Original paper

Some characteristics and observations on *Xiphinema vuittenezi* Luc, Lima, Weischer & Flegg, 1964 (Nematoda: Longidoridae) with comments on its discovery in Syria

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Summary. According to literary sources, the structure of the tubular part of the uterus is a matter of controversy in *Xiphinema vuittenezi* females: specifically, the presence or absence of spindle-shaped spines and Z-differentiation. By studying the freshly dissected genital tracts of female *X. vuittenezi*, the presence of spindle-shaped spines in variable number and size was confirmed, which are distributed throughout the tubular part of the uterus, but seem to be most concentrated proximally, next to the *pars dilatata uteri*. The spines have a central projection that allows them to attach to the wall of the uterus forming a "uterine network" that resembles a mixture of pentagons and hexagons. No *Z*-differentiation was observed. Intraspecific tail variability in *X. vuittenezi* was presented and illustrated. Based on morphological, morphometric and molecular data, it can be assumed that the discovery of *X. vuittenezi* in Syria refers to another, probably still undescribed species.

Key words: genital tract, intraspecific variability, spindle-shaped spines, Syria, tail shape, tubular part, uterus, Z-differentiation.

INTRODUCTION

Ali et al. (2024) reported four *Xiphinema* species from banana rhizospheres in Syria for the first time. One of these was *X. vuittenezi* Luc, Lima, Weischer & Flegg, 1964. Some details given in the paper for two populations that were found, raised doubts that this was really *X. vuittenezi*. These doubts were based primarily on some morphometric values, the structure of the genital tract in females, specifically the tubular part of the uterus, the structure and shape of the tail in females, as well as in the J3 and J4 juvenile stages, and phylogenetic relationships.

Luc et al. (1964), in the original description of *X. vuit-tenezi*, did not report the presence of spines in the uteri and such structures were not mentioned in the revised polytomous key to the species of *Xiphinema* by Loof and Luc (1990).

Mojtahedi et al. (1980) in their paper on *Xiphinema* species in Iranian vineyards for *X. vuittenezi* wrote: "In the females of all Iranian populations, distinct spines were observed in the long tubular portion of the uteri, between the enlarged distal portion and the ovijector (Fig. 2). The spindle-shaped structures of variable number and size have not been reported previously for *X. vuittenezi*, but have also been observed in paratype females of this species and in other European specimens." (p. 169).

Kruger (1988) in his paper on the uterine differentiation in *Xiphinema* suggested that the presence or absence of uterine differentiation should be confirmed for six *Xiphinema* species, one of them being *X. vuittenezi*.

Malan et al. (1997) described *Xiphinema petersmithi* Malan, Swart, Meyer & Heyns, 1997, a new short-tailed species of the genus from South Africa. Specimens of the new species were found in grapevine rhizospheres (rootstock var.

143 B) at Nietvoorbij experimental farm near Lutzville, Western Cape Province. Considering the genital tract of a new species, Malan et al. (1997) wrote: "Each branch consists of ... a tube-like uterus without Z-differentiation, Minute spines present in lining of uterus, indistinct in fixed specimens but more readily visible in those with a convoluted uterus and especially in freshly-dissected specimens." (p. 41). Barsi (2005) compared nine paratype specimens (seven females and two pre-adult juveniles) of X. petersmithi with numerous populations of X. vuittenezi, and based on the absence of any consistent character that could be used to differentiate these two species, it was considered that X. petersmithi Malan, Swart, Meyer & Heyns, 1997 is a junior synonym of X. vuittenezi Luc, Lima, Weischer & Flegg, 1964. The presence of small, relatively indistinct, spindle-shaped spines in the lining of uterus was confirmed, which were more easily visible in the specimen with a convoluted uterus.

Barsi and Lamberti (2000) observed small spindleshaped spines in all populations of *X. vuittenezi* from Serbia, and Barsi et al. (2000) reported the same for populations from Slovenia, Slovakia, Switzerland, Hungary, Germany and England. Paratype females of *X. vuittenezi* (fig. 1 in Barsi et al. 2000), and females of the same species collected near to the type locality at Guntersblum, Germany, revealed the presence of uterine spines in varying numbers (Barsi et al. 2000).

Kumari and Decraemer (2006) in their paper on a female of *X. vuittenezi* with two vulvae from the Czech Republic wrote: "anterior genital branch . . . composed of . . . bipartite uterus with a wider glandular part and a narrower muscular part filled with spines and crystalloids" (p. 943).

Groza et al. (2013) provided additional data on the occurrence and distribution of four *Xiphinema* non-*americanum* group species in Romania, one of them being *X. vuittenezi*. Concerning the genital tract Groza et al. (2013) wrote: "uterus tripartite, Z differentiation in the form of small globular bodies and spindle-like uterine spines, sometimes quadric in shape refractive structures observed at the proximal par of tubular region of uterus . . ." (p. 225–226). In Remarks they noticed that "Populations of *X. vuittenezi* studied exhibited interpopulation variability concerning some morphological characters, . . . the uterine spines are bigger and in a higher numbers in Murfatlar females, similar to those reported by Barsi and Lamberti (2000b), small globular bodies at the junction of pars dilalata and tubular part of uterus almost missing." (p. 226–227).

Vazifeh et al. (2019) illustrated the presence of spines in the uterus in *X. vuittenezi* from Iran (fig. 6J, L) and stated that "The present Iranian specimens correspond well with the earlier descriptions in their general morphology and morphometrics" (p. 16).

Tabolin et al. (2024) in their paper on X. vuittenezi from

vineyards in Armenia wrote: "Each [genital] branch consists of . . . *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)." (p. 2). It should be noted that in fig. 2 (Tabolin et al. 2024, p. 3) globular bodies were not illustrated although they were mentioned in the text.

Ali et al. (2024) in their paper about the genital tract of *X. vuittenezi* from Syria wrote: "[genital] . . . branches about equally developed without any Z-differentiation but containing crystalloid bodies distributed over the entire length (Fig. 3)." (p. 736).

According to the above data, the tubular part of the uterus in *X. vuittenezi* is still a subject of controversy. The first goal of the present study was to clarify this controversy regarding the structure of the tubular part of the uterus, and the second goal was to illustrate intraspecific variability of the tail in *X. vuittenezi*.

MATERIAL AND METHODS

For light microscopic study of *Xiphinema vuittenezi*, females and juvenile developmental stages (JDS) mounted on permanent slides from Serbia, females from England, and Germany, as well as fresh material from Serbia mounted on temporary slides were used. For the study of the fresh female reproductive organs, live specimens were extracted from a soil sample taken from the rhizosphere of black elder (*Sambucus nigra* L.) near the Department of Biology and Ecology at Novi Sad (lat 45.246456° lon 19.855028°) using Cobb's wet sieving technique. The reproductive organs were dissected in tap water using a razor blade, carefully placed in tap water on temporary slides covered with coverslips, and uterine morphology was immediately studied. Photographs were taken using a Zeiss Axio Imager A1 compound microscope equipped with an AxioCam MRc 5 digital camera.

RESULTS AND DISCUSSION

Females of X. vuittenezi from Serbia, Germany and England on permanent slides

Lip region rounded, separated from the rest of the body by a depression in all females (Fig. 1A1-A3, B1-B2, C1-C2). Reproductive system amphidelphic, with both genital branches equally developed and reflexed; vulva slit like, situated at mid-body (Fig. 1D). Each genital branch consists of an ovary, oviduct, *pars dilatata oviductus*, conspicuous sphincter muscle, bipartite uterus (*pars dilatata uteri* + tubular part) and ovejector (Fig. 1E). Spindle-shaped spines in variable number and size are distributed throughout the tubular part of the uterus, but they seem to be most concentrated proximally, next to the *pars dilatata* (Fig. 1F1-F2, G, H). The tubular part of the uterus without egg in it is generally not or only moderately expanded, so that spindle-shaped spines are seen in greater or lesser density along the entire length. Sometimes crystalloids can also be seen (Fig. 1H). When the egg passes through the uterus, due to its size, the tubular part expands and in different focuses of the objective, it is possible to see/observe spindle-shaped spines both at the level of the egg and in the free part of the uterus (Fig. 1I1-I3). Figure 1I2 suggests that the spines are somehow attached to the wall of the tubular part of the uterus.

Females from Serbia on temporary slides

Fresh, heat relaxed, but unfixed females showed the same characteristics as females on permanent slides (Fig. 2A). A freshly dissected genital tract in tap water enabled a more detailed morphological analysis of the structure of the tubular part (Fig. 2B). Initially, the tubular part was not expanded, so that the spindle-shaped spines of variable size appeared concentrated along the entire length, similar to fixed material on permanent slides (Fig. 2B, tpu). Later, due to the cover slip pressure and the reduced thickness of the water film, the fresh material expanded, so the tubular part expanded to a greater or lesser extent (Fig. 2C, D, E). A higher concentration of spines was observed proximally, next to the pars dilatata (Fig. 2C, D, E). It was easier to observe individual spines in this stage (Fig. 2E1). Despite the pressure on the tubular part, the spines could not be flushed out, which meant that they were attached to the wall of the uterus. It was observed that the spines have a central projection that probably enables them to attach to the wall of the uterus (Fig. 2F3). Further flattening of the tubular part revealed that spines were attached by a central projection (Fig. 2F3) in a "uterine network", which is probably formed by the cells of the uterine wall (Fig. 2F1, F2). The "uterine network" resembles a mixture of pentagons and hexagons.

No Z-differentiation was found in the uterus of *X. vuittenezi*.

Intraspecific variability of tail shape in X. vuittenezi

In females, tail is short, conoid, broadly rounded, with a peg which is sometimes absent (Fig. 3I1-I2, J1-J2, K); blind canal present except in pegless specimens. In juveniles (Fig. 3), tale (shape) is elongate-conoid in first stage (Fig. 3E) and conoid and subdigitate in second (Fig. 3F), third (Fig. 3G), and fourth (Fig. 3H) stage. In J4, rarely, the subdigitate projection is shortened to a small bulge at the tip of the tail (Fig. 3H).

On the validity of X. vuittenezi from Syria

Ali et al. (2024) reported *X. vuittenezi* from banana rhizospheres in Syria for the first time. Some details given in the paper for two populations that were found, raised doubts that this was really *X. vuittenezi*. These doubts were based primarily on some morphometric values, the structure of the genital tract in females, specifically the tubular part of the uterus, the structure and shape of the tail in female, as well as in the J3 and J4 juvenile stages, and phylogenetic relationships.

Ali et al. (2024, p. 737) in Remarks wrote: "The morphological features and morphometric measurements of the fixed females closely agree with those of the type population (Luc et al., 1964), in which their authors did not consider the presence of crystalloids in this species (Coomans et al., 2001). It matches well with other populations from Europe and Middle East . . ., except for minor differences in the body length and b ratio . . . which may be because of geographical intraspecific variability."

Body length affects the b value (b ratio), because it is calculated by dividing the body length by the pharynx length. A comparative overview of average body length, average b value and average pharynx length in 34 populations shows that the average length of the pharynx in the Syrian population (n = 4 females) is within the range of those values in 33 *X. vuittenezi* populations (n = 805 females) (Table 1). Since the average body length of the Syrian population is shorter compared to other populations, the value of b is correspondingly lower.

The intra- and inter-population variability of body and odontostyle lengths of 34 populations (Fig. 4A, B) shows that there is no "close agreement" between the Syrian and type population on the one hand, and other populations on the other hand with respect to body and odontostyle lengths.

Based on the material presented in the previous part of the paper, I would like to point out the existence of morphological differences between *X. vuittenezi* from Syria and *X. vuittenezi* from Serbia, Germany and England.

According to Ali et al. (2024, p. 736): "[genital] . . . branches about equally developed without any Z-differentiation but containing crystalloid bodies distributed over the entire length" (fig. 3G-I in Ali et al. 2024, p. 736). As presented earlier in the present paper, in *X. vuittenezi* spindleshaped spines are distributed throughout the tubular part of the uterus, but they seem to be most concentrated proximally, next to the *pars dilatata*. Sometimes crystalloids can also be seen (Fig. 1H).

In females "Tail short, broadly round, with a peg (Fig. 3).... J3 characterized by a conical tail with a rounded end subdigitate extension (Fig. 3). J4 characterized by a tail bluntly rounded, slightly dorsally convex-conoid with a terminal peg (Fig. 3)." (Ali et al. 2024, p. 736–737).

For X. vuittenezi tail shapes of juvenile stages (J1-J4)



Fig. 1. *Xiphinema vuittenezi*. **A1-C2**, Female head region from Serbia (**A1-A3**), Germany (**B1-B2**), and England (**C1-C2**); **D-E**, female genital tract (ova = ovary, ovd = oviduct, pdo = *pars dilatata oviductus*, sph = sphincter muscle, pdu = *pars dilatata uteri*, tpu = tubular part of uterus, ove = ovejector, vu = vulva; **F1-H**, part of *pars dilatata uteri* and tubular part of uterus with spindle-shaped spines in females from Serbia (**F1-F2**), Germany (**G**), and England (**H**) (crystalloids marked with black arrowheads); **I1-I3**, Details of tubular part of uterus with egg and spindle-shaped spines (marked with white arrowheads). (Scale bars: A1-C2 = 20 µm, D = 100 µm, E = 50 µm, F1 = 20 µm, F2 = 20 µm, G-H = 20 µm, I1-I3 = 20 µm.)



Fig. 2. *Xiphinema vuittenezi* (Novi Sad, Serbia), fresh material. **A**, Entire body of heat relaxed female (vu = vulva); **B**, Anterior genital branch (ova = ovary, ovd = oviduct, pdo = *pars dilatata oviductus*, sph = sphincter muscle, pdu = *pars dilatata uteri*, tpu = tubular part of uterus, part; **C-E**, part of *pars dilatata uteri* and tubular part of uterus in females; **E1**, Detail with spindle-shaped spines (marked with white arrowheads); **F1-F2**, Details of the uterine wall with "uterine network"; **F3**, Spindle-shaped spine attached to the uterine wall by a central projection (marked with white arrowhead). (Scale bars: A = 200 µm, B = 200 µm, C-E = 20 µm, E1 = 20 µm, F1-F3 = 20 µm.)



Fig. 3. *Xiphinema vuittenezi*, juvenile developmental stages and females. **A-D**, Anterior body region of J1 (**A**), J2 (**B**), J3 (**C**), and J4 (**D**) from Serbia; **E-H**, Tail region of J1 (**E**), J2 (**F**), J3 (**G**), and J4 (**H**) from Serbia; **I1-K**, Variability of tail regions in females from Serbia (**I1-I2**), Germany (**J1-J2**), and England (**K**). (Abbreviations: ost = odontostyle, oph = odontophore, rost = replacement odontostyle, a = anal opening.) (Scale bar: A-H = 50 µm, I1-K = 20 µm.)

	Average body length (µm)	Average b value	Average pharynx length (μm)	Locality, country (literary source)
1	3240	7.0	463	Guntersblum, Germany (Luc et al. 1964)
2	3280	7.3	449	Aiuges-Mortes, France (Luc et al. 1964)
3	3040	6.9	441	Narbone-Plage, France (Luc et al. 1964)
4	3130	6.9	454	East Malling, England (Luc et al. 1964)
5	3260	6.6	494	Lisboa, Portugal (Luc et al. 1964)
6	3100	7.3	425	Kostinbrod, Bulgaria (Lamberti et al. 1983)
7	3400	6.8	500	S. Cataldo, Italy (Lamberti et al. 1985)
8	3200	6.7	478	Monteporzio Catone, Italy (Roca et al. 1987)
9	3300	6.9	478	Gatteo a Mare, Italy (Roca et al. 1988b)
10	3500	7.4	473	Exilles, Italy (Roca et al. 1988a)
11	3400	7.3	466	Trabia, Italy (Roca et al. 1989)
12	3200	6.8	471	Avellino, Italy (Roca et al. 1991)
13	3500	7.0	500	Kostinbrod, Bulgaria (Lamberti et al. 1997)
14	3300	6.9	478	Senta, Serbia (Barsi 1994)
15	3400	6.9	493	Senta, Serbia (Barsi and Lamberti 2000)
16	3170	6.8	466	Novi Sad, Serbia (Barsi and Lamberti 2000)
17	3120	6.5	480	Debeljaca, Serbia (Barsi and Lamberti 2000)
18	3280	6.6	497	Becej, Serbia (Barsi and Lamberti 2000)
19	3360	6.9	487	Male Pijace-Horgos, Serbia (Barsi and Lamberti 2000)
20	3302	6.4	516	Bŕíštvi, Czech Republic (Kumari 2004)
21	3356	6.6	508	Chrást, Czech Republic (Kumari 2004)
22	3257	6.5	501	Kutná hora, Czech Republic (Kumari 2004)
23	3355	6.5	516	Slaný, Czech Republic (Kumari 2004)
24	3124	6.4	488	Slaný, Czech Republic (Kumari 2004)
25	3453	6.9	500	Trója-Praha, Czech Republic (Kumari 2004)
26	3379	6.9	490	Polešovice, Czech Republic (Kumari et al. 2005)
27	3240	6.8	476	Ostrov, Romania (Groza et al. 2013)
28	3130	7.0	447	Murfatlar, Romania (Groza et al. 2013)
29	3510	7.6	462	Sufiyan, Iran (Vazifeh et al. 2019)
30	3300	7.2	458	Parpi, Armenia (Tabolin et al. 2024)
31	3200	6.6	485	YU, Serbia, Slovenia (Barsi 1989)
32	3500	6.9	507	Molina di Ledro-Grerosa, Italy (Coiro et al. 1989)
33	3200	6.7	478	Monteforte d'Alpone-Ponsara, Italy (Coiro et al. 1992)
34	2626	5.6	469	Hraisson, Syria (Ali et al. 2024)

Table 1. Comparative overview of average body length, average b value and average pharynx length in 34 populations of *Xiphinema vuittenezi*.

Notes.

The average pharynx length was calculated by dividing the average body length by the average b value.

Population data from Syria are in bold.



Fig. 4. Intra- and inter-population variability of body and odontostyle lengths in females in populations of *Xiphinema vuittenezi*. Type population (1) and population from Syria (34) are marked with black arrowheads.

from Serbia and females from Serbia, Germany and England (Fig. 3), one female and J4-J3 stages from Syria (Ali et al. 2024, fig. 3B, C, D) show clear differences. Closer comparison of tail shape in female, J4 and J3 stages based on photomicrographs (original from Serbia; fig. 3B, C, D in Ali et al. 2024, Syria) and drawings (fig. 1F, J, I in Luc et al 1964, Lisbon, Portugal) found significant differences in tail shape in *X. vuittenezi* from Syria compared with those from Serbia and Portugal (Fig. 5D, E, F, H, I, J).

The phylogenetic relationship between *X. vuittenezi* and other *Xiphinema* species based on the sequences of the D2-D3 expansion segments of the 28S rRNA gene was presented by Ali et al. (2024) and Tabolin et al. (2024). Comparison of parts of the partial 28S rRNA gene phylogenetic trees (Fig. 6) of fig. 6 from Ali et al. (2024, p. 740) and fig. 3 from Tabolin et al. (2024, p. 5) revealed noticeable differences.

Tabolin et al. (2024) compared sequences of *X. vuittenezi* from Armenia with sequences of the same species listed in the GenBank database that originated from Hungary, Romania, Czech Republic and Iran (Fig. 6B). They found high similarities with sequences from these countries with a note that "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." (Tabolin et al. 2024, p. 3).

In addition to the original sequences of *X. vuittenezi* from Syria, Ali et al. (2024) used all sequences used in Tabolin et al. (2024) (marked in grey), as well as one new sequence originating from Hungary, two from Romania and three from Iran.

Ali et al. (2024) have stated that "The 28S sequences from Syrian populations of X. vuittenezi (OR196835-OR196836, OR428495-OR428496) matched closely (more than 99% of similarity) to an Iran poputation of the same species (MK905505), varying in 4-13 nucleotides and 0-5 indels. Our sequences matched also with other world isolates with 98-99% similarity (e.g. HG329724-HG329724, HG969310, AY601614, MK943733, MK957234, EF614266, MK957237, MK957239)." (p. 741). In the following, they stated that "The Syrian isolates of X. vuittenezi were closest to isolates of the same species from different world populations but not within the same clade (Fig. 6), mostly due to the intraspecific variations of the 28S sequences from isolates that distinguished Hungarian, Iranian, Romanian, Czech and Russian populations from the Syrian, yielding intraspecies cladogenesis with two subclades inside this species clade." (p. 741). It should be noted that in the previous quote, the Russian population actually refers to the Armenian population.

Based on all the mentioned morphometric and morphological differences between *X. vuittenezi* s.s. (33 popula-

tions and 805 females) and *X. vuittenezi* from Syria (4 females), as well as on the basis of molecular differences, it can be assumed that the discovery of *X. vuittenezi* in Syria refers to another, probably still undescribed species.

CONCLUSIONS

The results presented in the present paper show that females of *X. vuittenezi* have a bipartite uterus with spindle-shaped spines in variable number and size, which are distributed throughout the tubular part of the uterus; but seem to be most concentrated proximally, next to the *pars dilatata*. The spines have a central projection that allows them to attach to the wall of the uterus forming a "uterine network" that resembles a mixture of pentagons and hexagons. No Z-differentiation was observed. Based on morphological, morphometric and molecular data, it can be assumed that the discovery of *X. vuittenezi* in Syria refers to another, probably still undescribed species.

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Fig. 5. Comparison of *Xiphinema vuittenezi* based on illustrations. **A**, Tubular part of uterus with spindle-shaped spines (original, Serbia); **B**, Tubular part of uterus (fig. 3G, H, I in Ali et al. 2024, Syria); **C**, Anterior region, comparison (fig. 3A in Ali et al. 2024, Syria; line drawing, fig. 1B in Luc et al. 1964, Lisbon, Portugal); **D**, Female tail, comparison (fig. 3B in Ali et al. 2024, Syria; line drawing, fig. 1F in Luc et al. 1964, Lisbon, Portugal); **F**, J3 tail, comparison (fig. 3D in Ali et al. 2024, Syria; line drawing, fig. 1I in Luc et al. 1964, Lisbon, Portugal); **F**, J3 tail, comparison (fig. 1B in Luc et al. 1964, Lisbon, Portugal); **G**, Anterior region, comparison (original, Serbia; line drawing, fig. 1B in Luc et al. 1964, Lisbon, Portugal); **H**, Female tail, comparison (original, Serbia; line drawing, fig. 1J in Luc et al. 1964, Lisbon, Portugal); **J** tail, comparison (original, Serbia; line drawing, fig. 1J in Luc et al. 1964, Lisbon, Portugal); **J** tail, comparison (original, Serbia; line drawing, fig. 1B in Luc et al. 1964, Lisbon, Portugal); **H**, Female tail, comparison (original, Serbia; line drawing, fig. 13 in Luc et al. 1964, Lisbon, Portugal); **J** tail, comparison (original, Serbia; line drawing, fig. 14 tail, comparison (original, Serbia; line drawing, fig. 13 in Luc et al. 1964, Lisbon, Portugal); **J** tail, comparison (original, Serbia; line drawing, fig. 13 in Luc et al. 1964, Lisbon, Portugal); **J** tail, comparison (original, Serbia; line drawing, fig. 14 tail, comparison (original, Serbia; line drawing, fig. 13 in Luc et al. 1964, Lisbon, Portugal); **J** tail, comparison (original, Serbia; line drawing, fig. 14 tail, comparison (original, Serbia; line drawing, fig. 14 tail, comparison (original, Serbia; line drawing, fig. 15 in Luc et al. 1964, Lisbon, Portugal); **J** tail, comparison (original, Serbia; line drawing, fig. 14 tail, comparison (original, Serbia; line drawing, fig. 15 in Luc et al. 1964, Lisbon, Portugal); **J** tail, comparison (original, Serbia; line drawi



Fig. 6. Comparison of phylogenetic relationships of *Xiphinema vuittenezi* based on the sequences of the D2-D3 expansion segments of the 28S rRNA gene presented by Ali et al. (2024) and Tabolin et al. (2024). **A**, Ali et al. (2024, p. 740, fig. 6, part); **B**, Tabolin et al. (2024, p. 5, fig. 3, part).

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