Characterisation of *Crossonema civellae* (Steiner, 1949) Mehta & Raski, 1971 (Tylenchida: Criconematidae) from Armenia

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Summary. During a survey on plant-parasitic nematodes in the Yerevan Botanical Garden, Armenia, a population of *Crossonema civellae* was recovered from soil collected near the roots of *Campsis radicans* (L.). This nematode species is characterised by retrorse body annuli, each bearing a continuous fringe of spines, relatively long stylet and bluntly rounded tail profile. The nblast analysis of the D2-D3 segments of 28S rRNA gene sequence of the Armenian population of *C. civellae* revealed its identity with the sequences of *C. civellae* from Iran and Venezuela. The sequences of the 18S rRNA gene and the cytochrome c oxidase subunit I gene were similar to those of *Crossonema* sp. from USA and Nepal.

Key words: criconematids, morphometric data, nematode, phylogeny, taxonomy.

Criconema civellae was described by Steiner (1949) using nematode specimens collected near the roots of pomelo (*Citrus grandis* (L.) Osb.) growing in a greenhouse at Beltsville, Maryland, USA. Mehta and Raski (1971) proposed a new genus, *Crossonema* and due to an inadequate original description, they re-described *C. civellae* from new specimens collected at the type locality and designated it as the type species of this new genus.

According to Andrássy (2007), *C. civellae* is distributed in North and South Americas, Africa, Europe, Asia and Australia. However, some of these geographical records likely belong to other species. Moreover, some *Crossonema* species shows morphological variability in cuticular ornamentation and could be synonymised. Therefore, morphometric and molecular characterisation of species is very important for a future revision of the genus.

Karapetyan (1975) reported *C. civellae* in Armenia and briefly described two females collected in a glasshouse in the city of Kirovakan (now Vanadzor). Recently, specimens of *C. civellae* were found near the roots of trumpet vines (*Campsis radicans* (L.) Seemann & Bureau) in the Yerevan Botanical Garden of the Armenian National Academy of Sciences. The aim of this study is to provide morphometric and molecular characterisation of the Armenian population of *C. civellae*.

MATERIAL AND METHODS

Nematode sampling. Nematodes were extracted using a modification of the funnel method (Baermann, 1917) from soil samples collected near the roots of trumpet vines (*Campsis radicans* (L.) Seemann & Bureau) in the Yerevan Botanical Garden of the Armenian National Academy of Sciences, Yerevan (Armenia). The GPS location of the sampling site is 40.212472 N, 44.557639 E.

Morphological study. For morphological studies, the nematodes were killed with hot water, fixed in a 5% formaldehyde solution, and mounted in glycerin on slides using the Seinhorst technique (Seinhorst, 1959). Measurements of specimens were made using the microscope Mikmed-6 (LOMO, Russia). Abbreviations used in Table 1 are defined in Siddiqi (2000) and Ravichandra (2010), they are as follows: n – number of specimens, R – total body annules, Rst – annules between labial disc and base

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of stylet knobs, Roes – annules between labial disc and oesophago-intestinal valve, RV – number of annules from tail terminus to vulva, RB – breadth of one body annule, V – distance of vulva from anterior end divided by body length × 100, VL/VB – distance between vulva and posterior end of body divided by body width at vulva. Microphotographs were taken with the Omax A35140U camera. Permanent microscope slides with voucher specimens (nos 51/22 and 51/23) of *C. civellae* were deposited in the Nematode Collection of the A.N. Severtsov Institute of Ecology and Evolution, RAS, Moscow (Russia).

Molecular and phylogenetic study. All molecular studies were performed using the scientific equipment of the Core Research Facility of the 'Bioengineering' Centre (Moscow, Russia). For this work, nematodes frozen in distilled water were used. Their total DNA was extracted using the Wizard Kit (Promega, USA), according to the manufacturer's instructions. The forward Nem_18S_F (5'-CGC GAA TRG CTC ATT ACA ACA GC-3') and the reverse Nem_18S_R (5'-GGG CGG TAT CTG ATC GCC-3') primers (Floyd *et al.*, 2005) were used to amplify the fragment of the 18S rRNA gene. The D2-D3 expansion segments of the 28S rRNA gene were amplified using the forward D2A (5'-CAA GTA CCG TGA GGG AAA GTT G-3') and the reverse D3B (5'-TCG GAA GGA ACC AGC TAC TA-3') primers (Nunn, 1992). The partial cytochrome c oxidase subunit 1 gene was amplified with the forward primer COI-F5 (5'-AAT WTW GGT GTT GGA ACT TCT TGA AC-3') and the reverse primer COI-R9 (5'-CTT AAA ACA TAA TGR AAA TGW GCW ACW ACA TAA TAA GTA TC-3') (Powers *et al.*, 2014). The amplifications were performed using a 5X MasCFETaqMIX-2025 kit (Dialat, Russia) in a Tetrad thermal cycler (Bio-Rad, USA). PCR products were purified using the Wizard PCR Preps Kit (Promega, USA). The sequencing of the PCR products was carried out with the same primers using the genetic analyser ABI 3730 (Applied Biosystems, USA). The newly obtained sequences were submitted to the GenBank database under accession numbers: OP896745 (18S rRNA gene), OP896746 (28S rRNA gene) and OP913385 (*COI* gene).

The newly obtained sequences were aligned with other sequences of *Crossonema* and *Criconema* deposited in GenBank NCBI (https://www.ncbi.nlm.nih.gov/genbank/) by Clustal W using the default parameters (Thompson *et al.*, 1994). The outgroup taxon was chosen according to our previous study (Tabolin & Kolganova, 2022). Phylogenetic trees were 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 1,000 replicates was performed. Bootstrap support is given on appropriate clades for the ML tree.

RESULTS AND DISCUSSION

Crossonema civellae (Steiner, 1949) Mehta & Raski, 1971

Fig. 1

Measurements. See Table 1.

Female. Body slightly ventrally arcuate after fixation. Lip region with two annuli. First annulus with forward-directed margins and six well-developed lips, wider than the second. Body annuli retrorse, each bearing a continuous fringe of spines. The number of spines in the fringe is about 70 on the mid-body. Anastomoses are very rare. The arrangement of spines on the tail region varies greatly. Stylet moderately developed, knobs anteriorly concave, with marginal processes directed

Table 1. Morphometric data of *Crossonema civellae*. All measurements are in μm and in the form: mean ± s.d. (range).

Characters	Population from Yerevan, Armenia
n	15
Body length	481.9 ± 46.7 (360-550)
Maximum body width	64.2 ± 6.5 (55-77.5)
First annulus diameter	26.8 ± 1.9 (24-30)
Second annulus diameter	24.5 ± 2.0 (21-27.5)
Stylet length	91.8 ± 5.1 (87.5-100)
Stylet knobs width	12.6 ± 0.5 (11.75-13.75)
Pharynx length	139.4 ± 10.7 (120-150)
Vulva to tail terminus	43.8 ± 3.8 (37.5-47.5)
R	39.6 ± 2.4 (36-45)
Rst	9.0 ± 0.8 (8-10)
Roes	12.4 ± 0.8 (11-13)
RV	5.4 ± 0.8 (4-7)
RB	12.5 ± 0.5 (12-13.5)
V %	91.1 ± 1.3 (88.2-93.2)
St % L	19.0 ± 2.0 (15.9-24.2)
a	7.7 ± 1.0 (6-10)
b	3.7 ± 0.3 (3.3-4.2)
VL/VB	0.9 ± 0.1 (0.7-1.2)

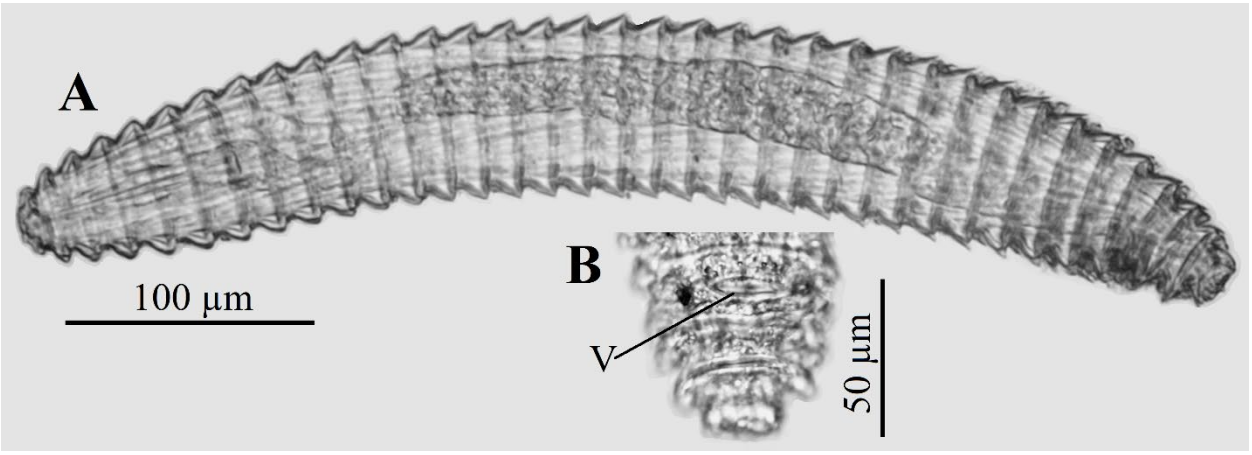

Fig. 1. Light microphotographs of *Crossonema civellae*. A: Entire female, B: Vulva region. V – vulva.

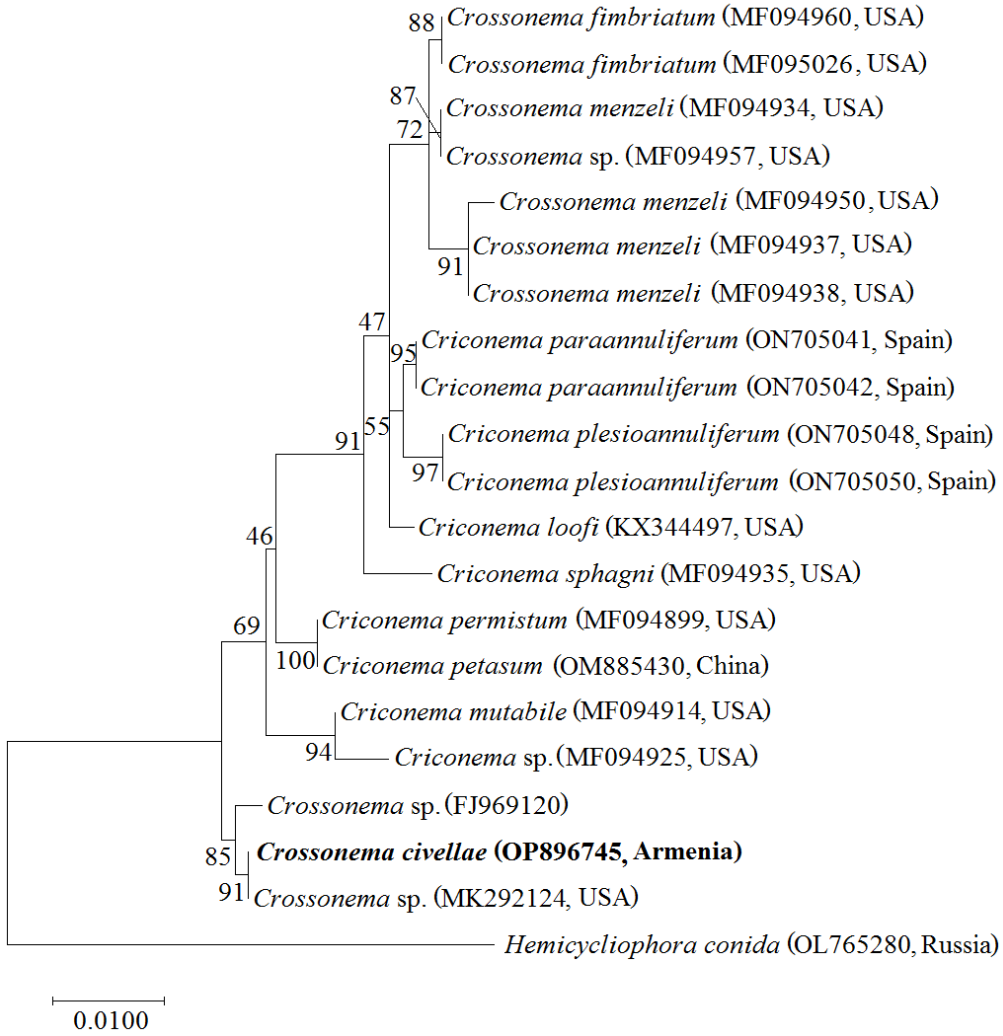

Fig. 2. Phylogenetic relationships of *Crossonema civellae* from Armenia with other *Crossonema* and *Criconema* species as inferred from the Maximum Likelihood method using the 18S rRNA gene sequences under the GTR + I + G model. The percentage of trees in which the associated taxa clustered together is shown next to the branches. The new sequence is indicated in bold.

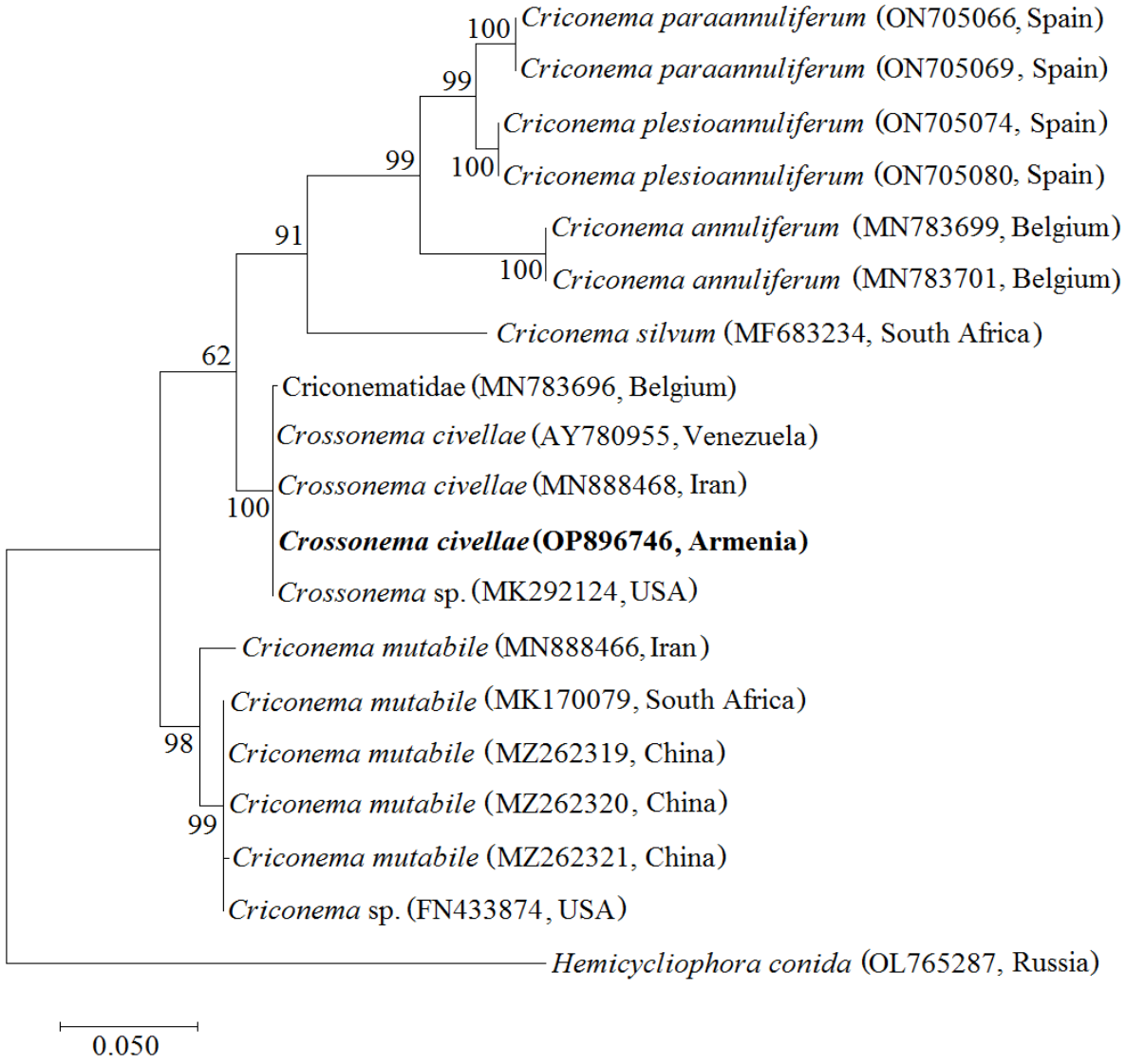

Fig. 3. Phylogenetic relationships of *Crossonema civellae* from Armenia with other *Crossonema* and *Criconema* species as inferred from the Maximum Likelihood method using the D2-D3 expansion segments of the 28S rRNA gene sequences under the GTR + I + G model. The percentage of trees in which the associated taxa clustered together is shown next to the branches. The new sequence is indicated in bold.

anteriorly. S-E pore not seen. The genital system is mono-prodelphic with an outstretched ovary reaching in several specimens the fourth annulus from the anterior end. Vulval aperture is transverse, its lips about equal in size. Anus is clearly visible only in one specimen, in which it is located on the third annulus from the posterior end. In other specimens, according to the position of the barely visible rectum, it can be assumed that the anus is located on the third or fourth annulus.

Male. Not found.

Remarks. The morphometric characters of the Armenian population of *C. civellae* (in the first place) differ from those in the description by Mehta and Raski (1971) by a lower number of total body annuli (36-45 *vs* 40-48), wider first and second annuli (24-30 µm and 21-27.5 µm *vs* 22-28 µm and 19-24 µm) and wider stylet knobs (11.75-13.75 µm *vs* 9-10 µm across). These differences are considered to be intraspecific variations.

The Armenian population of *C. civellae* could be differentiated from the most morphologically close species *Crossonema multisquamatum* (Kirjanova, 1948) by the RV value (4-7 *vs* 8).

Molecular characterisation. High-quality sequences were obtained in three samples, both

from forward and reverse primers. The sequences of the 18S rRNA gene, the D2-D3 expansion segments of the 28S rRNA gene and the *COI* gene obtained from different individuals in this study were identical within each marker. The sequences of the 18S rRNA gene of the studied specimens were identical to the sequence of *Crossonema* sp. from Beltsville (MD), USA (MK292124) and share a 99.64% similarity with *Crossonema* sp. (FJ969120). The similarity with other *Crossonema* sequences deposited in GenBank NCBI was less than 99%. In the partial 18S rRNA gene phylogenetic tree (Fig. 2), the sequence of the Armenian population of *C. civellae* clustered with *Crossonema* sp. from Europe (FJ969120) and *Crossonema* sp. from the USA (MK292124), while the sequences of *Crossonema fimbriatum* (Cobb in Taylor, 1936) Mehta & Raski, 1971 and *C. menzeli* (Stefanski, 1924) Mehta & Raski, 1971 were on other distant branches.

The sequence of the D2-D3 expansion segments of the 28S rRNA gene of the Armenian population was identical to the sequences of *C. civellae* from Iran (MN888468), *C. civellae* from Venezuela (AY780955) and *Crossonema* sp. from Beltsville (MD), USA (MK292124). In the partial 28S rRNA gene phylogenetic tree (Fig. 3), the sequence of the Armenian population of *C. civellae* formed the clade with these sequences with maximal (100%) bootstrap support.

The sequence of the *COI* gene of the Armenian population shared a 99.72% similarity with those of *Crossonema* sp. from Chalti, Nepal (MN710964), a 99.58% similarity with *Crossonema* sp. from Fairfax County (VA), USA (MN710950) and a 99.45% similarity with *Crossonema* sp. from Anderson County (SC), USA (MN710951). The similarity with other sequences of *Crossonema* deposited in GenBank (NCBI) was less than 90%.

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С.Б. Таболин, К.В. Акопян, Т.В. Колганова и В.Д. Мигунова. Характеристика *Crossonema civellae* (Steiner, 1949) Mehta & Raski, 1971 (Tylenchida: Criconematidae) из Армении.

Резюме. При изучении фитопаразитических нематод растений в Ботаническом саду г. Еревана в почве у корней *Campsis radicans* (L.) была обнаружена популяция *Crossonema civellae*. Этот вид нематоды характеризуется обращёнными назад кольцами тела, каждое из которых непрерывно покрыто щетинками, относительно длинным стилетом и тупо округленным профилем хвоста. Анализ участка D2-D3 гена 28S рДНК армянской популяции *C. civellae* выявил его идентичность с последовательностями этого вида из Ирана и Венесуэлы. Последовательности гена 18S и гена *COI* имели высокое сходство с видами *Crossonema* из США и Непала.