

Outbreak Reports

First Human Case of Diphyllbothriosis Due to *Dibothriocephalus dendriticus* Infection — China, November 2023

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Summary

What is already known about this topic?

Dibothriocephalus dendriticus (*D. dendriticus*) is a recognized causative agent of diphyllbothriosis, a worldwide fish-borne zoonosis affecting up to 20 million people. It is predominantly distributed in circumboreal regions, and no human infections have been previously reported in China.

What is added by this report?

This is the first human case of diphyllbothriosis caused by *D. dendriticus* in China. We report the clinical and epidemiological findings, as well as the morphological and genetic characteristics of the parasite. Retrospective investigation suggests this was an autochthonous case acquired in China.

What are the implications for public health practice?

The increasing demand for fish products and raw foods poses a growing risk of diphyllbothriosis and potential economic losses. Attention should be paid to preventing *D. dendriticus* from becoming an emerging disease in China due to the globalization of food trade and global integration.

Abstract

Objective: Human diphyllbothriosis is a global fish-borne zoonosis affecting approximately 20 million people. This study reports the first human case of *Dibothriocephalus dendriticus* (*D. dendriticus*) in China and explores its epidemiological and phylogenetic implications.

Methods: Morphological features of eggs and proglottids were examined. The mitochondrial *cox1* gene was sequenced for species identification. Phylogenetic analysis and epidemiological data were analyzed to trace the infection source.

Results: The expelled tapeworm measured 50 cm in length and 0.7 cm in width. The gravid proglottid was longer than wide, with a centrally positioned uterus. Eggs measured $63.29 \pm 1.17 \times 48.31 \pm 0.94 \mu\text{m}$ ($n=15$)

and had an operculum. The *cox1* gene (PQ169609) showed 99.87% homology with *D. dendriticus* (AM412738.2). Morphological and molecular analyses confirmed the parasite as *D. dendriticus*. Consumption of raw salmon in Hong Kong Special Administrative Region (May 2023) and raw trout in Beijing Municipality (August 2022) were identified as potential infection sources. Phylogenetic analysis linked the strain to one from UK fish (KY552870), suggesting a common origin.

Conclusion: This study reports the first human case of *D. dendriticus* in China. It highlights the emerging threat of *D. dendriticus* amid globalization and rising fish consumption. Strengthening food safety measures is essential to reducing infection risk.

Human diphyllbothriosis, a worldwide fish-borne zoonosis, is responsible for the most reported cestode infections in humans, with an estimated 20 million people affected globally (1). Infection occurs through consumption of raw or inadequately cooked fish containing plerocercoid larvae, resulting in symptoms such as diarrhea, abdominal pain, and vomiting. *Dibothriocephalus dendriticus* (*D. dendriticus*) is a prominent causative agent of human diphyllbothriosis, although human infections are considered occasional (2–3). The primary endemic regions include Northern Europe, Arctic and Subarctic North America, and Siberia, particularly the Lake Baikal region (2). However, no human *D. dendriticus* infections have been previously documented in China.

In November 2023, a white worm excreted by a child from Jiangsu Province, China, was submitted to the National Institute of Parasitic Diseases, China CDC for identification. Based on clinical and epidemiological findings, along with morphological and genomic analyses, the sample was identified as *D. dendriticus*.

This case highlights the importance of continued

vigilance against diphyllobothriosis caused by *D. dendriticus* in China and underscores challenges to public health and food safety amid the globalization of food trade, climate change, and cultural integration (4–5).

INVESTIGATION AND RESULTS

A 13-year-old boy from Jiangsu Province began experiencing unexplained weight loss in October 2022, accompanied by mild symptoms including diarrhea and abdominal pain. On November 24, 2023, a white worm was observed hanging from his anus during defecation and broke when his parents attempted to pull it out. The expelled tapeworm, measuring 50 cm in length and 0.7 cm in width, was submitted to the National Institute of Parasitic Diseases, Chinese Center for Disease Control and Prevention, for identification (Figure 1A). Morphological examination and molecular testing, including polymerase chain reaction (PCR) and sequencing, confirmed the parasite as *Dibothriocephalus dendriticus*. The patient was treated with praziquantel and a 3-day course of albendazole. Follow-up stool examination one month post-

treatment revealed no parasite eggs.

A retrospective investigation revealed that the patient had experienced pyrexia of 39 °C due to influenza A virus infection for three days prior to the worm's emergence on November 21, 2023. Laboratory findings showed low direct eosinophil count ($0.03 \times 10^9/L$) and percentage (0.00%), while procalcitonin (PCT, 0.07 ng/mL), C-reactive protein (CRP, 8.18 mg/L), interleukin-1 beta (IL-1 β , 18.14 pg/mL), interleukin-8 (IL-8, 30.87 pg/mL), and immunoglobulin E (IgE, 377.00 IU/mL) levels were elevated. These acute immunological changes likely contributed to the parasite's expulsion.

Epidemiologically, the patient had a preference for raw fish dishes such as sushi and sashimi. Three potential infection sources were identified: sashimi consumed in Japan (February 2020), raw trout (aquacultured in a reservoir) consumed in Beijing Municipality in August 2022, and raw salmon consumed in Hong Kong Special Administrative Region (SAR), China (May 2023). The first scenario was deemed highly unlikely, as the absence of proglottid segments over a three-year period would be improbable. Therefore, this case is considered an

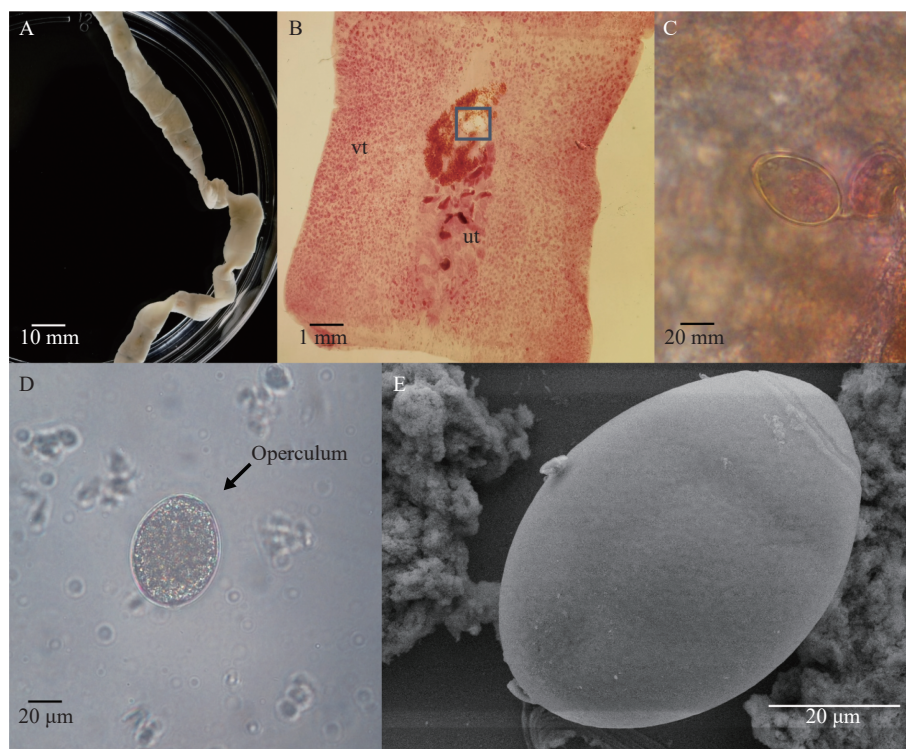


FIGURE 1. Morphological features of *D. dendriticus* from the first human case in China under different magnifications. (A) The worm; (B) Carmine-stained gravid proglottids; (C) Immature egg in the uterus (zoomed in on the square in B); (D) Eggs observed under a light microscope; (E) Eggs visualized using a SEM.

Abbreviation: ut=uterus; vt=vitellarium; *D. dendriticus*=*Dibothriocephalus dendriticus*; SEM=scanning electron microscope.

autochthonous infection acquired within China.

Dibothriocephalus eggs were collected and examined under 400× magnification, measuring $63.29 \pm 1.17 \times 48.31 \pm 0.94 \mu\text{m}$ ($n=15$). An operculum was clearly observed (Figure 1D, 1E), although the small knob was barely visible. Proglottids were stained with alcoholic hydrochloric acid-carmines to enhance visualization of internal structures, particularly the uterus, for definitive identification (Figure 1B).

Gravid segments of *D. dendriticus* are typically wider (0.82–10.0 mm) than long (0.13–2.1 mm) and contain a centrally positioned tubular uterus (ut), which forms 6–8 coils in a rosette-like shape (1). However, the proglottids in our specimen were longer than wide. Although the uterus remained centrally positioned (Figure 1B), the characteristic rosette-like coiling was less pronounced, likely due to mechanical distortion during extraction.

For species confirmation, the mitochondrial cytochrome c oxidase subunit 1 (*cox1*) gene was selected as a molecular marker due to the high morphological similarity among *Dibothriocephalus* spp. eggs. PCR (Figure 2) was performed using the forward primer: GTGTTTTCATTTGATGATGACCAGTC and reverse primer: ATGATAAGGGAYAGGRG CYCA. Sequencing was conducted by BGI Tech Solution (Beijing Liuhe) Co., Ltd. The resulting sequences were analyzed against the NCBI database, confirming the worm as *D. dendriticus*.

A total of 22 related species, including all available

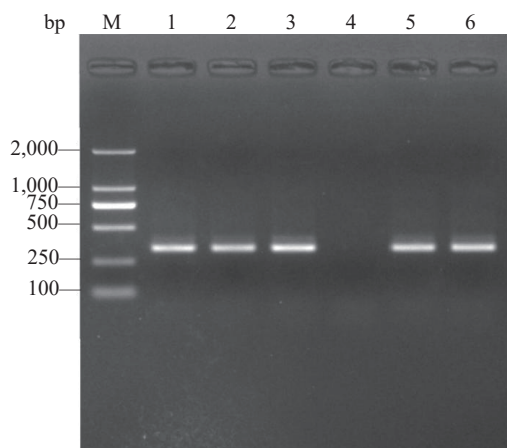


FIGURE 2. Electrophoresis analysis of *Dibothriocephalus dendriticus* using a partial *cox1* gene sequence.

Note: Lane M: molecular marker (bp); Lane 1–3: sample triplicate of *D. dendriticus*; Lane 4: NC; Lane 5–6: PC. Target bands are bright.

Abbreviation: NC=negative control; PC=positive control; *D. dendriticus*=*Dibothriocephalus dendriticus*.

human cases of *D. dendriticus* infection from the NCBI database (Table 1), were selected for phylogenetic analysis to elucidate the evolutionary relationships within the genus *Dibothriocephalus* and identify potential epidemiological connections.

A phylogenetic tree was constructed based on the *cox1* gene of *D. dendriticus*, using Maximum Likelihood (ML) and Bayesian Inference (BI) methods. Primers were designed according to Wicht et al. (6). Sequence alignment was performed in BioEdit 7.2.5 with *Spirometra mansoni* (LC498700) as the outgroup. For the ML analysis, the optimal model (TN+F+I) was selected based on Bayesian Information Criterion (BIC), and analysis was conducted in IQ-Tree 1.6.12 with 1,000 bootstrap replicates (7). For BI analysis, the best-fit model (GTR+I+G) was determined using MrModeltest 2.4, and analysis was performed in MrBayes 3.2.7 with Markov Chain Monte Carlo (MCMC) sampling using four chains over two million generations (8). Trees were visualized using iTOL v6 (9).

The phylogenetic tree (Figure 3) revealed close relationships among *D. latus*, *D. nihonkaiensis*, *D. ursi*, and *D. dendriticus*, with *D. ursi* forming a sister-group relationship with *D. dendriticus*. *D. dendriticus* was divided into two distinct clades: one consisting solely of “Group 1,” which includes strains from fish in Chile, and another containing all remaining groups.

Public Health Response

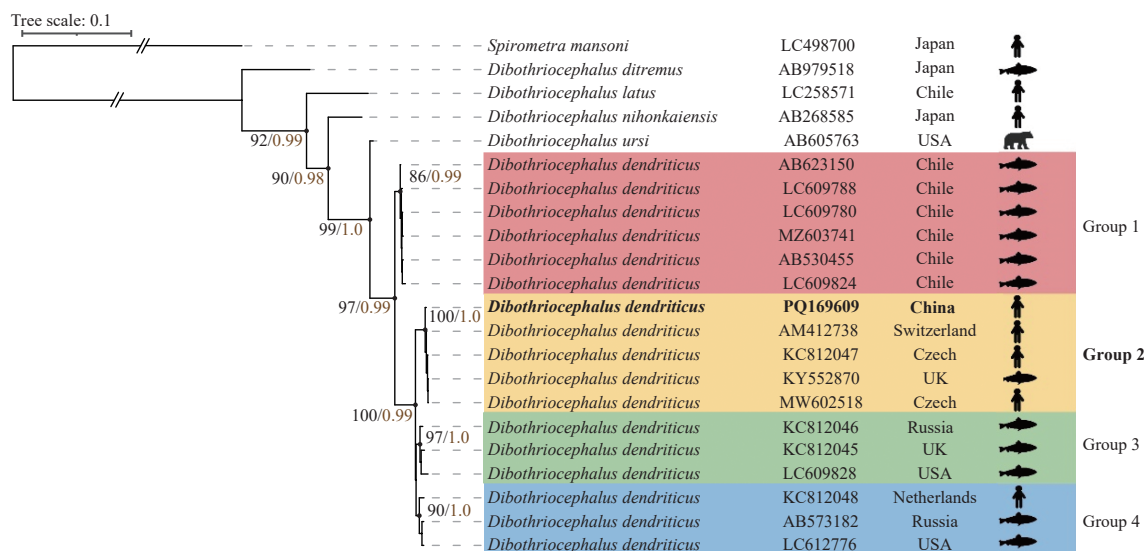
In response to this emerging public health concern, it is imperative to disseminate knowledge about diphyllbothriosis prevention. Public health efforts should promote the consumption of thoroughly cooked fish products and educate consumers about proper freezing techniques. According to U.S. FDA guidelines, freezing fish at -35°C for at least 15 hours effectively eliminates *Dibothriocephalus* larvae (10).

DISCUSSION

Fish represents an essential protein source for human consumption, with demand steadily increasing in recent years. The Food and Agriculture Organization (FAO) projects that production of fish and fish products will exceed 200 million tons by 2030. This rising demand, however, corresponds with an increased risk of diphyllbothriosis. China, previously considered a non-endemic area for *D. dendriticus*, now reports its first human case of diphyllbothriosis caused by this

TABLE 1. List of human *Dibothriocephalus dendriticus* infection cases available on the NCBI site, with GenBank accession numbers.

Country	Patient (age, years)	Clinical symptom	Suspected origin of infection	Accession numbers
Netherlands	Male (31)	None	Brazil (raw fish)	KC812048
Switzerland	Female (59)	Chronically relapsing courses of diarrhea	Alaska (fish), Canada (fish), Norway (fish), Switzerland (salmon)	AB412738
Switzerland	Male (4)	Abdominal cramps, loose stools	Norway (salmon), Asia (fish), Switzerland (perch)	HQ682067
Czech	Female (28)	None	Alaska (wild salmon)	KC812047
Czech	Unknown	Unknown	Canada	MW602518
China	Male (13)	Weight lose	Japan (raw fish), China (raw fish)	PQ169609

FIGURE 3. Maximum likelihood and BI phylogenetic analyses of the complete *D. dendriticus* *cox1* sequences (1,567 bp).

Note: *Spirometra mansoni* was selected as the outgroup. The TN+F+I model was used for ML analysis, while the GTR+I+G model was applied for the BI analysis. Branch support values are indicated on the tree, with ML=black and BI=brown. Newly obtained sequences are highlighted in bold.

Abbreviation: *D. dendriticus*=*Dibothriocephalus dendriticus*; ML=maximum Likelihood; BI=Bayesian Inference.

parasite.

The precise timing and location of infection remain uncertain. *D. dendriticus* typically has a short prepatent period, with peak egg shedding occurring within one year (11). Based on the retrospective investigation and the parasite's life cycle, the May 2023 exposure in Hong Kong appears more likely. However, infection from raw trout consumed in Beijing in August 2022 cannot be ruled out, as the patient reported weight loss beginning in 2022, which is a common symptom of infection.

The atypical morphological features observed in this *D. dendriticus* specimen may result from post-mortem changes or mechanical distortion during extraction by the patient's parents. Similar morphological anomalies have been documented in Swiss strains. De Marval et al. (12) suggested that such anomalies might indicate an Asian origin of infection, while Wicht et al. (6)

proposed they were artifacts resulting from stretching. Phylogenetic analysis reveals that the *D. dendriticus* strain from this case is closely related to strain KY552870 from the UK, suggesting a common origin.

In conclusion, this study documents the first human infection of *D. dendriticus* in China and highlights the emerging threat posed by this parasite. The increasing popularity of raw fish consumption, combined with air transportation without adequate freezing protocols, may elevate infection risk. Enhanced food safety measures and public health surveillance are essential to protect the population from *D. dendriticus* infection.

Conflicts of interest: No conflicts of interest.

Ethical statement: Authorized by the Ethics Committee of the National Institute of Parasitic Diseases, Chinese Center for Disease Control and Prevention (Ref No. 202209). Written informed consent was obtained from each patient or their proxy.

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