#### **— БИОРАЗНООБРАЗИЕ В УСЛОВИЯХ АНТРОПОГЕОГЕНЕЗА**

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# TO THE KNOWLEDGE OF PLANT PARASITIC NEMATODES OF DONBASS: HELICOTYLENCHUS DIGONICUS PERRY, 1959 (TYLENCHIDA: HOPLOLAIMIDAE)

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As a result of the studies of plant-parasitic nematodes of trees and shrubs on the territory of Donetsk Botanical Garden for the first time for Donbass and Ukraine was recorded *Helicotylenchus digonicus* Perry, 1959 (Tylenchida: Hoplolaimidae) – polyphagous root semi-endoparasite. The paper contains description, morphological and morphometric characteristics of recorded populations, trophical associations, and brief data on the distribution, biology, ecology and harmfulness of the species. The morphometrics of the Donbass populations generally are in agreement with topotypes, but in comparison with other populations regional isolates has some differences. The species is the dominant among all plant-parasitic nematodes. It was found in the rhizosphere of 29 species of plants. The highest population density was recorded on *Fraxinus excelsior* L., *Juglans regia* L., *Robinia pseudoacacia* L. and *Tilia europaea* L.

Key words: plant-parasitic nematodes, Donbass, first record, Helicotylenchus digonicus, morphometrics

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#### Introduction

The study of plant-parasitic nematodes is an essential part of modern plant-pathological research. Nematodes are one of the main components of soil ecosystems, and it is important to understand their role in biocenoses, to know trophical relationships and to assess harmfulness level for native and adventive floral components. This communication is a continuation of a series of works, devoted to the study of the fauna of plant-parasitic nematodes of Donbass and contains information about *Helicotylenchus digonicus* Perry, 1959 (Tylenchida: Hoplolaimidae) – first recorded for the fauna of Donbass and Ukraine.

## Research Aim and Objectives

The aim of the research was to identify the species composition of plant-parasitic nematodes in green spaces of Donbass. The objectives of research included the description of morphological and morphometric characteristics, elucidation of trophical relationships, initial assessment of populations den-

sity and the level of harmfulness of identified species in regional conditions.

## **Research Objects and Methods**

Soil samples were collected in September of 2016–2020 under the trees and shrubs from 0.5 m depth on the territory of Donetsk Botanical Garden (DBG), Donbass. Nematodes were extracted from soil using the modified Baermann funnel method, killed by heating (70 °C), fixed in triethanolamine formalin water solution (TAF), processed to glycerol by slow evaporation and mounted on microscope slides [1, 5, 6, 9]. Images were taken using Canon Power Shot A 640 camera attached to Carl Zeiss Primo Star microscope, and edited using Adobe Photoshop CS5. Measurements were made with AxioVision Rel. 4.7.

The abbreviations and their definitions for the de Man's ratios and other indices used in tables are as follows: n = number of specimens on which measurements are based, <math>L = overall body length,

a = body length divided by its maximum diameter (at vulval region), b = body length divided by the length from anterior to esophago-intestinal valve, c = body length divided by tail length, c' = tail length divided by anal diameter of body, V = ratio between distance from vulva to anterior end of body and total body length in %. All measurements are in  $\mu$ m.

## **Results and Discussion**

As a result of the studies for the first time for the territory of Donbass and Ukraine was recorded *Helicotylenchus digonicus* Perry, 1959 (Tylenchida: Hoplolaimidae) – polyphagous root semi-endoparasite. The following is description, morphological and morphometric characteristics of recorded populations, trophical associations, and brief data on the distribution, biology, ecology and harmfulness of the species.

*Helicotylenchus digonicus* Perry, 1959 Measurements: see Tables 1–2.

Description. Female (n=50): Body after relaxation spirally curved in open C- to spiral-shape, more so in posterior region, 646±54.3 (511–736) µm long (Fig. 1, Tables 1-2). Lip region truncate, 3.4±0.2 (3.0-3.8) µm high and  $5.9\pm0.4$  (5.3-7.7) µm wide, anteriorly flattened, with 4-5 annules. The labial framework is well sclerotized, with outer margins of the labial framework extending from basal plate (Figs. 2A–E). Stylet 22.9±0.6 (21.6–25.6) µm long, anterior faces of stylet knobs flattened or indented. Position of dorsal pharyngeal gland opening (DGO)  $9.8\pm2.1$  (7.2–14.0) µm anterior to stylet knobs. Median pharyngeal bulb oval to round, muscular. Orifice of dorsal esophageal gland at less than half stylet length behind stylet base. Esophageal glands partially surrounding anterior end of intestine, subventrals slightly longer than the dorsal. Excretory pore almost opposite esophago-intestinal junction. Hemizonid located 1 to 3 annules anterior or posterior to excretory pore. Hemizonion not seen. Cuticle annulated, 1.0 to 2.0 µm. Vulva a transverse

**Table 1.** Morphometrics of *Helicotylenchus digonicus* Perry, 1959: populations from USA, Iran and Donetsk (Donetsk Botanical Garden). All measurements are in µm and in the form: range (mean ± standard deviation)

	Locality and host				
Character	USA, WI, Madison, Poa pratensis L. [7] (Topotypes)	USA, KS [10]	Iran, Kerman, Baft, <i>Juglans</i> <i>regia</i> L. [8]	Donetsk, DBG, various plants [orig.]	
n (females)	20	10	7	50	
L	500-790	632–780 (689±46.8)	670-852 (736±61.3)	474–776 (578±57.2)	
a	23–33	23.5–29.7 (27.1±1.9)	25-29 (26.7±1.2)	22.5–32.0 (26.4±2.4)	
b	5.0-7.2	5.4-6.4 (5.8±0.3)	4.6-5.2 (4.9±0.2)	4.9-7.7 (6.3±0.2)	
c	41–63	37.8–50.6 (43.1±4.7)	40-57 (58.5±5.4)	38.4–64.8 (51.5±6.0)	
c'	0.7 – 0.9	$0.9-1.5 (1.2\pm0.2)$	$0.9-1.2 (1.1\pm0.1)$	$0.7-1.1 \ (0.95\pm0.1)$	
V, %	58-64	61.5-63.7 (62.6±0.7)	58-70 (63.6±3.7)	55.7–68.1 (62.3±2.4)	
Lip region width	_	5.9-6.4 (6.2±0.1)	$4-8 (5.6\pm1.2)$	5.3-7.7 (5.9±0.4)	
Lip region height	_	3.3-3.8 (3.7±0.2)	3.4-5 (4.1±0.4)	3.0-3.8 (3.4±0.2)	
Stylet length	24–28	25.0–27.5 (26.1±0.8)	23-28 (24.3±1.6)	21.6–25.6 (22.9±0.6)	
Vulval body diameter	_	23.8–27.5 (25.4±1.1)	25-32 (27.6±2.1)	19.0–25.0 (21.0±1.7)	
Anal body diameter	_	13.8–15.0 (14.0±0.4)	13-16 (14.4±1.2)	10.3–14.7 (11.8±0.9)	
Tail length	_	12.5–20.0 (16.3±2.6)	12-18 (15.3±2.0)	8.4–14.5 (11.3±1.3)	

**Table 2.** Morphometrics of *Helicotylenchus digonicus* Perry, 1959: populations from Donetsk (Donetsk Botanical Garden). All measurements are in  $\mu$ m and in the form: range (mean  $\pm$  standard deviation)

	Locality and host				
Character	Pinus nigra pallasiana (Lamb.) Holmboe [orig.]	Syringa vulgaris L. [orig.]	Thuja occidentalis L. [orig.]	Tilia europaea L. [orig.]	
n (females)	10	20	8	10	
L	540-595 (569±20.6)	474–645 (558±46.0)	570-695 (636±43.6)	511–736 (646±54.3)	
a	23.6–28.4 (25.0±1.9)	22.6–28.0 (25.6±1.8)	26.8–31.3 (29.6±1.5)	24.2–32.0 (27.7±2.2)	
b	6.0-6.4 (6.2±0.2)	6.1-6.6 (6.3±0.2)	6.2-6.6 (6.4±0.1)	4.9-7.7 (6.5±0.2)	
c	43.8–57.5 (50.5±4.2)	39.7–64.8 (50.3±6.1)	46.4–63.7 (55.7±5.7)	45.0–66.1 (56.7±6.4)	
c'	$0.9-1.1 (1.0\pm0.1)$	$0.8-1.1 (0.9\pm0.1)$	0.9-1.1 (1.0±0.1)	$0.7-1.1 (0.9\pm0.1)$	
V, %	59.8–68.1 (63.6±2.4)	58.1-65.8 (62.1±2.1)	61.4–66.0 (62.9±1.5)	59.7–66.3 (61.7±2.0)	
Lip region width	5.6-6.4 (6.0±0.3)	5.3-6.3 (5.7±0.4)	5.5-6.8 (6.2±0.5)	5.8-7.7 (6.9±0.5)	
Lip region height	3.3-3.8 (3.6±0.2)	3.0-3.8 (3.3±0.2)	3.2-3.8 (3.6±0.2)	3.3-5.3 (4.1±0.2)	
Stylet length	22.1-24.1 (23.1±0.7)	21.8–23.5 (22.4±0.4)	22.3–23.9 (23.1±0.6)	21.6–25.6 (23.9±0.8)	
Vulval body diameter	$19.2-24.8$ $(22.8\pm2.0)$	19.0–25.0 (21.8±1.7)	20.0-23.0 (21.5±1.1)	20.3–25.1 (23.4±1.5)	
Anal body diameter	10.5–12.6 (11.6±0.6)	10.4–13.4 (11.9±0.8)	10.3–13.0 (11.1±0.9)	$10.9-14.7$ $(12.4\pm0.9)$	
Tail length	9.5–13.6 (11.4±1.1)	9.5–13.8 (11.1±1.1)	9.6–13.3 (11.5±1.4)	8.4–14.5 (11.5±1.6)	



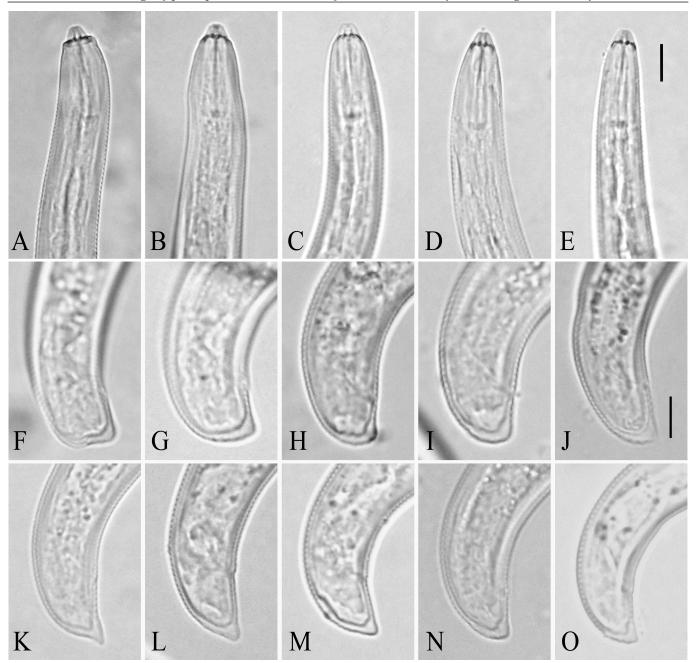
slit without protruding lips, located at  $61.7\pm2.0$  (59.7–66.3) % of body length. Reproductive system with two branches equally developed. Spermatheca offset and empty. Lateral field with 4 lines, areolated anterior and posterior to vulva and crenate on tail; inner two lines end on tail in u-shape pattern. Phasmids pore-like, located at anus level or two-three annuli anterior to anus. Rectum does not overlap anus. Tail  $11.5\pm1.6$  (8.4–14.5) µm long, usually slightly shorter or equal to anal body diameter:  $c'=0.9\pm0.1$  (0.7–1.1); shape more curved dorsally, with 8 to 16 ventral tail annules. Shape of tail and terminus of tail varies considerably (Figs. 2F–O).

Males: not found.

Juveniles: similar to female in the structure of head, stylet, esophagus and tail. Juvenile stages are

**Fig. 1.** Helicotylenchus digonicus Perry, 1959, female, entire view. Scale bar =  $10 \, \mu m$ 

**Рис. 1.** *Helicotylenchus digonicus* Perry, 1959, самка, внешний вид. Шкала = 10 мкм



**Fig. 2.** *Helicotylenchus digonicus* Perry, 1959, females: A–E: anterior region; F–O: posterior region. Scale bar = 10 µm **Puc. 2.** *Helicotylenchus digonicus* Perry, 1959, самки: А–Е: головной конец; F–O: хвостовой конец. Шкала = 10 µm

difference by the level of reproductive system development.

The morphometrics of Donbass populations generally are in agreement with topotypes [7], but in comparison with other populations [8, 10, 11, etc] Donbass isolates has some differences: higher 'b' values, lesser 'c" values (the average c' ratio≤1), a bit more posterior position of vulva values, shorter stylet, lesser vulval and anal body diameter, as well as shorter tail (Tables 1–2).

Type locality: Madison, WI, USA. The species has a wide global distribution. It is common in the

temperate zone of Europe (including Russia and Asian republics of the former USSR) and North America. Also known from some countries of Afrotropical, Australian, Neotropical and Oriental regions [2–4]. For the first time recorded for the fauna of Donbass and Ukraine.

The species is ectoparasite and semi-endoparasite of the plants roots. Helminthes are inactive, localized near the root hairs. Polyphagous. Type host: meadow bluegrass (*Poa pratensis* L.). Most often noted as a pest of cereals and fodder legumes. It is also registered as a pest of vegetables, fruit and berry

trees and shrubs and ornamental plants [2, 3]. Feeding results in cortical lesions, necrotic streaks and rots on the roots.

In Donbass *H. digonicus* is the dominant species among all plant-parasitic nematodes. It was found in the rhizosphere of 29 species of plants: Acer negundo L., Aesculus hippocastanum L., Cotinus coggygria Scop., Crataegus sp., Fraxinus excelsior L., F. pennsylvanica Marsh., Gleditsia triacanthos L., Juglans regia L., Lonicera tatarica L., Mahonia aquifolium (Pursh) Nutt., Malus domestica Borkh., Morus nigra L., Populus nigra L., Quercus robur L., Q. rubra L., Pinus nigra pallasiana (Lamb.) Holmboe, Prunus armeniaca L., Pseudotsuga menziesii (Mirb.), Rhus typhina L., Robinia pseudoacacia L., Salix alba L., Salix caprea L., Sorbus aucuparia L., Symphoricarpos albus (L.) S.F.Blake, Syringa vulgaris L., Taxus baccata L., Thuja occidentalis L., Tilia europaea L., Ulmus pumila L. The average population density was 26 specimens per 100 cm<sup>3</sup> of soil. The highest population density was recorded on F. excelsior (56 spec. per 100 cm<sup>3</sup>), J. regia (54 spec. per 100 cm<sup>3</sup>), R. pseudoacacia (65 spec. per 100 cm<sup>3</sup>) and *T. europaea* (70 spec. per 100 cm<sup>3</sup>).

The damage of *H. digonicus* on the growth and development to any of these hosts has not been established.

#### **Conclusions**

As a result of the studies of plant-parasitic nematodes of trees and shrubs on the territory of Donetsk Botanical Garden for the first time for the territory of Donbass and Ukraine was recorded *Helicotylenchus digonicus* Perry, 1959 (Tylenchida: Hoplolaimidae) – polyphagous root semi-endoparasite. The morphometrics of Donbass populations generally are in agreement with topotypes, but regional isolates has some differences. The species is the dominant among all plant-parasitic nematodes. It was found in the rhizosphere of 29 species of plants. The highest population density was recorded on *F. excelsior*, *J. regia*, *R. pseudoacacia* and *T. europaea*.

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# К ИЗУЧЕНИЮ ФИТОПАРАЗИТИЧЕСКИХ НЕМАТОД ДОНБАССА: HELICOTYLENCHUS DIGONICUS PERRY, 1959 (TYLENCHIDA: HOPLOLAIMIDAE)

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В результате изучения фитопаразитических нематод древесно-кустарниковых растений на территории Донецкого ботанического сада, впервые для Донбасса и Украины был зарегистрирован Helicotylenchus digonicus Perry, 1959 (Tylenchida: Hoplolaimidae) — полифаг, корневой семиэндопаразит. В статье приведено описание, морфологическая и морфометрическая характеристика гельминтов из зафиксированных популяций, сведения о трофических связях, а также краткие данные по распространению, биологии, экологии и вредоносности вида. Морфометрические показатели популяций из Донбасса в целом согласуются с топотипами, но в сравнении с другими популяциями имеют ряд отличий. Вид доминирует среди всех фитопаразитических нематод, зафиксирован в ризосфере 29 видов растений. Наибольшая плотность популяций отмечена на Fraxinus excelsior L., Juglans regia L., Robinia pseudoacacia L. и Tilia europaea L.

*Ключевые слова:* фитопаразитические нематоды, Донбасс, первое указание, *Helicotylenchus digonicus*, морфометрия

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