Molecular Diagnosis of a Hybridized Tapeworm (Between *Taenia Saginata* and *Taenia Asiatica*) Infection Case in Yunnan, China, 2019

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Case Study

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Abstract

Human taeniasis is still prevalent in many countries in the world and affecting human health. Three species of tapeworms in the genus *Taenia*, including *Taenia asiatica*, *Taenia saginata*, and *Taenia solium* are the most common pathogens for the diseases. The genetic relationships of *T. saginata* and *T. asiatica* are close. The natural hybridization between these two has not been clinically diagnosed in China. In this study, we report an 18-year-old male patient with taeniasis hospitalized in Yunnan, China, in 2019. The patient was treated with traditional Chinese medicine, and a tapeworm around 2.7 m long was expelled. The morphology of egg and gravid proglottid of the tapeworm were revealed. More interesting is that the tapeworm was identified as a *T. saginata-T. asiatica* hybridized by the molecularly and phylogenetic analysis. The case study reported the first human taeniasis caused by the *T. saginata* and *T. asiatica* hybridization and suggested that the new types of hybridized taeniases exist in China and further investigation and research on the pathogens in Yunnan is needed.

Main Text

Human taeniasis is caused by three species of tapeworms in the genus *Taenia*, including *Taenia asiatica*, *Taenia saginata*, and *Taenia solium*. Pigs are the intermediate host for *T. solium and T. asiatica* (mostly found in pig liver), and *T. saginata* mainly establishes in bovine striated muscles [1]. As the definitive host, humans are infected by ingesting raw or undercooked infected pork and beef [2]. *T. asiatica* is mainly confined to Asian countries, including Korea, China, Thailand, etc. *T. saginata* and *T. solium* are distributed all over the world [3–6]. The genetic similarity between *T. saginata* and *T. solium* are high. Here, we report a rare case caused by a *T. saginata-T. asiatica* hybridized tapeworm in the Yunnan Province of China.

On February 3, 2019, an 18-year-old boy was hospitalized in E'Shan, Yunnan, China, due to a long-lasting abdominal distension, and white tapeworm segments in his feces. Complete blood count and biochemical analysis were performed and the results showed the high eosinophil count (5.3×10^8/L). Traditional Chinese medicine was prescribed for the treatment with oral administration of pumpkin (*Cucurbita moschata*) seed power first; the extract of areca nut (*Areca catechu*) one hour after; followed by 30% hydrated magnesium sulfate ($MgSO_4$) solution half an hour later. A cestode of about 2.7 m long was expelled after 1.5 hours since the medicine was administrated.

The tapeworm specimen was collected and kept in a dish with phosphate buffer solution (Figure, panel A). Eggs of the tapeworm were found from the fecal sample and gravid proglottid (Figure, panel B). Tapeworm segments stained and and > 13 uterine segments were observed (Figure, panel C). Proglottids of the tapeworm were used to extract genomic DNA using a TIANamp Genomic DNA kit (TIANGEN, Beijing, China). The full-length mitochondrial cytochrome c oxidase 1 (*cox1*) gene and NADH dehydrogenase 1 (*nadh1*) gene, and nuclear 18S ribosomal RNA (18S rRNA) gene were amplified. The primers were designed as follows: Cox1F (5'-TTA GAG GAA ATT GTG AAG TTA CTG CT-3') and Cox1R (5'-TTA TAA GAA TCC ACC AAG CAT GAT GC-3') for *cox1*, Nadh1F (5'-CTC AGG AGA ACT CTT TAT GTG GAG

C-3') and Nadh1R (5'-CAC ACG ACT ATA ATG GTA CCT AAC-3') for *nadh1*, and 18SF (5'-CTT CAC AGC CAC TGC TGC TAA CAC-3') and 18SR (5'-TCC TGC CAG TAG TCA TAT GCT TGT CT-3') for 18 s rRNA. All the replicons were ligated into T-vectors and full-length sequenced.

The complete 1620 bp *cox1* gene sequences showed 99.1–99.9% nucleotide (nt) identity to that of *T. saginata*, 95.7–96.1% to *T. asiatica*, and 88.8–89.1% to *T. solium* (Figure, panel E). The complete 912 bp *nadh1* gene sequences showed 99.2% nt identity to that of *T. saginata*, 95.7–95.9% to *T. asiatica*, and 87.1%-87.4% to *T. solium* (Figure, panel D). However, 2 different sequences of 18S rRNA gene were identified. One sequence was 2604 bp which showed 99.4% nt identity to that of *T. saginata*, and 97.8% to that of *T. asiatica*. The other sequence was 2579 bp and showed 98.3% nt identity to that of *T. asiatica*, and 97.5% to that of *T. saginata* (Figure, panel F). According the *cox1* gene and *nadh1* gene, this specimen was most close to *T. saginata* tapeworms reported in Thailand. But, the 18S rRNA gene of this specimen showed hybridization between *T. saginata* and *T. asiatica*, and indicated that the tapeworm was a heterozygote (Figure, panel F and G). The result was confirmed by PCR detecting 2 genotypes of 18 s rRNA gene with 25 nt deletion (Figure, panel H).

Morphological characteristics of the adult worms, larvae and ova have been used for cestodes identification. However, the phenotypic methods are time-consuming and require special expertise. Now, PCR amplification-sequencing and real-time-PCR have been employed to determine the species of cestodes [7]. The targets are conserved regions in mitochondrial genes (*nadh1* and *cox1*) and ribosomal RNA genes (18S and 28S). Most of the previous reports used *nadh1* and *cox1*. However, the present case indicates that sequencing the mitochondrial genes only may not able to identify the hybridization of close related species, like *T. asiatica* and *T. saginata*, because of the matrilineal inheritance of mitochondrial genes [8]. Thus, amplifying and sequencing mitochondrial and ribosomal genes together will be better for species determination.

The parents of this patient were examined and showed negative of tapeworms. The life history of this patient revealed his dietary preference of barbecue, which is suspected as the source of the tapeworm. Even with the improvement of sanitation and dramatically changes in dietary habits. Taeniasis is still prevalent in some rural areas in Yunnan. A current investigation in 4 townships in Yunnan reported a 16.71% infection rate in the total population and all the cases were diagnosed as *T. asiatica* infection [5]. But this case suggested that *T. saginata* and even new types of hybridized taeniases may exist in the region and further investigation and research on the pathogens of human taeniases in Yunnan is needed.

Conclusion

The present study reported an investigation of a young male patient with taeniasis case in Yunnan, China, in 2019. The genetics and evolution of the tapeworm was identified by both of the mitochondrial genes *cox1* and *nadh1*, and the nuclear 18S rRNA gene. The sequencing and phylogenetic analysis of the nuclear 18S rRNA gene indicated that the tapeworm was a heterozygote of *T. saginata* and *T. asiatica*. This case study gave the first evidence of hybridization between *T. saginata* and *T. asiatica* in China. In

addition to mitochondrial genes, nuclear gene information is very important to understand the genetic characteristics of pathogens in the future.

List Of Abbreviations

Taenia asiatica, *T. asiatica*

Taenia saginata, *T. saginata*

Taenia solium, *T. solium*

cytochrome c oxidase 1, *cox1*

NADH dehydrogenase 1, *nadh1*

nuclear 18S ribosomal RNA, *18S rRNA*

Declarations

Ethics approval and consent to participate:

This research was approved by Medical Ethics Committee of Dali University under number: DLDXLL2018008.

Consent for publication:

Not applicable

Availability of data and materials:

Gene sequences obtained from this study were deposited in GenBank under accession numbers: MN452861-MN452864.

Competing interests:

The authors declare that they have no competing interests.

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Authors' contributions:

YZZ treated the patient and collected the sample; CHL, ZY, YQ, and JL did the gene amplification, cloning and sequencing; ZSH, WF, and JJH performed the morphological investigation; XYG coordinated and designed the experiment; XYG, HYL, PD, YQ, and YZZ wrote the manuscript.

Acknowledgements:

Not applicable

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Figures

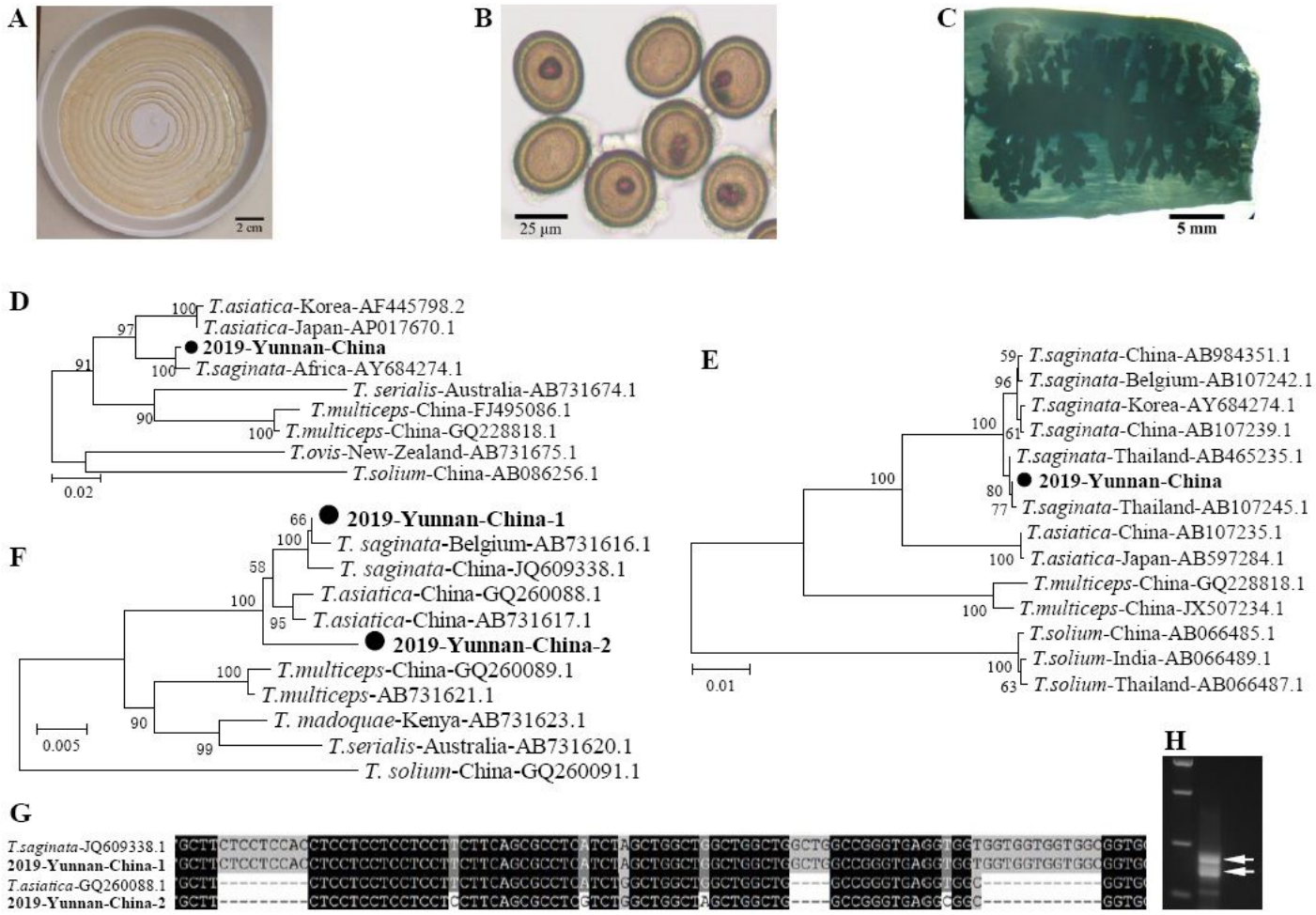
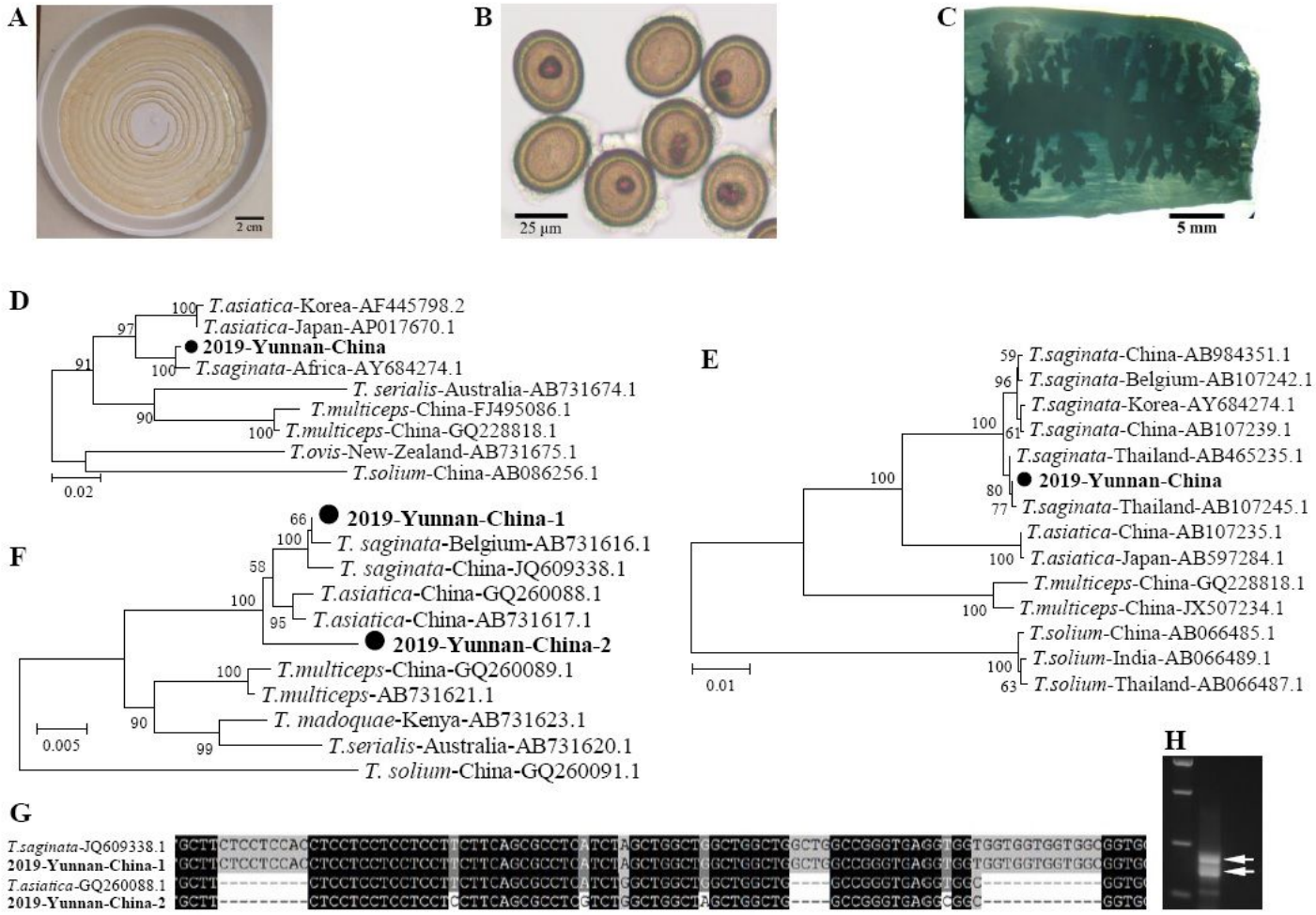


Figure 1

Human taeniasis caused by a hybridized tapeworm between T. asiatica and T. saginata in a 18 year-old boy in Yunnan, China, 2019. A The tapeworm recovered from the patient (about 2.7 m in length). B) Eggs of the tapeworm derived from feces and proglottid. C) A gravid proglottid showing main lateral branches. D) Phylogenetic relationships between the nadh1 nucleotide sequences of the case and those of T. asiatica, T. saginata, T. solium, and T. multiceps tapeworms from various countries. E) Phylogenetic analysis of cox1 gene. F) Phylogenetic analysis of the 2 different nucleotide sequences of the 18S rRNA gene obtained from the case. G) Alignment of the short region in 18S rRNA gene containing 2 deletions. H) Detection 2 genotypes of 18s rRNA gene by PCR. Genes obtained from this study were in bold and indicated by solid circles and the sequences were deposited in GenBank under accession numbers: MN452861-MN452864.



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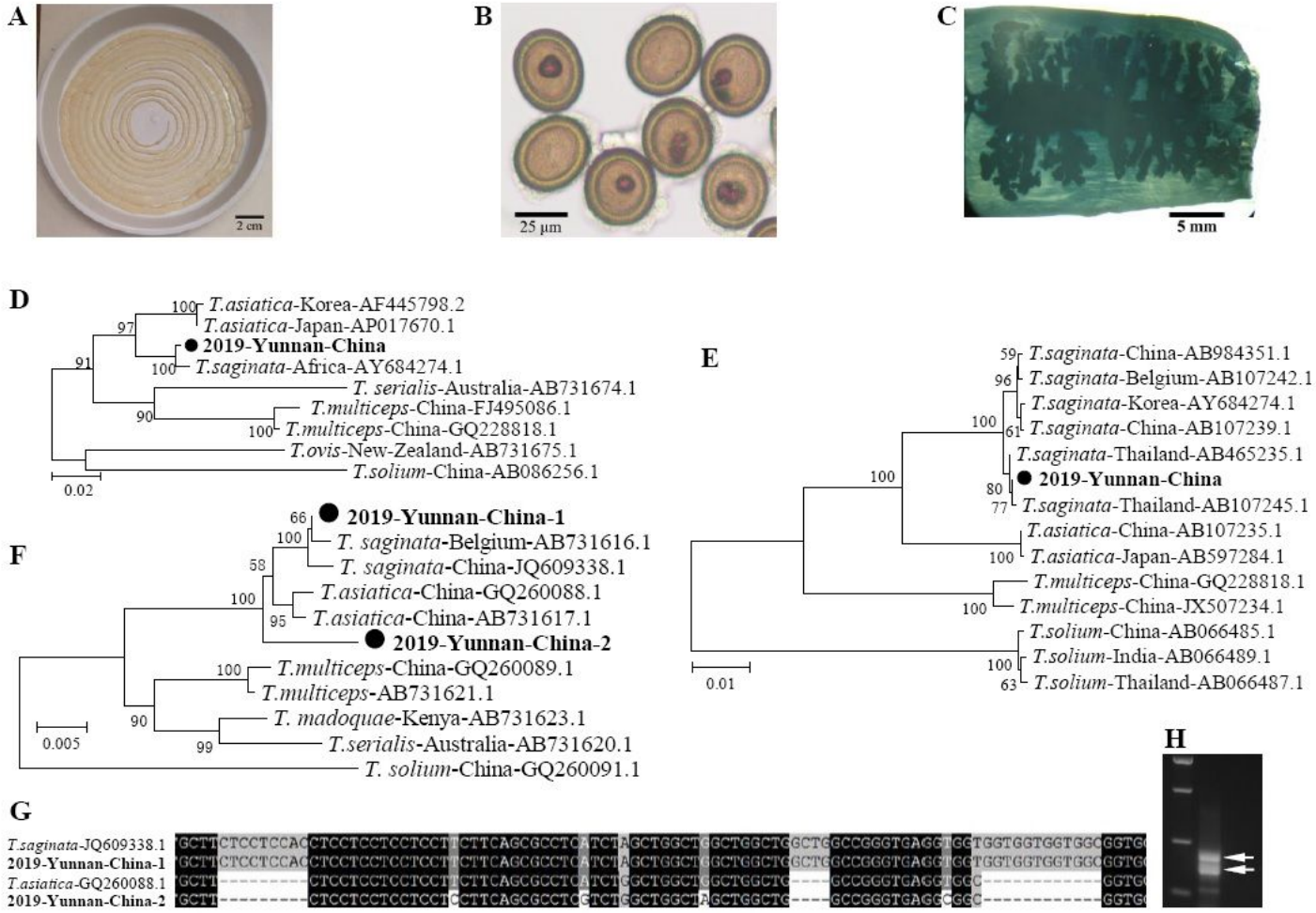

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