

virus infections in Hangzhou (online Technical Appendix Figure). Prompt control of A(H7N9) infection outbreaks and vaccination against seasonal influenza viruses could reduce the potential for co-infections with A(H7N9) virus and seasonal viruses.

Taken together with the previous finding of human co-infection with A(H7N9) virus and A(H3N2) virus (1), our results show that human co-infection with A(H7N9) virus and each of the 3 seasonal influenza viruses currently circulating worldwide can occur. Avian influenza viruses, including A(H7N9), preferentially replicate in the lower respiratory tract of humans (8,9). In contrast, seasonal influenza viruses preferentially infect the upper respiratory tract of humans (10). Coexistence of A(H7N9) virus with either A(H1N1)pdm09 virus or influenza B virus in the pharyngeal swab samples from 2 patients suggests that the upper respiratory tract could provide a location for the A(H7N9) virus to reassort with other influenza viruses. The possibility that seasonal influenza viruses might provide some gene segments that increase the human-to-human transmissibility of possible new reassortants is cause for concern. For detection of such new influenza virus reassortants, extensive surveillance to identify influenza virus co-infections is necessary.

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Misidentification of *Diphyllobothrium* Species Related to Global Fish Trade, Europe

To the Editor: *Diphyllobothriosis*, infection by tapeworms of the genus *Diphyllobothrium* (Cestoda: Diphylllobothriidae) (1), is a well-known disease of humans. In Europe, infections caused by 3 species of *Diphyllobothrium* have recently been reported in humans: *D. latum* is considered to be the principal species infecting persons in Europe (1); 4 cases of *D. dendriticum* infection and 6 cases of *D. nihonkaiense* infection have also been reported (2,3). Except for those caused by *D. latum*, which is autochthonous in northeastern Europe and subalpine lakes, most of the cases in Europe have been imported or caused by consumption of fish imported from areas to which the parasites are endemic (1,3,4).

Diphyllobothriosis is not endemic to Spain, but 7 cases of *D. latum*

infection have been reported there (online Technical Appendix Table, <http://wwwnc.cdc.gov/EID/article/20/11/14-0996-Techapp1.htm>). Most recently, Pastor-Valle et al. confirmed, using molecular tools, an imported case of infestation by *Diplogonoporus balaenopterae* and 3 imported cases of diphyllobothriosis caused by *D. pacificum* (5), a tapeworm endemic to the Pacific coast of South America (1,4).

Specific identification of most human-infecting *Diphyllobothrium* tapeworms based on clinical material is virtually impossible (1,3); the only exception is identifying the Pacific broad tapeworm, *D. pacificum*. This tapeworm can be easily distinguished from other human-infecting diphyllobothriids by the presence of pits alongside the median line on the ventral surface of its proglottids; smaller, more spherical, eggs; and the almost equatorial position of the genital pore, a feature that is markedly pre-equatorial in other species (online Technical Appendix Figure 1, <http://wwwnc.cdc.gov/EID/article/20/11/14-0996-Techapp1.htm>). Several hundred cases of infection by this species have been reported from Peru, and a few reports have been made from Ecuador, Chile, and Japan (1). The life cycle of *D. pacificum* is not completely known, but several species of marine fish have been identified as sources of human infection in Peru (4).

We critically examined all recent records of diphyllobothriosis in Spain to clarify species identification because published morphologic data indicated misdiagnosis (online Technical Appendix Table). Tapeworms detected in 2 recent human cases reported by Colomina et al. (6) and Esteban et al. (7), described as *D. latum*, resembled those of *D. pacificum* because of the morphology of proglottids and eggs (6,7). Therefore, we requested material of these cestodes for scrutiny. Morphologic and molecular evaluation (partial *IsrDNA* and *cox1* gene sequences; multiplex PCR testing by Wicht et al. (8), (Figure,

online Technical Appendix Figures 1, 2) actually confirmed that *D. pacificum* was misidentified as *D. latum* in both cases, despite the molecular identification through multiplex PCR.

No voucher specimens for re-identification were available for another 2 alleged cases of *D. latum* infection (online Technical Appendix Table). However, the eggs reported in the study by Gil-Setas et al. were more similar in shape and size to those of *D. nihonkaiense* or *Diplogonoporus balaenopterae* than to those of *D. latum* (9).

D. latum is the principal causative agent of human diphyllobothriosis; its fish intermediate hosts are perch, pike, burbot, and ruff in Europe (1,4). Other fish, such as salmonids and marine fish, cannot transmit this parasite and serve as intermediate hosts of other species of *Diphyllobothrium* and *Diplogonoporus* (4).

The information on the spectrum of fish intermediate hosts of *D. pacificum* is limited. From very scarce anamnestic data about individual case-patients infected with *D. pacificum* in Spain, it is not possible to unravel the actual source of their infection. However, it

is obvious that the recent emergence of diphyllobothriosis caused by nonendemic species such as *D. pacificum*, *D. dendriticum* (3), *D. nihonkaiense* (2), and *D. balaenopterae* (5) is related to the global importation of fish that have not been frozen. If the fish are merely chilled, plerocercoids of diphyllobothriids may survive for several days (10).

Spain is the third largest importer of fish and seafood in the world; the value of fish products imported from >104 countries reached \$7 billion (US) in 2011 and increases continuously. More than 200,000 tons of fresh or chilled fish, which may serve as source of human fishborne diseases if eaten raw or undercooked, are imported to Spain every year. The fourth largest importer is Ecuador, the sixth is Chile, and the seventh is Peru; *D. pacificum* is endemic to each of these countries (4).

In the present study, we confirmed human infections with the Pacific broad tapeworm, *D. pacificum*, in Europe, but it is highly probable that this species can be introduced anywhere through the importation of fresh or chilled fish from the Pacific coast of South America. This has implications

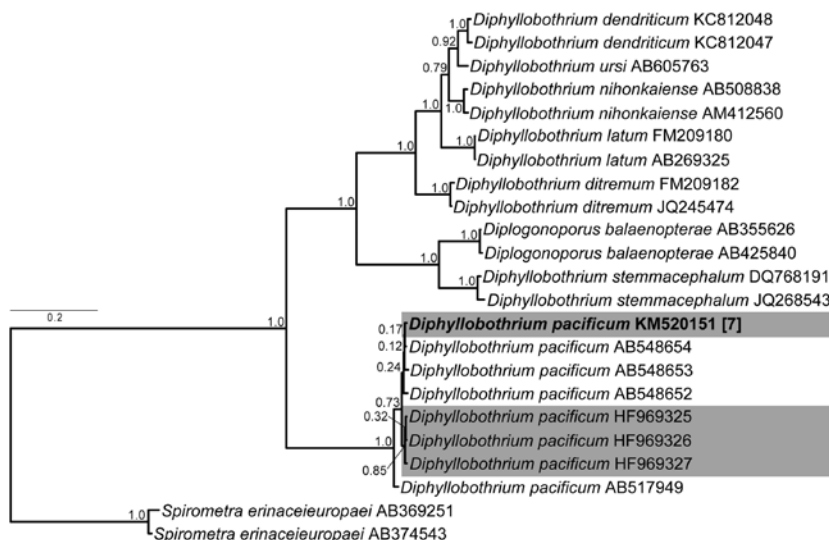


Figure. Bayesian inference phylogenetic analysis of selected human diphyllobothriids based on *cox1* gene analyzed as 3 independent data parts according to the nucleotide coding positions by using (GTR+G)(HKY)(GTR+G) evolutionary model setup in MrBayes (mrbayes.sourceforge.net). Topologies sampled every 1,000th generation over 4 runs and 20,000,000 generations, burn-in 25%. *Diphyllobothrium pacificum* identified in Spain marked in gray; new sequence is in bold type. Scale bar indicates nucleotide substitutions per site.

for food safety rules and human health risk measures taken by national health and veterinary agencies. Regarding adequate processing of clinical samples and their preservation for morphologic and genetic evaluation, we strongly recommend fixation of positive fecal samples with eggs or segments (proglottids) immediately with 96%–99% molecular grade (i.e., not denatured) ethanol for future molecular diagnosis (1,4,8).

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Drug Resistance in *Salmonella enterica* ser. Typhimurium Bloodstream Infection, Malawi

To the Editor: *Salmonella enterica* serotype Typhimurium is one of the most common causes of bloodstream infection in sub-Saharan Africa (1). Among adults, the principal risk factor for invasive nontyphoidal *Salmonella* (iNTS) disease is advanced HIV infection; up to 44% of HIV-infected patients experience bacteremic recurrence through recrudescence of the original infection (2,3). Epidemics of iNTS disease in sub-Saharan Africa have been associated with a novel genotype of *S. enterica* ser. Typhimurium of multilocus sequence type (ST) 313 that is rarely seen outside the region and is associated with multidrug resistance (MDR) to chloramphenicol, cotrimoxazole, and ampicillin (4,5). As a consequence, ceftriaxone has become a key agent in the empirical management of nonfocal sepsis in Malawi (6).

In March 2009, a 40-year-old HIV-infected and antiretroviral therapy-naïve woman sought care in Blantyre, Malawi, with an MDR *S. enterica* ser. Typhimurium bloodstream infection. She was treated with ceftriaxone (2 g intravenously once daily) and discharged with oral ciprofloxacin (500 mg twice daily) for 10 days. She was readmitted 1 month later with recurrent fever. At this time, she had an MDR *S. enterica* ser. Typhimurium bloodstream infection with additional resistance to ceftriaxone and ciprofloxacin. In the absence of a locally available effective antimicrobial drug, she was treated with ceftriaxone, gentamicin, and high-dose ciprofloxacin but died shortly thereafter.

To help clarify how this extended MDR *S. enterica* ser. Typhimurium emerged, we determined the molecular mechanisms underpinning this disturbing pattern of antimicrobial resistance

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Technical Appendix

Technical Appendix Table. Survey of cases of *Diphyllobothrium diphyllbothriosis* and *diplogonoporias* infections, Spain, 1983–2014*

Tapeworm species, subspecies	No. cases	Study	Remarks
<i>D. latum</i> †	1	Zerolo et al. 1983 (1)	No data or material available‡
<i>Diplogonoporus balanopterae</i>	1	Clavel et al. 1997 (2)	Indicated by proglottid morphology
<i>D. nihonkaiense</i> or <i>Diplogonoporus balanopterae</i> †	1	Gil-Setas et al. 2004 (3)	Indicated by egg morphology; no material available
<i>D. pacificum</i>	1	Colomina et al. 2002 (4)	Re-identified as ' <i>D. latum</i> ' by morphology
<i>D. pacificum</i>	1	Esteban et al. 2013 (5)	Re-identified as ' <i>D. latum</i> ' by DNA
<i>D. pacificum</i>	3	Pastor-Valle et al. 2014 (6)	DNA identification‡
<i>Diplogonoporus balanopterae</i>	1	Pastor-Valle et al. 2014 (6)	DNA identification‡

* *D. Diphyllbothrium.*

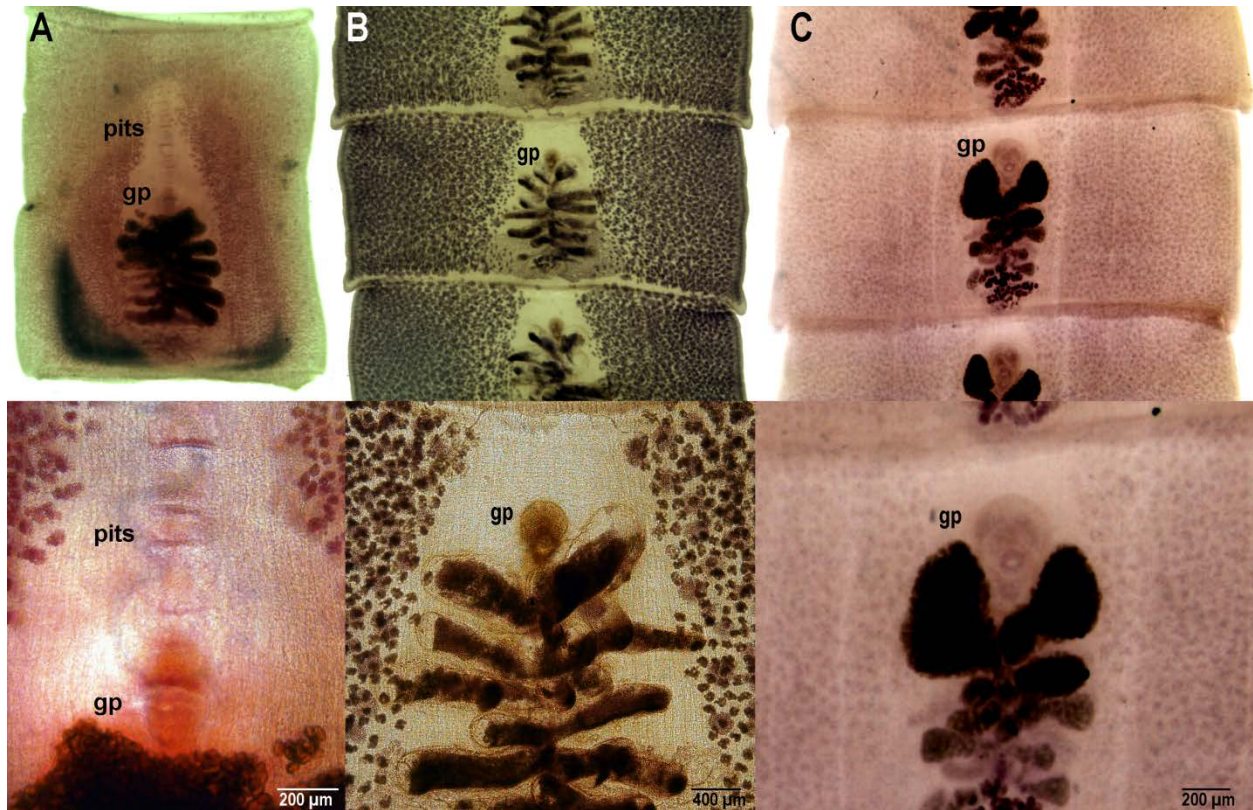
†Confirmation of identity not possible.

‡No morphological data provided.

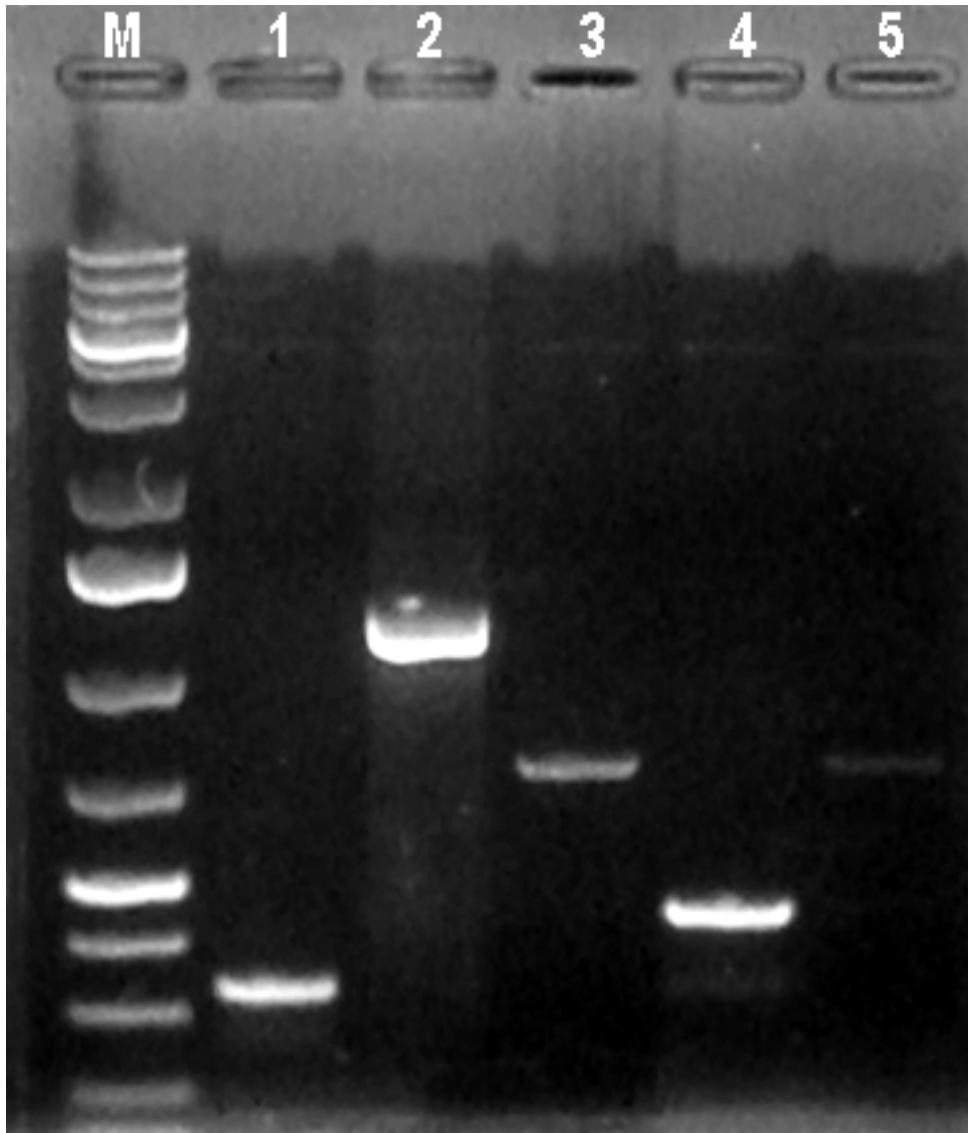
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Technical Appendix Figure 1. Photomicrographs of segments of diphyllobothriids from human. A) *Diphyllobothrium pacificum* reported by Esteban et al. (2013); B) *D. latum* (experimental human infection); C) *D. nihonkaiense* (case from Japan). gp, genital pore; pits, longitudinal pits anterior to the genital atrium typical of *D. pacificum*.



Technical Appendix Figure 2. Differential diagnosis of human-infecting *Diphyllobothrium* spp. by multiplex PCR after Wicht et al. 2010. Lane M, GeneRuler 1kb Plus Ladder (ThermoScientific); lane 1, *D. dendriticum* positive control (318 bp); lane 2, *D. nihonkaiense* positive control (1232 bp); lane 3, *D. pacificum* positive control (727 bp); lane 4, *D. latum* positive control (437 bp); lane 5; Esteban et al. (2013) amlicon.