

FIRST RECORD OF *Xiphinema hunaniense* Wang & Wu, 1992 (Dorylaimida: Longidoridae) ASSOCIATED WITH TEA (*Camellia sinensis* (L.) Kuntze) IN THANH HOA, VIETNAM

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ABSTRACT

The dagger nematodes, *Xiphinema* spp., are migratory root-ectoparasitic nematodes that cause damage to a wide range of wild and cultivated plants over the world. In Vietnam, this nematode group has been studied mainly based on morphological characterizations. During a survey of pathogens associated with tea, a plant with many medicinal and therapeutic potentials, a population of *Xiphinema hunaniense* was recorded. This study provides the first morphological and molecular characterizations of *Xiphinema hunaniense* found on Tea in Vietnam. The 28S rDNA, and 18S rDNA phylogenetic trees of the genus *Xiphinema* are also provided. 18S rDNA sequence of *X. hunaniense* is also submitted to GenBank for the first time.

Keywords: 18S rDNA, 28S rDNA, molecular, phylogeny, taxonomy.

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INTRODUCTION

Plant-parasitic nematodes may cause damage to all plant parts, including stems, leaves, flowers, fruits, and roots. These nematodes, especially root-ectoparasites, are one of the most damaging pests on crops (Perry & Moens, 2013). They can cause a direct impact on plants through the absorption of nutrients from plant tissues that affects the growth and development of plants. Besides, some nematode genera also inflict wounds on plant tissues, altering host plant tissues and affecting the productivity as well as the quality of host plants (Perry & Moens, 2013; Sikora et al., 2018). On tea, forty nematode species belonging to twenty genera have been reported from different tea growing areas worldwide (Sikora et al., 2018). Mamun et al. (2011) reported that plant-parasitic nematodes caused yield loss up to 20% on tea in Bangladesh. In Vietnam, Nguyen and Nguyen (2000) reported thirty-two species associated with tea, among them, three species of the genus *Xiphinema* were recorded, including one species of the *Xiphinema americanum*-group (*Xiphinema brevicollum* Lordello & da Costa, 1961) and two species of the *Xiphinema non-americanum*-group (*X. elongatum* Schuurmans Stekhoven and Teunissen, 1938 and *X. radicolica* Goodey, 1936). Remarkably, some species of the genus *Xiphinema* are able to transfer plant viruses. Thus, accurate identification of *Xiphinema* spp. is of crucial importance.

In a survey of pathogen associated with tea in Thanh Hoa (Vietnam), a species belonging to the *Xiphinema radicolica* group (within the *Xiphinema non-americanum*-group) was recorded. The combination of morphology and molecular characterization of 18S and 28S rDNA indicated that the *Xiphinema* population recorded in our survey belongs to *X. hunaniense* Wang & Wu, 1992. Herein, we provide morphological and molecular characterizations of this species as a first report of *X. hunaniense* on tea in Vietnam.

MATERIALS AND METHODS

Sampling

Soil and root samples were collected from the tea growing area in Trieu Son district, Thanh Hoa Province. The detritus layer at the rhizosphere of each tea plant was removed before taking approximately 1 kg of soil and 10 g of roots using a shovel. Samples were put in nylon bags and brought to the Department of Nematology, Institute of Ecology and Biological Resources for analysis.

Extraction

250 g of soil or 5 g of roots were used to extract nematodes following the method described by Nguyen (2003). The reference code for *Xiphinema* population in this study was IEBR-NEMA 4776.

Molecular characterization

Fresh nematodes were killed by hot water (60–70 °C) and fixed in TAF for at least 4–5 days. Subsequently, specimens were dehydrated and transferred to glycerine to make permanent slides following Nguyen (2003). Measurements and microphotographs of nematodes were taken using Carl Zeiss Axio Lab A1 microscope (Nguyen et al., 2017).

Molecular characterization

Nematode DNA was extracted following Nguyen et al. (2019). 18S and 28S rDNA regions were amplified using D2A/D3B (5'-ACAAGTACCGTGAGGGAAAGTTG-3'/5'-CCCGGAAGGAACCAGCTACTA-3') and 18F/18R (5'-TCTAGAGCTAATACATGCAC-3'/5'-TACGGAAACCT TGTTACGAC-3') primers (De Ley et al., 1999; Nguyen et al., 2019). The thermal profile to amplify 18S and 28S rDNA regions were: 1 cycle of 95 °C for 2 min, followed by 40 cycles of 95 °C for 35s, 50–55 °C for 30s, 72 °C for 45s, and finished at 10 °C for 10 min. PCR product was purified using the kit GenJet PCR Purification (Thermo Scientific- Germany). Forward and reverse sequences were assembled using Chromas pro. BLAST was used to search for closely related sequences from GenBank. The selected sequences were aligned using

ClustalW. Best fit model was chosen and phylogenetic trees were built using Mega 7.0 (Tamura et al., 2013).

RESULTS AND DISCUSSION

Xiphinema hunaniense Wang and Wu, 1992

Locality: Binh Son commune, Trieu Son district, Thanh Hoa Province, Vietnam.

Measurements: ♀♀ (n=5): L=2203±109 (1905–2289) µm, V%=26±0.8 (25–27), Odontostyle=123±7 (119–131) µm, Odontophore=70±3 (69–75) µm, Stylet=193±8 (189–203) µm, Max. body

diam. =49±2 (47–51) µm, Anal body diam. =23±1 (22–24) µm. Tail length=62±5 (56–67) µm, a=38.5±2 (36–41) c=31±2.5 (29–34), c'=2.5±0.3 (2.4–2.7).

Morphological characterization: Females of *Xiphinema hunaniense* in this study can be characterized by having a body curved ventrally, hemisphere lip region slightly offset from body contour, guiding ring located at 1/5 odontostyle length, lack of anterior genital branch, vagina directed slightly backward, and a digitate tail (Fig. 1).

Host: Tea (*Camellia sinensis* (L.) Kuntze).

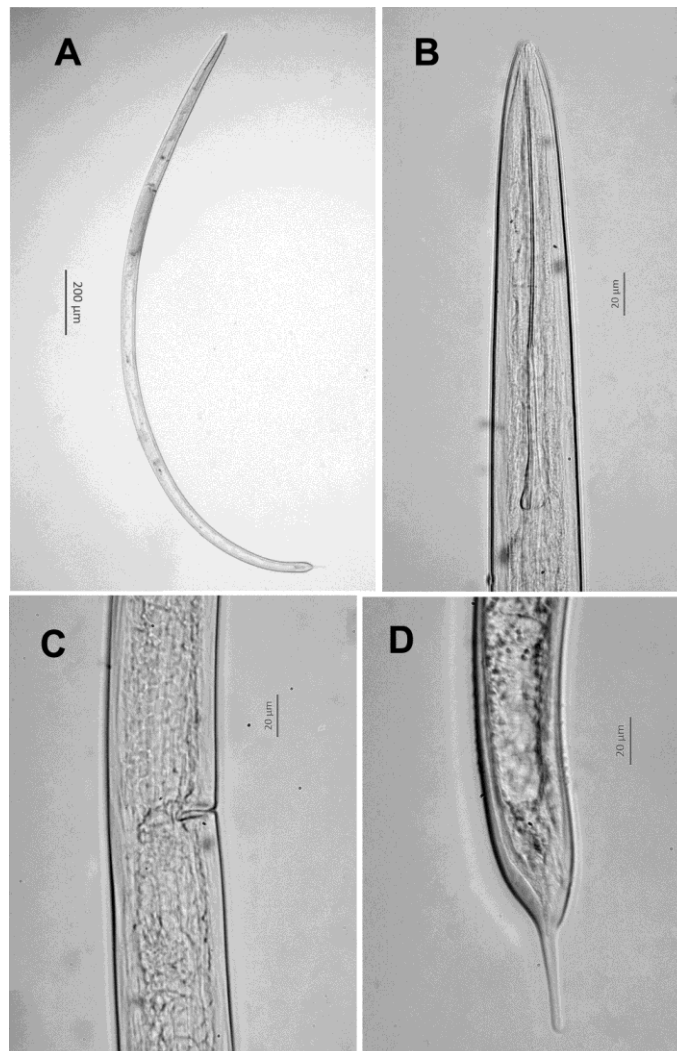


Figure 1. Female of *Xiphinema hunaniense* on tea in Vietnam, A: Entire body, B: Lip region, C: Vulva, D: Tail region

Molecular characterizations:

28S rDNA region

28S rDNA sequence of *Xiphinema hunaniense* in this study was 99.6% similar to the sequence of *X. hunaniense* from China (EF188839) and 99% similar to *X. hunaniense* on turmeric in Vietnam (MT513135, MT513136). Intraspecific variation to *X. hunaniense* was from 0 to 1%. The phylogenetic tree in Fig. 2 clearly showed 2 clades, clade I includes species belonging to the *Xiphinema non-americanum*-group and clade II consists of species belonging to the *Xiphinema americanum*-group. The 28S rDNA

sequence of *Xiphinema hunaniense* in this study, placed together with other *X. hunaniense* sequences from GenBank, has a sister relationship to the sequences of *Xiphinema chambersi* Thorne, 1939, *X. naturale* Lamberti, De Luca, Molinari, Duncan, Agostinelli, Coiro, Dunn & Radicci, 2002, *X. elongatum* Schuurmans Stekhoven & Teunissen, 1938, *X. insigne* Loos, 1949 with relatively high bootstrap support (91%) (Fig. 2). However, the 28S rDNA sequence of *X. hunaniense* in this study (MT846409) was only 86.63–89.11% similar to the sequences in this sister group (AY601617, DQ299515, KU680965, KF430802, AY601619, MT193455).

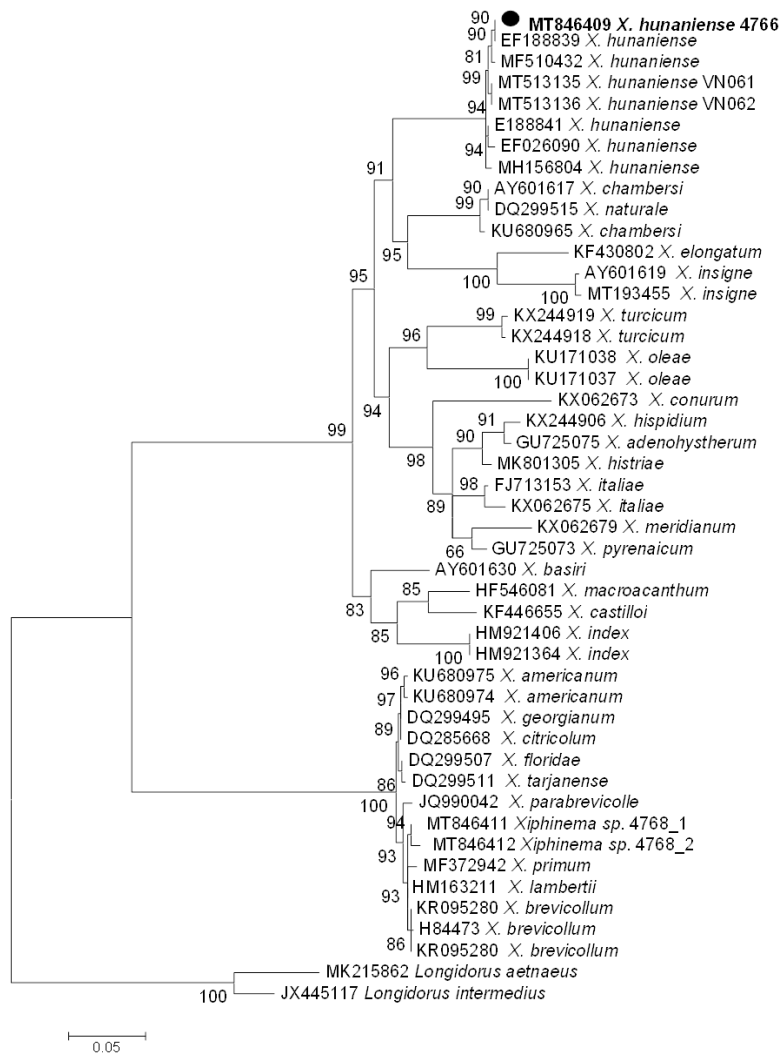


Figure 2. Phylogenetic tree generated from 28S rDNA sequences

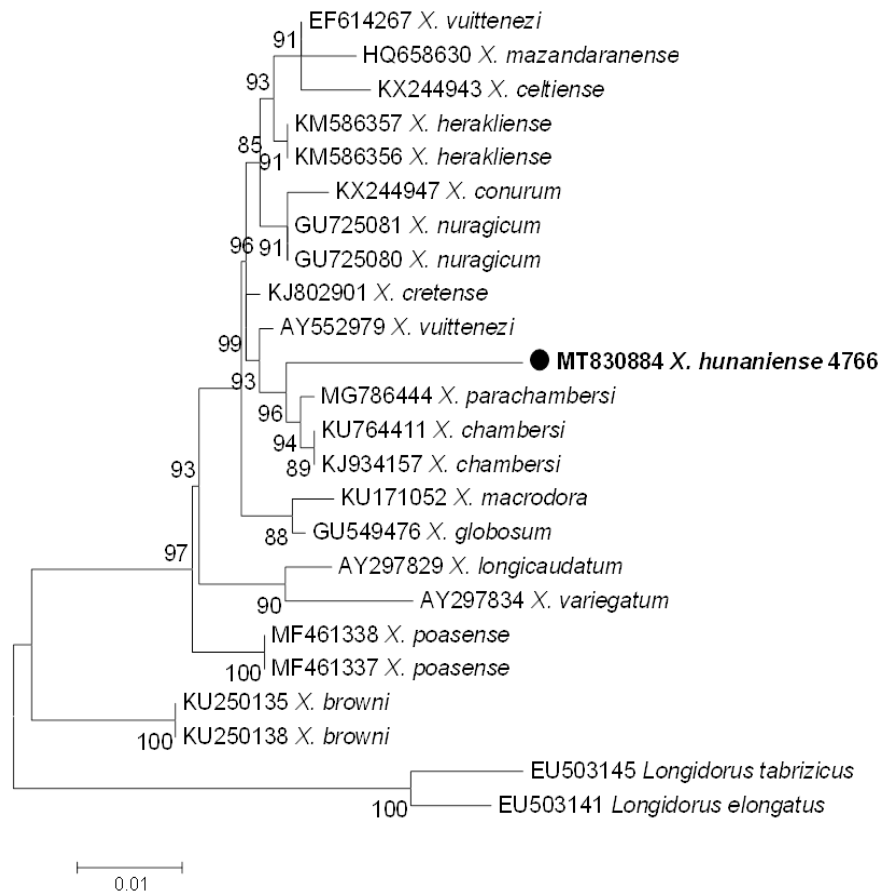


Figure 3. Phylogenetic tree generated from 18S rDNA sequences

18S rDNA region

18S rDNA sequence of *X. hunaniense* in this study was 904 bp long (accession number: MT830884). The interspecific variation between *X. hunaniense* in this study and other *Xiphinema* spp. was 95 to 98%. The phylogenetic tree showed that the 18S rDNA sequence of *X. hunaniense* was separated from all other species and has a sister relationship to sequences of *X. parachambersi* Maria, Ye, Yu & Gu, 2018 and *X. chambersi* (Fig. 3). However, the 18S rDNA sequence of *X. hunaniense* in this study (MT830884) was 98% similar (16 bp difference) to sequences of *X. parachambersi* and *X. chambersi* (MG786444, KU764411, KJ934157).

Discussion:

Although morphological identification of *Xiphinema* species has been frequently used,

there exist very similar species such as species of the *X. americanum*-group (e.g. *X. parapachydermum* Gutiérrez-Gutiérrez, Cantalapiebra-Navarrete, Decraemer, Vovlas, Prior, Rius & Castillo, 2012, *X. paratenuicutis* Gutiérrez-Gutiérrez, Cantalapiebra-Navarrete, Decraemer, Vovlas, Prior, Rius & Castillo, 2012) or some species of the *X. non-americanum*-group (e.g. *X. radicolica*, *X. brasiliense*, *X. hunaniense*) (Gutiérrez-Gutiérrez et al., 2012; Long et al., 2014). Therefore, study on molecular diversity of *Xiphinema* spp. is needed for quick and accurate identification. Especially, 18S, 28S, and ITS rDNA regions were the most frequently used molecular barcodes in identifying *Xiphinema* spp. (Nguyen et al., 2020; Orlando et al., 2016; Wang et al., 2003; Wu et al., 2007). This study was able to amplify 18S and 28S rDNA sequences of *X.*

hunaniense on tea in Vietnam and deposited to GenBank. These sequences can be very useful in future studies of taxonomy and DNA barcoding.

According to the dichotomous identification key of *Xiphinema* species in Vietnam of Nguyen and Nguyen (2000) and other polytomous keys provided by Loof et al. (1996), the *Xiphinema* population IEBR-NEMA 4776 was most similar to *X. radicumicola*, *X. brasiliense*, and *X. hunaniense* by an offset lip region from body contour, vulva anteriorly, gonad single and extending posteriorly, vagina directed slightly backward, and a digitate tail. However, this population can be differentiated from *X. radicumicola* by the stylet length (189–203 vs 143–175 μm) and c' value (2.4–2.7 vs 1.5–2.2). They can be distinguished from *X. brasiliense* by the c' value (2.4–2.7 vs 0.65–0.9). The *Xiphinema* population IEBR-NEMA 4776 was largely in agreement with the morphology of *X. hunaniense* from turmeric in Vietnam, except for odontophore (69–75 vs 70–71 μm), V% (25–27 vs 24.2–25), and tail length (56–67 vs 44–46 μm). Interestingly, variations of the above indices were also shown in the type population and other recorded populations of *X. hunaniense* from China, Nigeria, Australia, Malaysia, and Thailand (Long et al., 2014; Wang & Wu, 1992; Wu et al., 2007). Although the type population of *X. hunaniense* was found on tea (Wang & Wu, 1992), this study provides the first report of *X. hunaniense* on tea in northern Vietnam, added to the total of four species of the genus *Xiphinema* on tea in Vietnam (Nguyen & Nguyen, 2000).

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