Morphological and molecular characterisation of *Xiphinema vuittenezi* Luc, Lima, Weischer & Flegg, 1964 (Nematoda: Dorylaimida) from vineyards in Armenia

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Summary. *Xiphinema vuittenezi* is a plant-parasitic nematode widely distributed in the Republic of Armenia. Until recently, morphological and morphometric features of the Armenian population of *X. vuittenezi* have been poorly studied and data concerning molecular characteristics were not available. The study was conducted on the population of *X. vuittenezi* isolated from the rhizosphere of grapes in vineyards of the Aragatsotn region. Nematodes were extracted from soil using the decanting and sieving method and identified by morphological and morphometric parameters. The morphological and morphometric data of *X. vuittenezi* were compared with the original and other descriptions. The sequences of the D2-D3 expansion segments of the 28S rRNA gene and the *COI* gene of the Armenian population of *X. vuittenezi* showed similarity with those from Iran and the Czech Republic.

Key words: 28S rRNA, *COI* gene, grapevine, Longidoridae, morphometric data, *Vitis vinifera.*

Grapevine (*Vitis vinifera* L.) is a traditional crop for the Armenian agriculture. There has been significant progress in the rehabilitation and development of viticulture and winemaking over the past years, due to comprehensive and voluminous projects implemented in Armenia. The vineyards gradually expand, new ones are planted, and wine production increases (Hovhannisyan *et al.*, 2017). Many soil-borne pathogens can significantly reduce the yield and quality of crops. Among the phytopathogens affecting agriculture in Armenia, the ectoparasitic nematodes are one of the most important factors (Karapetyan *et al.*, 2011).

Nematodes of the genus *Xiphinema* are migratory root ectoparasites that feed mainly on the root tips of a wide range of wild and cultivated plant species (Hunt, 1993) and several species also transmit nepoviruses (Taylor & Brown, 1997). To date, several *Xiphinema* species, *X. artemisiae* Chizhov, Tiev & Turkina, 1986, *X. bakeri* Williams, 1961, *X. brevicolle* Lordello & da Costa, 1961, *X. diversicaudatum* (Micoletzky, 1927), Thorne, 1939, *X. index* Thorne & Allen, 1950, *X. pachtaicum* (Tulaganov, 1938), Kirjanova, 1951, *X. tarjani* Luc, 1975 and *X. vuittenezi* Luc, Lima, Weischer, Flegg, 1964 were reported in Armenia (Akopyan, 1991; Karapetyan *et al.*, 2011; Tabolin *et al.*, 2016). Currently, there are some doubts about the correct identification of *X. bakeri* and *X. tarjani* and these reports require confirmation.

It has been known that *X. vuittenezi* is distributed in Europe, Asia, North and South Americas, and Australia (Andrássy, 2007) and although it is considered as a polyphagous pest, this species prefers grapevine and stone fruits (Romanenko, 1993). The aim of this research was to conduct the morphometric and molecular characterisation of the Armenian population of *X. vuittenezi*.

MATERIAL AND METHODS

Nematode sampling. Soil samples were collected using a shovel and borer from 20 to 60 cm depth in the rhizosphere of the grapevine of Aragatsotn marz (village Parpi, 40°19'21.8" N, 44°18'38.6" E) in May 2017. Nematodes were extracted from the soil samples using a modification of the decanting and sieving method (Flegg, 1967).

Morphological study. For morphological studies, the nematodes were killed with hot water,

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fixed in a 5% formalin solution, and mounted in glycerin on slides using the Seinhorst's technique (Seinhorst, 1959). The measuring of specimens was made using the microscope Mikmed-6 (LOMO, Russia) and attached camera A35140U (Omax, China). The identification to species level was done by using cladograms (Coomans *et al.*, 2001) and the original description of this species (Luc *et al.*, 1964).

Molecular and phylogenetic study. The total DNA of live or fixed in ethanol nematodes was extracted from single individuals using the Proba express kit (Syntol LLC, Russia) according to the manufacturer's instructions. Amplification was conducted in a 2720 Programmable Thermal Cycler (Applied Biosystems, USA) under the following conditions: 95°C for 5 min and then 45 cycles of denaturation (95°C, 10 s), annealing (60°C, 15 s), extension (72°C, 1 min) and final extension (72°C, 10 min). The primers: D2A (5′- ATG AGC GGG ATG AGC TGT G -3′) and D3B (5′- ATA CTC CCG TCC GCA GAT C-3′) were used for the amplification of the D2-D3 expansion segments of the 28S rRNA gene (Nunn, 1992). The partial *COI* gene was amplified with the forward primer JB3 (5′- TTT TTT GGG CAT CCT GAG GTT- 3′) and the reverse primer JB5 (5′- AGC ACC TAA ACT TAA AAC ATA ATG AAA ATG -3′) (Derycke *et al*., 2005). PCR products were purified with the EvroGen PCR Clean-Up Kit (EvroGen LLC, Russia) following the manufacturer's protocol. Sequencing of amplicons was carried out with the genetic analyser ABI PRISM 3500 (Applied Biosystems, USA). New sequences were submitted to the GenBank database under numbers: MH484521 and OQ699231.

Phylogenetic analysis. New sequence of the D2-D3 of 28S rRNA gene was aligned using Clustal W with some published sequences of *Xiphinema*. The phylogenetic tree was inferred using the maximum likelihood (ML) method under the GTR + I + G model of evolution using the program MEGA 7 (Kumar *et al*., 2016). To obtain an estimate of the support for each node, a bootstrap analysis using 1000 replicates was performed. Bootstrap supports are given on appropriate clades for the ML tree.

RESULTS

During our surveys in Armenia, *X. vuittenezi* was found near the roots of grapes (*Vitis vinifera* L.) in the villages Parpi (Aragatsotn marz), Areni (Vayots Dzor marz), Lukashin (Armavir marz), Myasnikyan (Armavir marz), near the roots of quince trees (*Cydonia oblonga* Mill.), plum trees (*Prunus domestica* L.), apple trees (*Malus domestica* Borkh.), walnut trees (*Juglans regia* L.) and peach trees (*Prunus persica* (L.) Batsch) in the village of Norashen (Ararat marz) and near the roots of peach trees (*Prunus persica* (L.) Batsch) in the village of Myasnikyan (Armavir marz). The population density of *X. vuittenezi* in the rhizosphere of grapevine of village Parpi was 8±3 nematodes per 100 g of soil in May 2017.

Xiphinema vuittenezi Luc, Lima, Weischer & Flegg, 1964
(Figs 1 & 2, Table 1)

Female. The bodies of fixed specimens have an open C shape with the posterior region of the body more strongly curved. Cuticle finely transversely striated. The lip region is flatly rounded, slightly offset by depression. The amphidial aperture is almost as broad as the lip region. The odontostyle, odontophore and guiding ring are typical of the genus. The pharynx is longidoroid. The reproductive system is didelphic-amphidelphic with the anterior branch slightly longer than the posterior one. Each branch consists of a reflexed ovary, not reaching the oviduct–uterus junction; oocytes are arranged first in several rows and then in a single row; an oviduct with developed *pars dilatata oviductus* is located near the sphincter, *pars dilatata uteri* and cylindrical uterus, which is convoluted to a greater or lesser degree, with conspicuous globular bodies and spindle-shaped structures (Z-differentiation) (Fig. 2). Uterine eggs (n = 10): one or two at the same time, 188 ± 7.7 (174-198) × 38 ± 3.6 (33.3-46) μm. The vulva is slit-like, situated near mid-body. The tail is dorsally convex conoid, digitated with a ventral peg of variable length. There are two to three pairs of caudal pores on the tail.

Male. Not found.

The morphological and morphometric characteristics of the Armenian population of *X. vuittenezi* are very close to those of holotype females from Guntersblum, Germany (Luc *et al.*, 1964). The most notable difference is the position of the vulva, which is usually slightly pre-equatorial in the Armenian population (V = 48.4 (44.8-52.3)%) and slightly post-equatorial in the German population (V = 50.3 (46-55)%). However, the pre-equatorial position of the vulva of *X. vuittenezi* was also observed in Romanian and Iranian populations (Table 1).

Molecular characterisation. The sequences of the D2-D3 expansion segments of the 28S rRNA

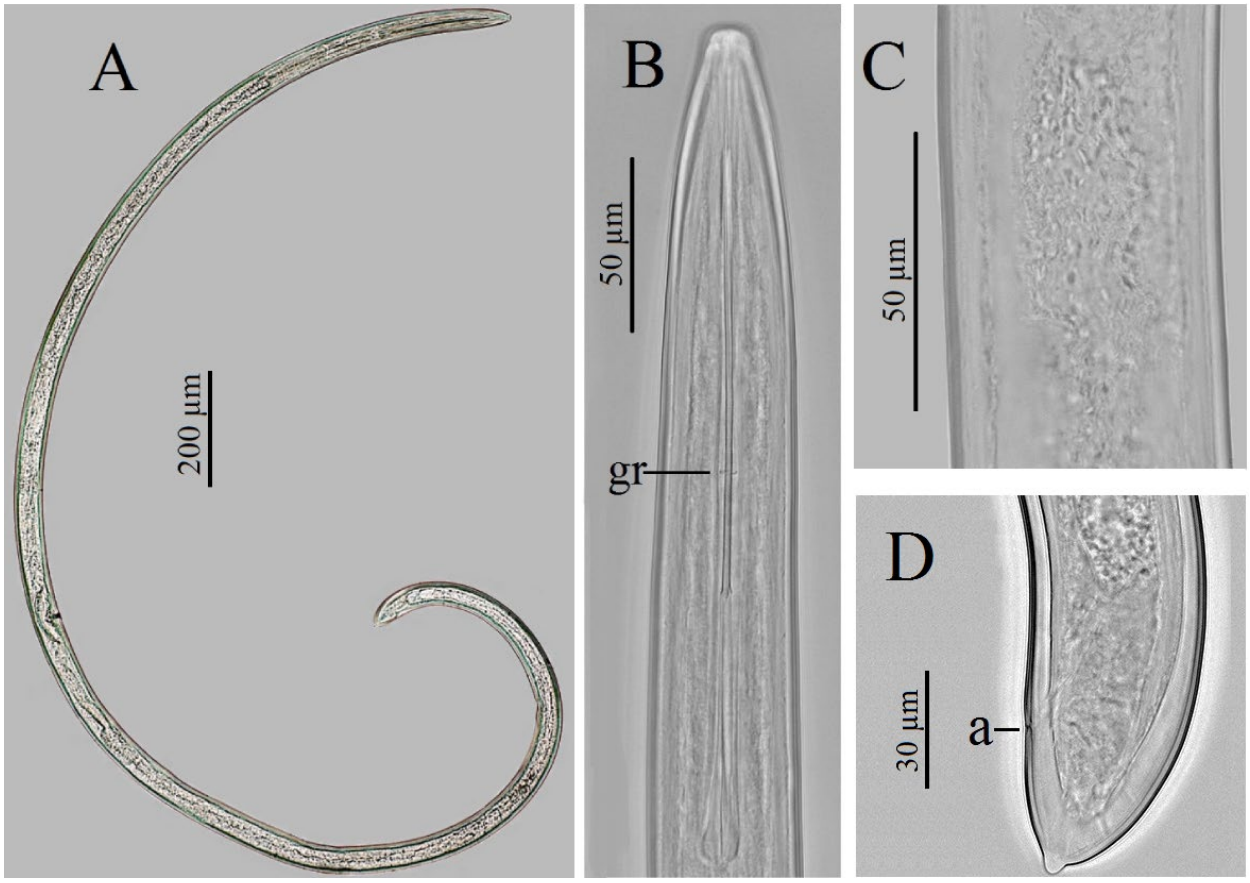

Fig. 1. Light microphotographs of *Xiphinema vuittenezi*. A: Entire female; B: Anterior region; C: Tubular part of uterus with spindle-shaped spines; D: Tail region; gr – guiding ring, a – anus.

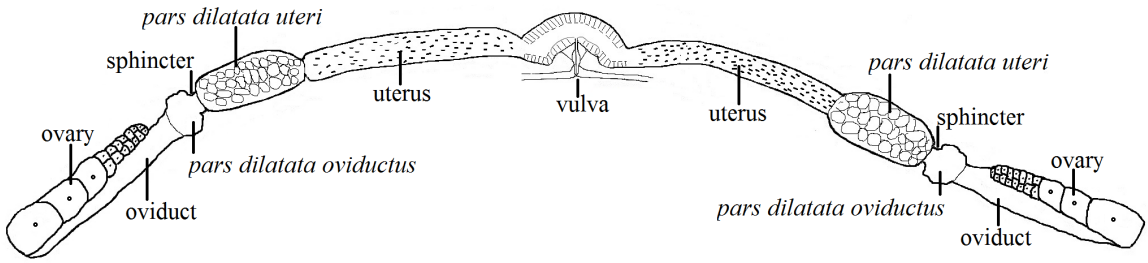

Fig. 2. The female genital system of *Xiphinema vuittenezi*.

gene and the *COI* gene obtained from three samples were identical within each marker. The sequences of the D2-D3 expansion segments of the 28S rRNA gene of the studied specimens showed a 99.86% similarity to the sequence of *X. vuittenezi* from Iran (MK957239), a 99.73% similarity to the sequence of *X. vuittenezi* from the Czech Republic (EF614266) and a 99.46% similarity to the sequences of *X. vuittenezi* from Romania (HG329724). In the partial 28S rRNA gene phylogenetic tree (Fig. 3), the sequence of the Armenian population of *X. vuittenezi* formed a clade with the above-mentioned sequences with a high bootstrap support. The sequences of *X. vuittenezi* from Hungary and Romania are clustered on a separate branch, since they have two identical substitutions that distinguish them from sequences from Armenia, the Czech Republic and Iran. The sequence of the *COI* gene of the Armenian population of *X. vuittenezi* shared a 97.19% similarity with *X. vuittenezi* from the Czech Republic (EF614265). The similarity with other

Table 1. Measurements of *Xiphinema vuittenezi* females from Parpi (Armenia) and their comparison with those from the literature.

Characters Locality	Parpi village, Armenia (this study)	Guntersblum, Germany (Luc *et al*., 1964)	Slaný, Czech Republic (Kumari & Decraemer, 2006)	Murfatlar, Romania (Groza *et al*., 2013)	Sufiyan, Iran (Vazifeh *et al*., 2019)
n	27	35	30	5	6
L (mm)	3.3 ± 0.2 (2.9-3.8)	3.24 (2.66-3.84)	3.35 ± 0.18 (3.00-3.75)	3.13 ± 0.24 (2.84-3.35)	3.51 ± 0.16 (3.25-3.76)
a	64.7 ± 5.9 (50.3-76.0)	66 (57-79)	61.5 ± 3.4 (52.2-67.6)	61.1 ± 4.3 (55.1-64.9)	69.0 ± 5.0 (63.0-78.0)
b	7.2 ± 0.58 (6.2-8.44)	7 (5.7-9.0)	6.5 ± 0.5 (5.6-7.4)	7.0 ± 0.5 (6.4-7.5)	7.6 ± 0.1 (7.3-7.9)
c	82.1 ± 7.8 (72.0-98.1)	93 (70-120)	85.8 ± 6.7 (75.0-97.8)	98.6 ± 7.7 (92.4-108.6)	89.0 ± 3.6 (77.0-95.0)
c’	1.1 ± 0.1 (0.9-1.3)	0.95 (0.8-1.1)	0.97 ± 0.1 (0.83-1.09)	0.9 ± 0.1 (0.8-1.0)	1.10 ± 0.08 (1.00-1.20)
V %	48.4 ± 1.7 (44.8-52.3)	50.3 (46-55)	49.2 ± 0.9 (47-51)	46.3 ± 1.1 (44.5-47.4)	49.0 ± 0.4 (47.0-50.0)
G1%	13.5 ± 0.2 (13.2-14)	14.5 (13-17)	–	13.9 ± 1.2 (13.0-15.6)	12.6 ± 0.3 (12-13)
G2%	12.8 ± 0.6 (12-14)	15 (12-18)	–	13.6 ± 1.1 (12.2-15.3)	12.0 ± 0.8 (11-13)
Odontostyle	128.1 ± 4.8 (120.0-138.0)	131 (124-135)	126 ± 4.5 (118-134)	135.0 ± 5.4 (129.5-142)	122.0 ± 3.7 (118.0-128.0)
Odontophore	75.9 ± 5.7 (65.0-85.0)	74 (65-82)	77 ± 2.5 (73-83)	80.6 ± 2.2 (78-83)	68.0 ± 4.1 (51.0-75.0)
Oral aperture to guiding ring	119.9 ± 7.2 (100.0-130.0)	113 (105-124)	120 ± 4.6 (109-129)	128.3 ± 2.7 (124-131)	108.0 ± 4.3 (100.0-113.0)
Tail	39.8 ± 2.4 (36.2-44.0)	34 (30-41)	39 ± 2.6 (35-45)	32.5 ± 2.6 (30-36)	39.0 ± 1.4 (38.0-41.0)
J (hyaline portion of tail)	15.1 ± 1.6 (13.0-18.0)	≤15	11 ± 1.8 (9-16)	11.6 ± 0.9 (10-13)	14.0 ± 1.8 (12.0-16.0)
Terminal peg	3.9 ± 1.5 (1.0-6.0)	2.7 (0-4.4)	–	–	–
Body diam. at lip region	13.6 ± 1.2 (12.0-16.8)	14 (13.5-16)	14 ± 1.3 (12-19)	13.2 ± 1.4 (11-15)	13.0 ± 0.7 (12.0-14.0)
Body diam. at guiding ring	39.5 ± 2.1 (38.0-41)	–	40 ± 1.8 (36-43)	38.7 ± 1.9 (37-41)	–
Body diam. at base of pharynx	46.0 ± 6.2 (38.0-55.0)	–	49 ± 3.0 (43-58)	43.7 ± 0.9 (42-44)	41.0 ± 2.7 (37.0-45.0)
Body diam. at mid-body or vulva	50.6 ± 4.4 (42.0-62.5)	49 (45-52)	55 ± 4.2 (48-66)	51.1 ± 0.7 (50-52)	49.0 ± 5.3 (41.0-56.0)
Body diam. at anus	38.3 ± 2.6 (32.5-42.0)	38 (35-42)	40 ± 2.0 (36-44)	37.0 ± 3.4 (31-39)	35.0 ± 2.1 (32.0-38.0)

Note: All measurements are in μm (mean ± SD) excluding L (mm).

sequences of *Xiphinema* deposited in GenBank was less than 80%. Thus, the results of the study confirmed the presence of the plant-parasitic nematode *X. vuittenezi* in the rhizosphere of grapevine in Armenia. Further research is needed to characterise other species of dagger nematodes occurring in Armenia, especially virus-transmitting species.

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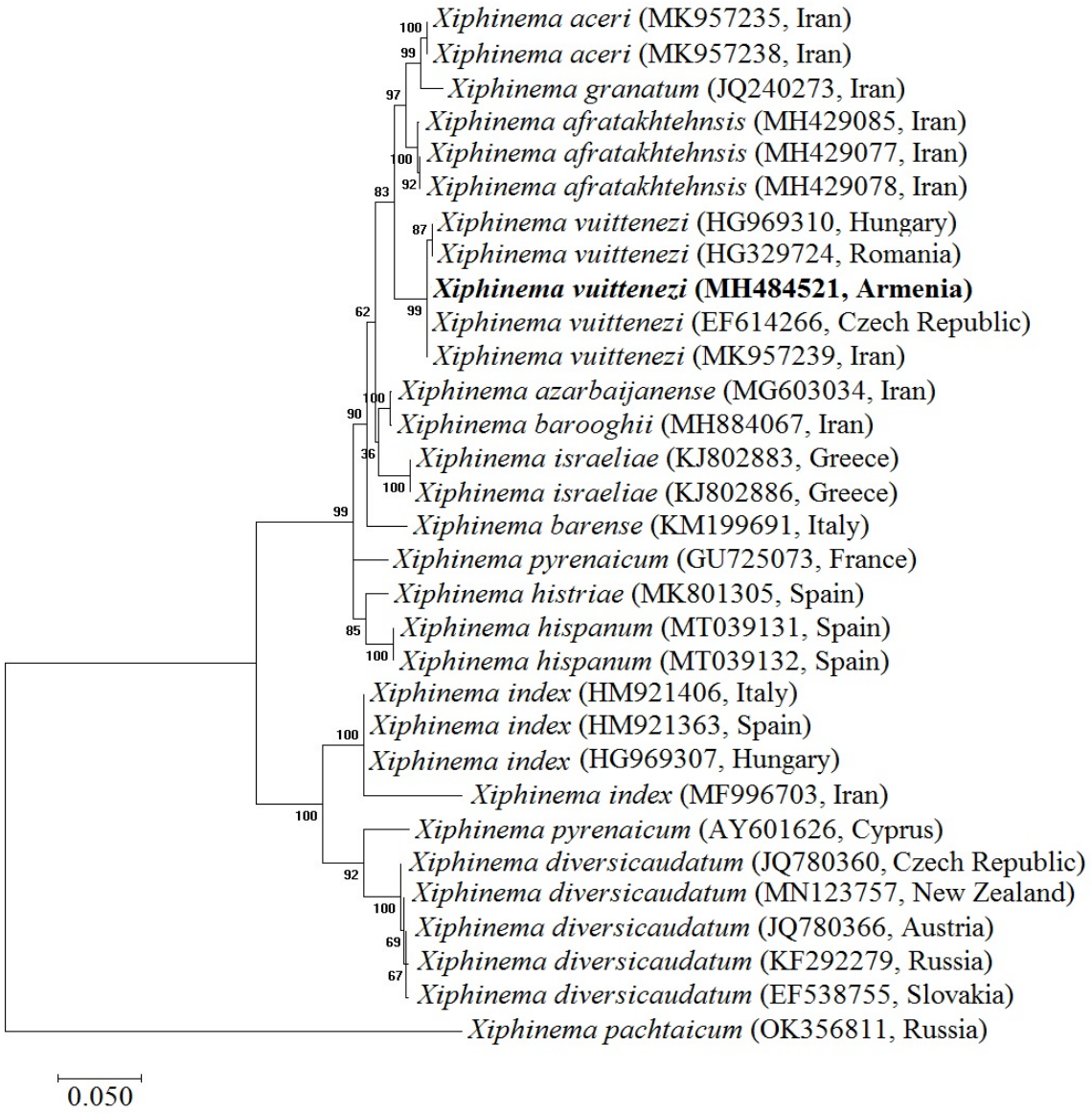

Fig. 3. Phylogenetic relationships of *Xiphinema vuittenezi* from Armenia with other *Xiphinema* species as inferred from the maximum likelihood method using the D2-D3 expansion segments of the 28S rRNA gene sequences under the GTR + I + G model. Bootstrap supports are given for appropriate clades. The new sequence is indicated in bold.

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С.Б. Таболин, К.В. Акопян и В.Д. Мигунова. Морфологическая и молекулярная характеристика *Xiphinema vuittenezi* Luc, Lima, Weischer & Flegg, 1964 (Nematoda: Dorylaimida) из виноградников Армении.

Резюме. Фитопаразитическая нематода *Xiphinema vuittenezi* широко распространена в Республике Армения. Морфологические и морфометрические особенности армянской популяции *X. vuittenezi* не были представлены в литературе, то же касается молекулярных данных. Настоящее исследование было проведено на популяции *X. vuittenezi*, выделенной из ризосферы винограда Арагацотнского марза. Идентификация нематод проводилась по морфологическим и морфометрическим признакам с последующим анализом D2-D3 участка гена 28S рДНК и митохондриального гена субъединицы I цитохромоксидазы с. Морфологические и морфометрические признаки *X. vuittenezi* были схожи с таковыми опубликованными в первоописании. Последовательности участка D2-D3 гена 28S рДНК и гена *COI* популяции *Xiphinema vuittenezi* из Армении имели высокое сходство с *X. vuittenezi* из Ирана и Чешской Республики.