Original Research Article

Investigating Developmental Stages and Morphological Characterization of *Xiphinema* speciesJuveniles(Nematoda: Longidoridae)

Abstract

The present research delves into a comprehensive study of the developmental stages and morphological features exhibited by *Xiphinema* speciesat juvenile stages 1, 2, and 3. Through meticulous examination and analysis, this study sheds light on the intricate transformations and adaptive traits exhibited by these nematodes as they progress through their early life stages.We conducted an examination of morphological traits and gathered essential measurements from the specimens.A noteworthy observation is the occurrence of replacement odontostyle in the pharyngeal region across all three juvenile stages, denoted as J1, J2, and J3. The initial juvenile stage of *Xiphinema* exhibited a body length ranging from 750.1-1104.8 µm, followed by the second juvenile stage with a length of 1166.1-1705.1 µm and the third juvenile stage spanning from 1752.8-2339.2 µm.In the juvenile stage, the length replacement of odontostyle occurs within specific ranges, first stage between 55.1-80.1 µm, second stage between 81.1-105.0 µm and third stage between 107.7-126.1 µm. In-depth morphological observations, complemented by microphotographs, provide insights into the structural changes that occur during each developmental phase.

Key words: Identification, Juvenile stages, Development, Nematode, *Xiphinema*

1. Introduction

The genus *Xiphinema* Cobb, 1913 (family Longidoridae), commonly known as dagger nematode, includes more than 275 species that feed as an ectoparasite of plants (Xu*et al*., 2019). It has worldwide distribution and causes damage to diverse wild and cultivated crops and transmitting plant viruses (Taylor and Brown, 1997). The genus comprises the largest number of species among the genera of the order dorylaimida and perhaps of the phylum Nematoda.The clear identification of juvenile and adult nematodes within the genus *Xiphinema* relies significantly on the discernible presence of a substitute odontostyle within the esophageal corpus(Bos*et al.*, 1984). Particularly, the distinctive positioning of the replacement odontostyle, where its tip coincides with the base of the odontophore, serves as a defining characteristic for distinguishing

Comment [A1]: Interesting theme. Well-structured article, however, some adjustments need to be made.

Comment [A2]: The information, arguments, and results of a scientific research do not require words (adjectives) that imply the idea of emphasis and induction to become firm. What brings scientific rigor to the reporting of scientific research is the detailed description of the theoretical foundation, the performed methodological procedures, and the faithful recording of observations and concluded analyses.
For example: what is intriguing to me may not be for another...

Comment [A3]: Scientific ideas should be firm and categorical. Thus, there is no need to use adjectives to emphasize scientific aspects. I suggest reviewing the use of adjectives throughout the text first-stage juveniles from other developmental stages(Robbins *et al*., 1996).Throughout the molting process, the operational odontostyle is shed, making way for the forward relocation of the replacement odontostyle to occupy its functional role. Notably, a novel replacement odontostyle takes shape in all stages except the adult phase, underscoring a unique developmental sequence (Alkemade *et al*., 1989). Consequently, the length of the operative odontostyle in any given stage closely mirrors that of the replacement odontostyle in the antecedent stage, further emphasizing this intricate developmental pattern (Yuste *et al*., 2016).

2. Materials and methods

2.1 Sampling, nematode extraction

The measured quantity of sub-sample (200 cc) from the composite sample was washed by Cobb's decanting and sieving technique (Cobb, 1918); the sieves of 20, 60, 200 and 325 mesh size were used to catch the target nematode populations. The catches of 60, 200 and 325 were further cleaned through modified Baermann's technique (Schindler, 1961). Here the residues passed through a double layer tissue paper supported on an aluminium wire-gauge, and the entire assembly was kept over a glass Petriplate of 10 cm diameter filled with tap water. It was left undisturbed for overnight at room temperature. Nematode suspension thus collected was labeled with details of host, date of collection, locality and collector's name and kept properly for further studies.

2.2 killing and fixing

Several fixatives have been recommended for killing and fixing the nematodes for dorylaimids. However, TAF (7.6 ml formalin, which contains 37% formaldehyde, 2 ml Tri-ethylamine, 90.4 ml Distilled water for 100 ml solution) was used for killing and fixing of nematode specimens. Mass fixation of nematode in the suspension was done with the help of hot TAF at 60°C (for simultaneously killing and fixing). The fixed nematodes were then observed understereobinocular compound microscope and identified the target nematode genera based on morphological characters from the mixed populations(Seinhorst*et al*., 1959).

2.3Statistical Analysis

Morphometric data recorded for the populations of *Xiphinema* were further analyzed on Microsoft Excel for determining values of De Man's, maximum-minimum (range), mean, standard deviation (SD) etc.

3. Results

The research findings reveal intriguing insights into the developmental stages of *Xiphinema* nematodes, particularly in terms of their morphological characteristics and the presence of replacement odontostyle.Of notable interest is the occurrence of replacement odontostyle observed in the pharyngeal region across all three juvenile stages (J1, J2, and J3). This intriguing phenomenon underscores the dynamic nature of *Xiphinema* nematode development. The specific localization of these replacement structures offers distinctive markers for each developmental stage, providing a basis for accurate identification and classification. The juveniles similar to adult female, but small in size and absence of reproductive organs. Replacement odontostyle are seen in pharyngeal region of all three juveniles (J1, J2 and J3) stages.

Description (Fig. 1, for measurement sees Tables 1)

3.1 Juvenile stage 1: In the case of the juvenile stage 1 (J1), the identification criterion hinges on the precise positioning of the replacement odontostyle. Notably, the anterior tip of the replacement odontostyle is subtly projected at the juncture between the functional odontostyle and the odontophore. This distinctive trait serves as a pivotal characteristic for categorizing nematodes within this developmental stage. Body 750.1-1104.8µm and Replacement of odontostyle55.1-80.1 µm long(Fig. 1, ATables 1).

3.2 Juvenile stage 2: As the nematodes progress to the juvenile stage 2 (J2), a compelling shift in the positioning of the replacement odontostyle becomes evident. Specifically, the anterior tip of the replacement structure becomes embedded within the basal flanges. This notable transformation further emphasizes the intricacies of *Xiphinema* nematode development and highlights the importance of meticulous morphological observations. Body 1166.1-1705.1µmand Replacement of odontostyle81.1-105.0µm long(Fig. 1, B Tables 1).

3.3 Juvenile stage 3: The journey culminates in the juvenile stage 3 (J3), where an intriguing spatial arrangement of the replacement odontostyle anterior tip is observed. Unlike the preceding stages, the anterior tip of the replacement odontostyle is situated at a discernible distance from the flanges. This unique positioning offers a distinct characteristic that aids in the accurate classification of nematodes in this advanced developmental stage. Figure 1 provides a visual representation of this distinct morphological feature.Body 1752.8-2339.2µmand Replacement of odontostyle107.7-126.1µm long(Fig. 1, C Tables 1).

4. Discussion:

The findings of this research shed light on the intricate developmental stages of *Xiphinema* species, specifically focusing on the morphological characteristics associated with their juvenile phases(Brown*et al*., 2016). The presence of replacement odontostyle across all three juvenile stages (J1, J2, and J3) holds significant implications for understanding the adaptive strategies of *Xiphinema*species(Fadakar*et al*., 2021). The distinct positioning of the replacement odontostyle in each stage serves as a reliable marker for characterizing the developmental phases (Loof, 1993 and loof, 1996).

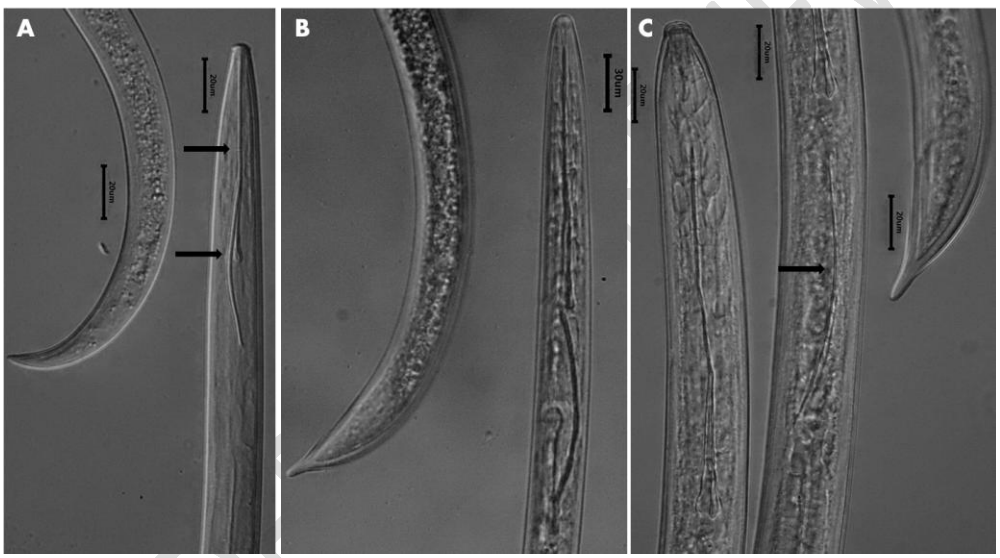

Fig.1 Light microphotographs of *Xiphinema*species (juveniles J1-J3) **A-C**, **A** First stage juvenile-anterior & posterior body region with arrows pointing position of guiding ring and flanges **B** Second stage juvenile- anterior and posterior region along with replacement of odontostyle **C** Third stage juvenile-anterior and posterior body region with arrow pointing replacement of odontostyle. (Scale bars: A, B & C = 20 µm)

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Table 1. Measurements of juveniles of *Xiphinema basiri* (mean ± SD, with range). All measurements are in µm

Characters	J1 (n=16)	J2(n=21)	J3 (n=17)
L	900.9±95.7	1490.9±162.1	2009.39±172.1

	(750.1-1104.8)	(1166.1-1705.1)	(1752.8-2339.2)
a	49.8±5.8 (39.3-59.0)	56.6±10.1 (38.90-71.1)	52.05±4.0 (46.9-59.7)
b	4.0 ±0.4 (3.1-4.7)	5.4±0.8 (4.1-7.0)	5.42±0.7 (4.7-7.1)
c	32.7 ±4.8 (23.4-43.0)	35.3±5.8 (24.2-47.1)	36.41±3.6 (30.2-42.4)
c'	2.2±0.3 (1.9±2.7)	2.4±0.5 (1.7-3.3)	2.12±3.6 (1.8-2.4)
Odontostyle length	57.6±6.9 (47.7-67.7)	75.0±3.8 (68.4-79.3)	100.26±8.7 (81.5-110.7)
Odontophore length	35.3±2.4 (32.2-39.1)	44.2±3.6 (36.1-49.0)	49.82±10.1 (32.3-60.0)
Total Stylet length	93.0±93.0 (79.9-104.6)	119.2±6.0 (104.5-127.5)	150.08±17.3 (113.8-164.6)
Head to guiding ring	41.3±3.7 (35.1-48.1)	62.8±4.1 (55.6-69.5)	86.66±12.2 (70.7-100.0)
Flanges width	6.9±0.1 (6.8-7.1)	7.8±0.6 (7.0-9.0)	8.71±0.6 (7.7-10.0)
Pharangeal length	223.7±10.1 (206.6-241.5)	277.4±22.4 (241.2-309.1)	373.49±35.0 (304.5-426.0)
Pharangeal bulb length	55.0±4.6 (47.7-61.3)	71.2±5.5 (61.1-79.5)	85.85±4.2 (79.9-92.3)
Pharangeal bulb dia.	10.7±0.8 (10.0-12.1)	14.0±0.7 (13.0-15.0)	17.41±1.0 (15.2-18.5)
Replacement of odontostyle	66.9±6.0 (55.1-80.1)	90.9±7.7 (81.1-105.0)	118.06±5.9 (107.7-126.1)
Body diam. at lip region	6.7±0.7 (6.1-7.7)	8.0±0.4 (7.5-8.5)	10.35±1.1 (9.0-11.5)
Mid body width	18.2±2.0 (16.1-21.5)	26.7±2.6 (22.0-30.0)	38.73±3.5 (32.7-45.0)
Body diam. at anus	12.6±0.8 (11.1-13.8)	18.7±2.4 (15.0-22.0)	26.29±1.8 (23.1-28.3)
Hyaline tail region	4.5±0.8 (3.8-6.2)	8.9±1.5 (7.0-12.0)	16.71±2.0 (13.8-20.0)
Tail length	27.8±2.4 (25.1-33.0)	42.8±4.9 (35.0-50.0)	55.41±4.1 (49.2-63.1)

The precision with which these markers distinguish one stage from another emphasizes the importance of meticulous morphological observations in taxonomic studies and ecological research. Furthermore, this study contributes to the broader knowledge of nematode biology, offering a foundation for more in-depth investigations into the functional significance of these morphological changes.

While this research delves into the morphological aspects of *Xiphinema* speciesjuvenile development, there remain intriguing questions about the underlying mechanisms driving these changes. Future studies could explore the genetic and molecular factors that regulate the development of replacement odontostyle and how these changes influence the nematodes' interactions with their surroundings.

5. Conclusion:

In conclusion, the investigation into the developmental stages of *Xiphinema* specieshas revealed valuable insights into the morphology and adaptive traits exhibited by these organisms during their juvenile phases. The presence of replacement odontostyle and their distinctive positioning provides a novel perspective on the growth and development of these nematodes. The intricate changes observed across stages underscore the dynamic nature of *Xiphinema* speciesdevelopment, potentially influenced by environmental cues and interactions with host plants.These findings contribute to our understanding of nematode biology and their potential implications for agricultural and ecological systems. The accurate classification facilitated by the localization of replacement odontostyle provides a practical tool for researchers and taxonomists. Furthermore, the study opens avenues for further research into the functional roles of these morphological changes and their significance in nematode adaptation and evolution.Ultimately, this research advances our comprehension of the complex life history of *Xiphinema* species, offering a foundation for continued exploration into the mechanisms driving their growth, development, and interactions within their ecosystems.

References:

Alkemade, J. R. M.& Loof, P. A. A. (1989). Observations on the ontogeny of some species of *Xiphinema* (Nematoda: Dorylaimida). *Mededelingen-Universiteit Gent, FaculteitLandbouwkundigeenToegepasteBiologischeWetenschappen*, **54**: 1177-1186.

Bos, W. S. & Loof, P. A. A. (1984). Nigerian species of the genus Xiphinema Cobb, 1913 (Nematoda: Dorylaimida): part I. *Nematologica*, **30**(4): 395-418.

Brown, D. J. F., Luc, M.&Purbadi, I. (1981). A description of some juveniles stages of *Xiphinema vulgare* (Nematoda: Dorylaimoidea). *NematologiaMediterranea*, **9**: 205-210

Fadakar, S., Pourjam, E., Barsi, L. & Pedram, M. (2021). A new species of the genus *Xiphinema* Cobb 1913 (Nematoda: Longidoridae) from north and northwest Iran. *European Journal of Plant Pathology*, DOI: 10.1007/s10658-021-02264-1.

Loof, P. A. A. & Luc, M. (1993). A revised polytomous key for the identification of species of the genus *Xiphinema* Cobb, 1913 (Nematoda: Longidoridae) with exclusion of the *X*. americanum-group: Supplement 1. *Systematic Parasitology*, **24**(3): 185-189.

Loof, P. A. A., Luc, M. &Baujard, P. (1996). A revised polytomous key for the identification of species of the genus *Xiphinema* Cobb, 1913 (Nematoda: Longidoridae) with exclusion of the *X*. americanum-group: Supplement 2. *Systematic Parasitology*, **33**: 23-29.

Robbins, R. T., Brown, D. J., Halbrendt, J. M.&Vrain, T. C. (1996). Compendium of juvenile stages of *Xiphinema* species (Nematoda: Longidoridae). *Russian Journal of Nematology*, **4**: 163-172.

Schindler, A.F. (1961). A simple substitute for Baermann funnel. *Plant Disease Reporter*, **45**(9): 747-748

Seinhorst, J. W. (1959). A rapid method for the transfer of nematodes from fixative to anhydrous glycerin.*Nematologica*, **4**(1): 67-69.

Taylor, C. E. & Brown, D. J. (1997). Nematode vectors of plant viruses. CAB- International, UK.

Xu, Y. M. & Zhao, Z. Q. (2019). Longidoridae and Trichodoridae (Nematoda: Dorylaimida and Triplonchida). *Fauna of New Zealand*, **79**: 360

Yuste, A.A., Cortés, J.A.C, Navarrete. C. C., Palomares-Rius, J.E. & Castillo, P. (2016). Cryptic diversity and species delimitation in the *Xiphinema americanum*-group complex (Nematoda: Longidoridae) as inferred from morphometrics and molecular markers. *Zoological Journal of Linnean Society*,**176**: 231-265.