

ASIAN TAENIA: SPECIES OR SUBSPECIES?

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Abstract. Asian *Taenia* is a human tapeworm found in Asian countries where people eat undercooked visceral organs of pigs contaminated with metacestodes. It was designated as a new species in Taiwan due to its unique lifecycle, which was confirmed by experimental infections; but described as a genotype, strain, or subspecies of *T. saginata* (*T. saginata asiatica*) due to molecular phylogenetic analyses. Several non-armed adult worms, which had been collected from humans in non-sympatric endemic areas, were examined. First, the mitochondrial DNA was typed by multiplex PCR and sequencing; and then a nuclear gene, elongation factor-1 alpha (EF-1 alpha) gene, was sequenced. Although intraspecific variation of EF-1 alpha gene was not detected, the sequence of EF-1 alpha gene of Asian *Taenia* was distinctly different from that of *T. saginata*. There was no phylogenetic discrepancy between the mitochondrial genes and that from EF-1 alpha gene. These results suggest that the hybridization between Asian *Taenia* and *T. saginata* has not occurred as far as we examined. Recently, it was reported that there were endemic areas where sympatric distribution of *Taenia solium*, *Taenia saginata*, and *Taenia asiatica* was confirmed. In the future, we intend to examine samples from such sympatric zones.

INTRODUCTION

Asian *Taenia* is a human tapeworm found in Asian countries where people eat the undercooked visceral organs of pigs contaminated with metacestodes. It was first recognized in Taiwan, and subsequently in many other Asian countries including China, South Korea, Indonesia, Philippines, and Vietnam (Simanjuntak *et al*, 1997; Ito *et al*, 2003; Eom, 2006). The morphological features of Asian *Taenia* resemble those of *T. saginata*; therefore, it has been equated for a long time with *T. saginata*. However, the life cycle of this cestode was known to be different from classical *T. saginata* Goeze (1782) in its intermediate host as well as in the infected organs (Fan, 1988; Fan *et al*, 1988; Eom *et al*, 2002). Unlike classical *T. saginata*, which infects the skeletal muscle of cattle, Asian *Taenia* in its larval stage infects visceral organs of the pig, such as the

liver, omentum, serosa, and lungs. Based on these differences, the Asian *Taenia* has been tentatively named as *T. saginata* (*taiwanensis* strain) (Cross and Murrell, 1991). The close relationship with *T. saginata* was also supported by molecular approaches (Zarlenga *et al*, 1991; Bowles and McManus, 1994). Eom and Rim (1993) described it as a new species (*T. asiatica* sp. n.), based on more detailed studies of the morphology and life cycle, which was reported by Fan (1988). However, some researchers insist that it should be regarded as a genotype, strain, or subspecies of *T. saginata* (*T. saginata asiatica*) because of the morphological similarity of the adult stage and the phylogenetically close relationship (Ito *et al*, 2003; McManus, 2006). There is considerable ongoing debate regarding the taxonomic position of Asian *Taenia* and whether it should be regarded as a genotype, strain, subspecies, or sister species of *T. saginata*.

In this report, we discussed what is known about Asian *Taenia* and considered its taxonomic position.

TERMINOLOGY

In the literature concerning Asian *Taenia*, the following terms are often used:

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- Strain: variants within a species that have been characterized by using one or more criteria;
- Subspecies: a taxonomic subdivision of a species consisting of an interbred, usually geographically isolated population;
- Sister species: species derived from a common ancestral species shared by no other species; and
- Sibling species: species that are extremely similar in appearance but are nonetheless reproductively isolated from one another.

Although these four terms describe relationships between closely related organisms, the meanings of the first two terms are clearly different from those of the latter two terms. The former terms imply the “same species,” while the latter terms imply “distinct species.”

MORPHOLOGY

Eom and Rim (1993) described the Asian *Taenia* as *T. asiatica* based on four morphological characteristics: 1) the existence of a rostellum on the scolex of the adult, 2) the posterior protuberance in gravid proglottids, 3) the large number of uterine twigs, and 4) wart-like formations on the scolex. However, Fan *et al* (1995) compared the morphological characteristics of the adults and cysticerci of both cestodes and claimed that these four characteristics were not specific for Asian *Taenia*: 1) a rostellum was observed in only 33% of the scolices, 2) posterior protuberance of the gravid proglottid was also observed in *T. saginata*, 3) uterine twigs and main branches were similar in number between Asian *Taenia* and *T. saginata*, and 4) wart-like formations were observed in the larvae of both tapeworms. Fan *et al* (1995) concluded from their findings that the Asian *Taenia* was a subspecies of *T. saginata* (*T. saginata asiatica*). The adult worm of Asian *Taenia* is very similar to that of *T. saginata*. If an adult worm from a human was provided, one could not identify, using only morphological criteria, whether it was Asian *Taenia* or *T. saginata*. However, this fact does not confirm that Asian *Taenia* is the same species

as *T. saginata*. There is a possibility that the Asian *Taenia* is a sibling species of *T. saginata*. In the light of this, it is impossible to determine by morphology alone whether Asian *Taenia* is a species distinct from *T. saginata*.

HOST SPECIFICITY AND ORGANOTROPISM

As described above, the main intermediate host of Asian *Taenia* is a domestic pig, and cysticerci are usually found in the visceral organs. Conversely, the cysticerci of classical *T. saginata* are found in the muscle of cattle (beef). Therefore, it seems that host specificity of Asian *Taenia* is quite different from that of *T. saginata*. However, the metacestodes of Asian *Taenia* were also found in the liver of cattle that were experimentally infected with eggs (Fan, 1988; Fan *et al*, 1988; Eom *et al*, 2002). In addition, the cysticerci of classic *T. saginata* were also found in the liver of slaughtered cattle (Belino, 1975; van Veen, 1979), but never found in the liver of pigs.

There are several strains of *Echinococcus granulosus*, and the host specificity of some strains is quite different from that of other strains (eg, G1: sheep strain vs G4: horse strain). In the case of the genus *Taenia*, Azuma *et al* (1995) examined the infectivity of four laboratory-reared isolates of *T. taeniaeformis* in various rodents. Each of four isolates was most infective to the rodent species from which the original metacestode had been isolated in the field: the SRN and KRN isolates were most infective to the rat; the BMM isolate, to the mouse; and the ACR isolate, to the gray red-backed vole (Fig 1). Furthermore, intraspecific variation of infectivity to intermediate hosts is widely recognized among taeniid cestodes. However, based only on differences of infectivity between Asian *Taenia* and *T. saginata*, we seem to have no rationale to distinguish them as species.

GENETIC DIFFERENCE

Molecular biological studies were also applied to the debate regarding the taxonomic position of Asian *Taenia*. Bowles and McManus

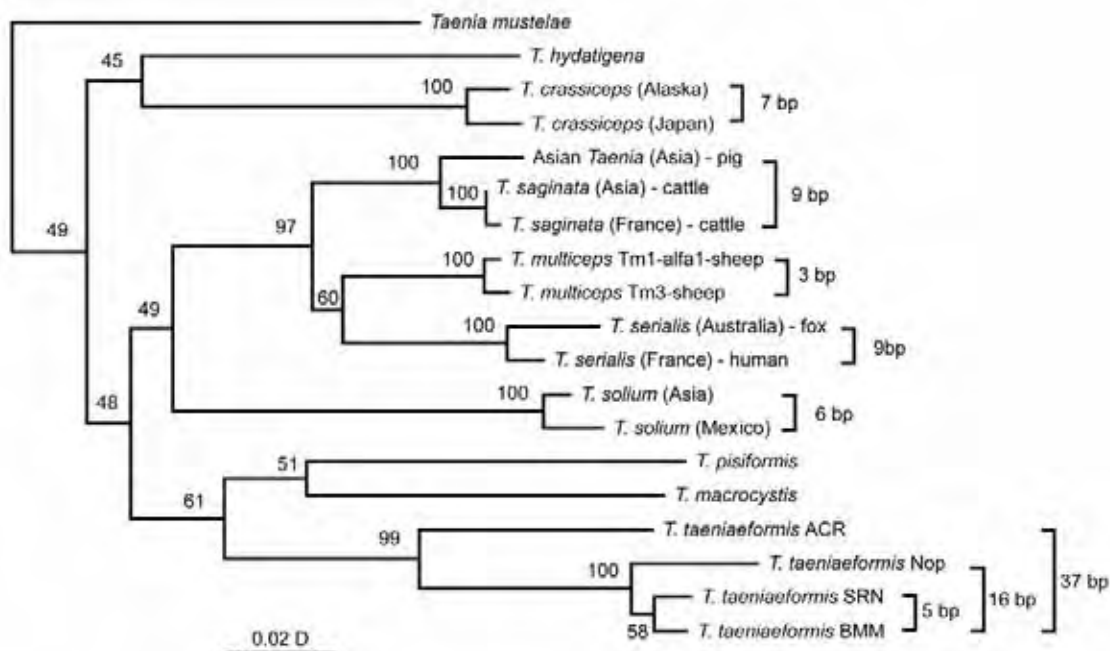


Fig 1- Phylogenetic relationships of the genus *Taenia* inferred from the mitochondrial CO1 gene sequences.

(1994) examined partial sequences of the CO1 gene from several species of the genus *Taenia*, including Asian *Taenia* and *T. saginata*, and inferred the phylogenetic relationships of taeniid cestodes. They concluded that Asian *Taenia* is a genetically distinct entity but is closely related to *T. saginata*, and its taxonomic placement as a subspecies or strain is a more appropriate than a formal designation as a new species. After their report, sequences of the same region of the CO1 gene have been reported from several species of taeniid cestodes. Then we constructed the phylogenetic tree of the genus *Taenia* from the CO1 gene sequence data (Fig 1).

DNA sequence data were aligned using the CLUSTAL W computer program (Thompson *et al*, 1994). The evolutionary distances were computed by Kimura's two-parameter method (Kimura, 1980), and the phylogenetic tree was constructed by the neighbor-joining method (Saitou and Nei, 1987) using the PHYLIP 3.63 computer program. Bootstrap values (Felsenstein, 1985) expressed in percentages out of 1,000 replicates are given near each branch. *Catenotaenia* sp from Hokkaido, Japan was used as an outgroup species

to root the tree. Intermediate host species from which each isolate was isolated are as follows: *T. crassiceps* (Alaska): tundra vole (*Microtus oeconomus*), *T. crassiceps* (Japan): Japanese grass vole (*Microtus montebelli*), *T. taeniaeformis* ACR: gray red-backed vole (*Clethrionomys rufocanus bedfordiae*), *T. taeniaeformis* Nop: Japanese field mouse (*Apodemus argenteus*), *T. taeniaeformis* SRN: Norway rat (*Rattus norvegicus*), *T. taeniaeformis* BMM: house mouse (*Mus musculus*).

Because the length of the CO1 gene sequence used for phylogenetic analysis was not very long (391 bp), the bootstrap value of each node of species was not always high. Therefore, the relationships between taeniid species are obscure in this tree. However, the bootstrap value of the node that divides Asian *Taenia* and *T. saginata* is very high. In addition, nucleotide substitutions between Asian *Taenia* and *T. saginata* occur in only 9 out of the 391 nucleotide positions examined. This difference is comparable to intraspecific variations found in other *Taenia* species. These indicate that Asian *Taenia* is more closely related to *T. saginata*. When using

other DNA sequences, almost the same results are obtained. How do we evaluate these facts?

According to Eom (2006) and Hoberg (2006), recent molecular evidence strongly suggests that *T. asiatica* is a sister-species of *T. saginata*, and therefore a distinct species. McManus (2006), however, asserts that molecular studies support the opinion that the classification of Asian *Taenia* as a subspecies or strain of *T. saginata* is more appropriate than its designation as a separate species. Which stance is more reasonable? The taxonomic status, species or subspecies, could not be determined from DNA sequence data. Compared with interspecific variations among *Taenia* species, the nucleotide difference between Asian *Taenia* and *T. saginata* is very small. The nucleotide difference between two species is proportional to the time since speciation. There may be very few differences between the nucleotide sequences of the two species that have speciated more recently. Although comparison of sequence data is objective, inferring the taxonomic status from such data is quite subjective, as is doing so from the morphological or biological data.

THE BIOLOGICAL SPECIES CONCEPT

When considering the issue of species or subspecies, we must define what constitutes a species. There are several competing theories or “species concepts” (Mayden, 1997). The most widely accepted are the morphological species concept, the biological species concept, and the phylogenetic species concept. The biological species concept defines a species as a group of individuals that are actually, or potentially, interbreeding, and that are reproductively isolated from other such groups. Mayr’s definition is one of the more commonly used definitions because it has an intuitive appeal to what we think species are, and because it addresses the biological reality that reproduction is at least in part a key to what species are. According to Mayr (1969), “species” are groups of populations (which are groups of individuals living together that are separated from other such groups) that can potentially interbreed or are actually interbreeding, and that can successfully produce viable, fertile offspring

(without the help of human technology).

Although “subspecies” means a taxonomic subdivision of a species consisting of an interbreeding, usually geographically isolated population of organisms, a sympatric distribution of these two tapeworms is known in some endemic zones. If Asian *Taenia* is the same species as *T. saginata*, hybridization between Asian *Taenia* and *T. saginata* should occur in sympatric zones. However, no hybrids have been so far identified. In this regard, the number of isolates that have been examined from sympatric endemic areas is limited.

Then, we tried the cross breeding experiments. We can easily obtain cysticerci of both cestodes from immunodeficient, *SCID* mice (Ito *et al*, 1997; Ito and Ito, 1999). It has been demonstrated that cysticerci developed in *SCID* mice are fully developed and capable of infecting humans (Nakaya *et al*, 2006). In February 2006, we carried out experimental infections. Three volunteers ingested the cysticerci. Two persons were positive controls. One person ingested each cysticercus. However, only *T. saginata* successfully infected its host, and crossbreeding experiments have not yet succeeded. Therefore, examination of field samples is very important at this time.

How could we detect the hybridization? As described above, data from mitochondrial DNA sequences indicate that Asian *Taenia* is genetically distinct from *T. saginata*. This suggests that Asian *Taenia* has been isolated from *T. saginata* for some time. Therefore, Asian *Taenia* and *T. saginata* each should have its own type of nuclear DNA. If the hybridization did not occur, phylogeny from mtDNA would be consistent with that from any nuclear DNA. Conversely, if the hybridization has occurred in the past, there would be heterozygous individuals that carry two different alleles (one is derived from Asian *Taenia* and the other is derived from *T. saginata*) in some nuclear loci. Perhaps there are homozygous individuals who carry another type of nuclear genes that is different from that of the mitochondrial gene. However, hybrids have not yet been identified. Therefore, we usually use mtDNA for diagnosis of species (Yamasaki *et al*, 2004). In order to determine

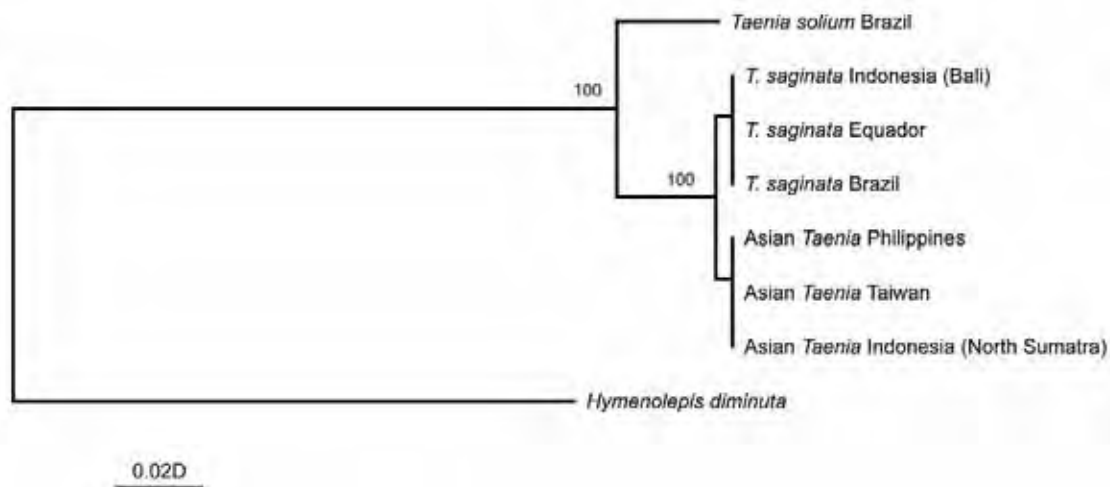


Fig 2- Phylogenetic relationships of the genus *Taenia* inferred from the nuclear EF-1 alpha gene sequences.

whether the hybridization has occurred or not, the mitochondrial DNA type should be determined first, and then nuclear DNA type might be examined. We have selected the nuclear elongation factor 1 alpha gene (EF-1 alpha gene) as a target gene. Although EF-1 alpha is a highly conserved ubiquitous protein involved in translation, it is a single gene and therefore suitable for our purpose.

Several non-armed adult worms, which had been collected from human in non-sympatric endemic areas, were examined. First, mitochondrial DNA were typed by multiplex PCR (Yamasaki *et al*, 2004) and sequenced, and then the EF-1 alpha gene was sequenced. Although intraspecific variation of EF-1 alpha gene was not detected, the sequence of the EF-1 alpha gene of Asian *Taenia* was distinctly different from that of *T. saginata*. The phylogenetic relationship of human *Taenia* that was inferred from the EF-1 alpha gene sequences is shown in Fig 2. Phylogenetic analysis used was same as Fig 1. *Tentacularia* sp (AF124799) was used as an outgroup species to root the tree. There was no discrepancy between the phylogeny from mitochondrial genes and that from EF-1 alpha gene. These results suggest that the hybridization between Asian *Taenia* and *T. saginata* has not occurred, as far as we have examined.

Recently, it was reported that there are

endemic areas where sympatric distribution of *Taenia solium*, *Taenia saginata* and *Taenia asiatica* have been confirmed (Eom *et al*, 2002). In the future, we intend to examine samples from such sympatric zones.

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