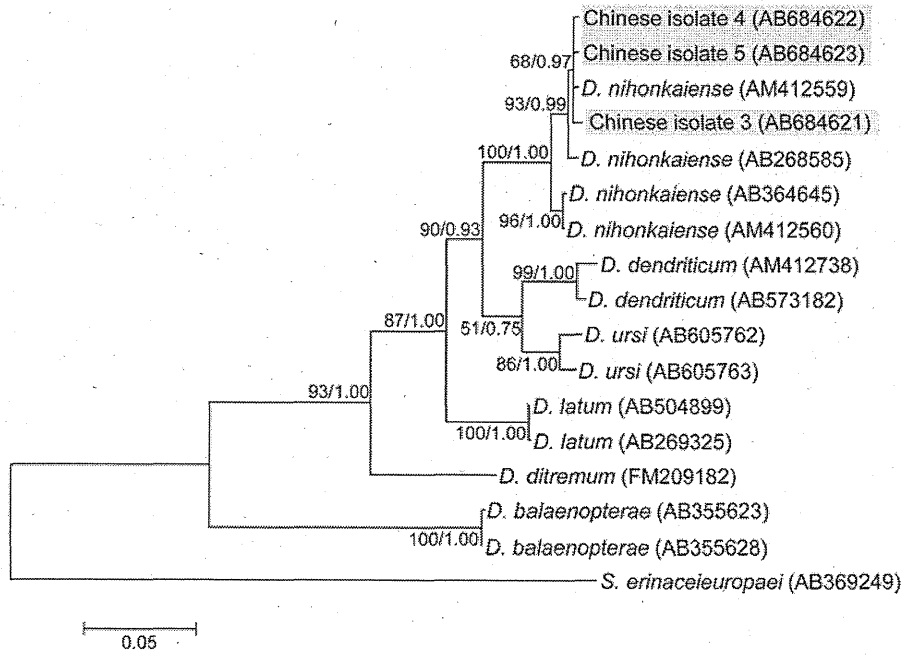

Figure 2. Phylogenetic tree constructed by using the maximum likelihood algorithm (Kimura's 2-parameter model) on the basis of the complete *cox1* sequences of isolates from *Diphyllobothrium* species found in persons in China and related *Diphyllobothrium* species. Numbers at nodes are bootstrap values (1,000 replicates) and posterior probabilities (10^6 generations) for maximum likelihood and Bayesian inference, respectively. *Spirometra erinaceieuropaei* was used as an outgroup. Scale bar indicates the number of base substitutions per site.

we cannot determine with certainty whether previous diphyllobothriosis cases in mainland China were caused by *D. latum* or *D. nihonkaiense*, identification of *Diphyllobothrium* spp. should be performed with care. In addition, studies on the distribution and sources of infection of *D. latum* and *D. nihonkaiense* on mainland China should be undertaken.

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