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**TO THE KNOWLEDGE OF PLANT PARASITIC NEMATODES OF DONBASS:  
*ROTYLENCHUS BUXOPHILUS* GOLDEN, 1956 AND  
*R. FALLOROBUSTUS* SHER, 1965 (TYLENCHIDA: HOPLOLAIMIDAE)**

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As a result of the studies of plant-parasitic nematodes of trees and shrubs in the territory of Donetsk Botanical Garden two species of *Rotylenchus* Filipjev, 1936 (Tylenchida: Hoplolaimidae) were recorded: *R. buxophilus* Golden, 1956 was registered for the first time for Donbass and Ukraine, *R. fallorobustus* Sher, 1965 – for the first time for Donbass and Left-bank Ukraine. Both species are polyphagous root semi-endoparasites. *Rotylenchus buxophilus* was registered in 2020 in rhizosphere of common privet (*Ligustrum vulgare* L.), and *R. fallorobustus* – in 2019 in rhizosphere of larch (*Larix* sp.). The paper contains descriptions, 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 of both species generally are in agreement with literature data, but regional isolates have some differences.

**Key words:** plant-parasitic nematodes, Donbass, first record, *Rotylenchus*, 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 *Rotylenchus buxophilus* Golden, 1956 and *R. fallorobustus* Sher, 1965 (Tylenchida: Hoplolaimidae) – first recorded for the fauna of Donbass.

### **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 density and the level of harmfulness of identified species in regional conditions.

### **Research Objects and Methods**

Soil samples were collected in September of 2019–2020 under the trees and shrubs from 0.5 m depth in 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 [5, 10, 11, 14]. 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, 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  $\mu\text{m}$ .

### Results and Discussion

As a result of the studies in the territory of Donetsk Botanical Garden two species of *Rotylenchus* Filipjev, 1936 (Tylenchida: Hoplolaimidae) were recorded: *R. buxophilus* Golden, 1956 was registered for the first time for Donbass and Ukraine, *R. fallorobustus* Sher, 1965 – for the first time for Donbass and Left-bank Ukraine. 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.

#### *Rotylenchus buxophilus* Golden, 1956

Measurements: see Table 1.

Description. Female (n=6): Body after relaxation spirally curved in open C, rarely almost fully stretched out,  $848 \pm 113$  (761–1047)  $\mu\text{m}$  long (Fig. 1A, Table 1). Cuticle clearly annulated. Lateral field with 4 lines at mid-body, areolated at esophageal region, terminating in loop close to tail terminus. Lip region hemispherical (Figs. 2A–E),  $6.0 \pm 0.5$  (5.5–6.7)  $\mu\text{m}$  high and  $10.4 \pm 1.0$  (9.2–11.7)  $\mu\text{m}$  wide, not distinctly offset, with 4 annules about as wide as body annules. The labial framework is well sclerotized. Stylet robust,  $33.7 \pm 0.8$  (32.4–34.9)  $\mu\text{m}$  long, basal knobs rounded. Median pharyngeal bulb rounded to oval, muscular. Esophageal glands sacciform, partially surrounding anterior end of intestine. Dorsal gland overlapping intestine by distance less than body is wide. Excretory pore anterior to esophago-intestinal junction level. Hemizonid located anterior to excretory pore. Vulva a transverse slit, not protruding from the contour of the body, with double distinct epiptygma, located at  $55.6 \pm 2.2$  (52.7–59.1) % of body length. Spermatheca inconspicuous, without sperms. Reproductive system didelphic-amphidelphic, branches equally developed. Rectum not conspicuous, partially overlapped by intestine. Phasmids small, pore-like, located 5 to

15 annuli anterior to anus level. Tail dorsally convex-conoid shape,  $18.4 \pm 1.6$  (16.0–19.7)  $\mu\text{m}$  long, and usually equal to anal body diameter:  $c' = 1.0 \pm 0.1$  (0.9–1.1); with 10 to 20 ventral tail annules. Tail terminus often with short projection, annulated. Shape of tail and terminus of tail varies considerably (Figs. 2F–J).

Males: not found.

Juveniles: similar to female in the structure of head, stylet, esophagus and tail. Juvenile stages differ by the level of reproductive system development.

The morphometrics of Donbass populations has some differences in comparison with other populations [4, 8, 16] – first of all lesser body length, as well as stylet length, vulval body diameter and especially shorter tail (Table 1).

Type locality: Deale, MD, USA. The species has a wide distribution in North America. Also it is common in the temperate zone of Europe and European part of Russia, known from many countries of Asia and New Zealand [2–4, 7, 8, 12, 16]. 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: common boxwood (*Buxus sempervirens* var. *suffruticosa* L.) [4]. Most often registered in rhizosphere of various woody plants, in particular ornamental [7, 12]. It is also recorded as a pest of vegetables, fruit and berry plants [2, 3]. Feeding results in cortical lesions, necrotic streaks and rots on the roots. The symptoms expressed on boxwood are (strong) growth reduction and the presence of small root lesions [13].

In Donbass *R. buxophilus* was registered in 2020 in rhizosphere of common privet (*Ligustrum vulgare* L.). The average population density was quite significant – 81 specimens per 100  $\text{cm}^3$  of soil. The damage of the species on the growth and development of host plants has not been established.

#### *Rotylenchus fallorobustus* Sher, 1965

Measurements: see Table 2.

Description. Female (n=10): Body after relaxation spirally curved,  $890 \pm 51$  (836–990)  $\mu\text{m}$  long (Fig. 1B, Table 2). Cuticle clearly annulated. Lateral field with 4 lines at mid-body, areolated only anteriorly. Lip region hemispherical (Figs. 3A–E),  $6.3 \pm 0.3$  (6.0–6.7)  $\mu\text{m}$  high and  $10.2 \pm 0.4$  (9.6–10.7)  $\mu\text{m}$  wide, slightly or not set off, with 6 annules. The labial

**Table 1.** Morphometrics of *Rotylenchus buxophilus* Golden, 1956: populations from USA, New Zealand, Belgium and Donetsk (Donetsk Botanical Garden). All measurements are in  $\mu\text{m}$  and in the form: range (mean  $\pm$  standard deviation)

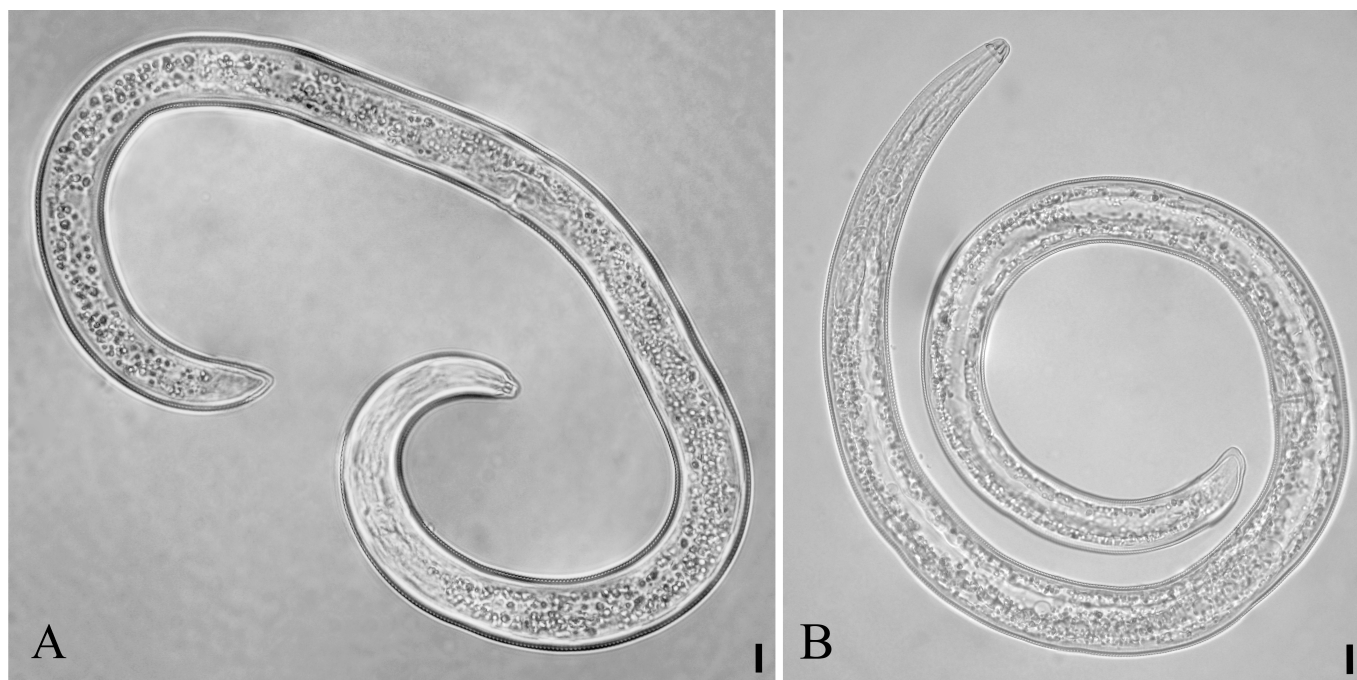
Character	Locality and host			
	USA, MD, Deale, <i>Buxus</i> <i>sempervirens</i> var. <i>suffruticosa</i> L. [4] (Paratypes)	New Zealand, Nelson, <i>Erica arborea</i> L. [16]	Belgium, Ghent, <i>Dioscorea tokoro</i> Makino ex Miyabe [8]	Donetsk, DBG, <i>Ligustrum vulgare</i> L. [orig.]
n (females)	20	6	15	6
L	1020–1150	1020–1250 (1170 $\pm$ 80)	818–1065 (972 $\pm$ 87)	761–1047 (848 $\pm$ 113)
a	31	32–43 (39 $\pm$ 3.8)	27.2–32.5 (30.1 $\pm$ 1.9)	25.8–34.1 (29.5 $\pm$ 2.9)
b	7.0	7.3–8.7 (8.0 $\pm$ 0.53)	5.3–8.2 (7.0 $\pm$ 0.8)	5.9–7.5 (6.8 $\pm$ 0.6)
c	43	41–58 (49 $\pm$ 5.9)	34.6–49.8 (40.1 $\pm$ 4.1)	42.4–54.5 (46.2 $\pm$ 4.3)
c'	–	0.8–1.2	1.0–1.4 (1.2 $\pm$ 0.1)	0.9–1.1 (1.0 $\pm$ 0.1)
V, %	55	55–58 (56 $\pm$ 0.8)	48–58 (53 $\pm$ 3)	52.7–59.1 (55.6 $\pm$ 2.2)
Lip region width	–	–	10–12 (11 $\pm$ 0.6)	9.2–11.7 (10.4 $\pm$ 1.0)
Lip region height	–	–	6–8 (7 $\pm$ 0.3)	5.5–6.7 (6.0 $\pm$ 0.5)
Stylet length	33.5	38–40 (39 $\pm$ 0.9)	32–38 (36 $\pm$ 1.6)	32.4–34.9 (33.7 $\pm$ 0.8)
Vulval body diameter	–	27–37 (30 $\pm$ 3.4)	29–37 (32 $\pm$ 2)	26.2–30.7 (28.7 $\pm$ 1.8)
Anal body diameter	–	16–23 (18.4 $\pm$ 2.42)	19–24 (21 $\pm$ 1.6)	17.2–20.1 (18.6 $\pm$ 1.0)
Tail length	–	20–28 (24 $\pm$ 2.9)	18–28 (24 $\pm$ 3.1)	16.0–19.7 (18.4 $\pm$ 1.6)

framework is well sclerotized. Stylet robust, 34.3 $\pm$ 1.4 (32.4–36.1)  $\mu\text{m}$  long, basal knobs rounded, with indentation of anterior surface. Median pharyngeal bulb rounded to oval, muscular. Esophageal glands sacciform, partially surrounding anterior end of intestine. Dorsal gland overlapping intestine by distance less than body is wide. Excretory pore at level of esophageal glands. Hemizonid located anterior to excretory pore. Vulva a transverse slit, not protruding from the contour of the body, with single or double epiptygma, located at 55.0 $\pm$ 1.3 (52.8–57.1) % of body length. Spermatheca inconspicuous, without sperms. Reproductive system didelphic-amphidelphic, branches equally developed. Rectum not

conspicuous, partially overlapped by intestine. Phasmids pore-like, located 2 to 8 annuli anterior to anus level. Tail dorsally convex-conoid shape, 12.9 $\pm$ 2.2 (10.0–17.0)  $\mu\text{m}$  long, equal to or slightly longer than anal body diameter: c'=1.1 $\pm$ 0.08 (1.0–1.3); with 10 to 15 ventral tail annules. Shape of tail and terminus of tail varies considerably (Figs. 3F–J).

Males: not found.

Juveniles: similar to female in the structure of head, stylet, esophagus and tail. Juvenile stages are different in the level of reproductive system development.

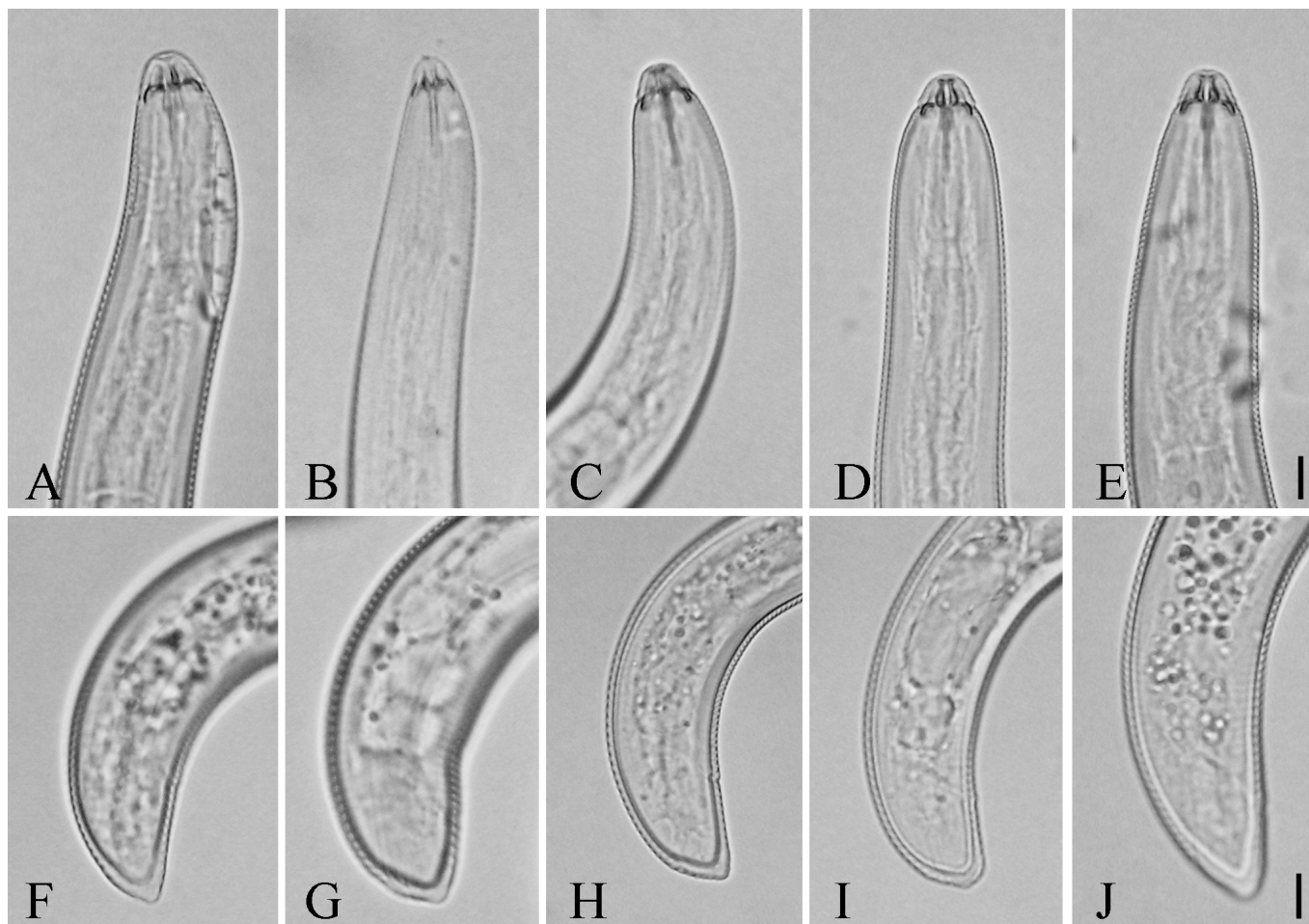


**Fig. 1.** *Rotylenchus* spp., females, habitus: A: *R. buxophilus* Golden, 1956; B: *R. fallorobustus* Sher, 1965. Scale bar = 10  $\mu$ m  
**Рис. 1.** *Rotylenchus* spp., самки, внешний вид: А: *R. buxophilus* Golden, 1956; В: *R. fallorobustus* Sher, 1965. Шкала = 10 мкм

**Table 2.** Morphometrics of *Rotylenchus fallorobustus* Sher, 1965: populations from Netherlands, Spain and Donetsk (Donetsk Botanical Garden). All measurements are in  $\mu$ m and in the form: range (mean  $\pm$  standard deviation)

Character	Locality and host		
	Netherlands, Leiden, Hoogmade, undefined grass [12] (Paratypes)	Spain, Andalusia, Arroyo Frio <i>Populus nigra</i> L. [1]	Donetsk, DBG, <i>Larix</i> sp. [orig.]
n (females)	20	13	10
L	920–1250	830–1070 (970 $\pm$ 160)	836–990 (890 $\pm$ 51)
a	20–29	23.7–29.4 (26.6 $\pm$ 2.0)	26.6–29.6 (28.1 $\pm$ 1.0)
b	7.0–8.7	5.5–6.6 (5.9 $\pm$ 0.3)	7.2–8.3 (7.8 $\pm$ 0.4)
c	44–78	57–94.3 (77.0 $\pm$ 11.6)	39.6–46.0 (42.3 $\pm$ 2.3)
c'	–	0.4–0.7 (0.5 $\pm$ 0.08)	1.0–1.3 (1.1 $\pm$ 0.08)
V, %	52–58	53–59 (56 $\pm$ 1.6)	52.8–57.1 (55.0 $\pm$ 1.3)
Lip region width	–	–	9.6–10.7 (10.2 $\pm$ 0.4)
Lip region height	–	–	6.0–6.7 (6.3 $\pm$ 0.3)
Stylet length	33–37	31–35 (33.8 $\pm$ 1.2)	32.4–36.1 (34.3 $\pm$ 1.4)
Vulval body diameter	–	–	29.0–36.0 (31.7 $\pm$ 2.3)
Anal body diameter	–	–	17.3–21.2 (18.8 $\pm$ 1.2)
Tail length	–	10–17 (12.9 $\pm$ 2.2)	18.6–23.4 (21.1 $\pm$ 1.5)





**Fig. 2.** *Rotylenchus buxophilus* Golden, 1956, females: A–E: anterior region; F–J: posterior region. Scale bar = 10  $\mu\text{m}$   
**Рис. 2.** *Rotylenchus buxophilus* Golden, 1956, самки: А–Е: головной конец; F–J: хвостовой конец. Шкала = 10 мкм

The morphometrics of Donbass populations generally are in agreement with literature data [1, 12], but differs by significantly longer tail (the average  $c'$  ratio 1.0–1.3) (Table 2).

Type locality: Hoogmade, Leiden, Netherlands. The species is common in the temperate zone of Europe, known from USA and Canada [1, 7, 12]. It is widespread in European part of Russia – especially in Central and Northwest regions [9]. Also, *R. fallorobustus* was registered in Carpathians and Transcaucasia [6, 15]. For the first time recorded for the fauna of Donbass and Left-bank Ukraine.

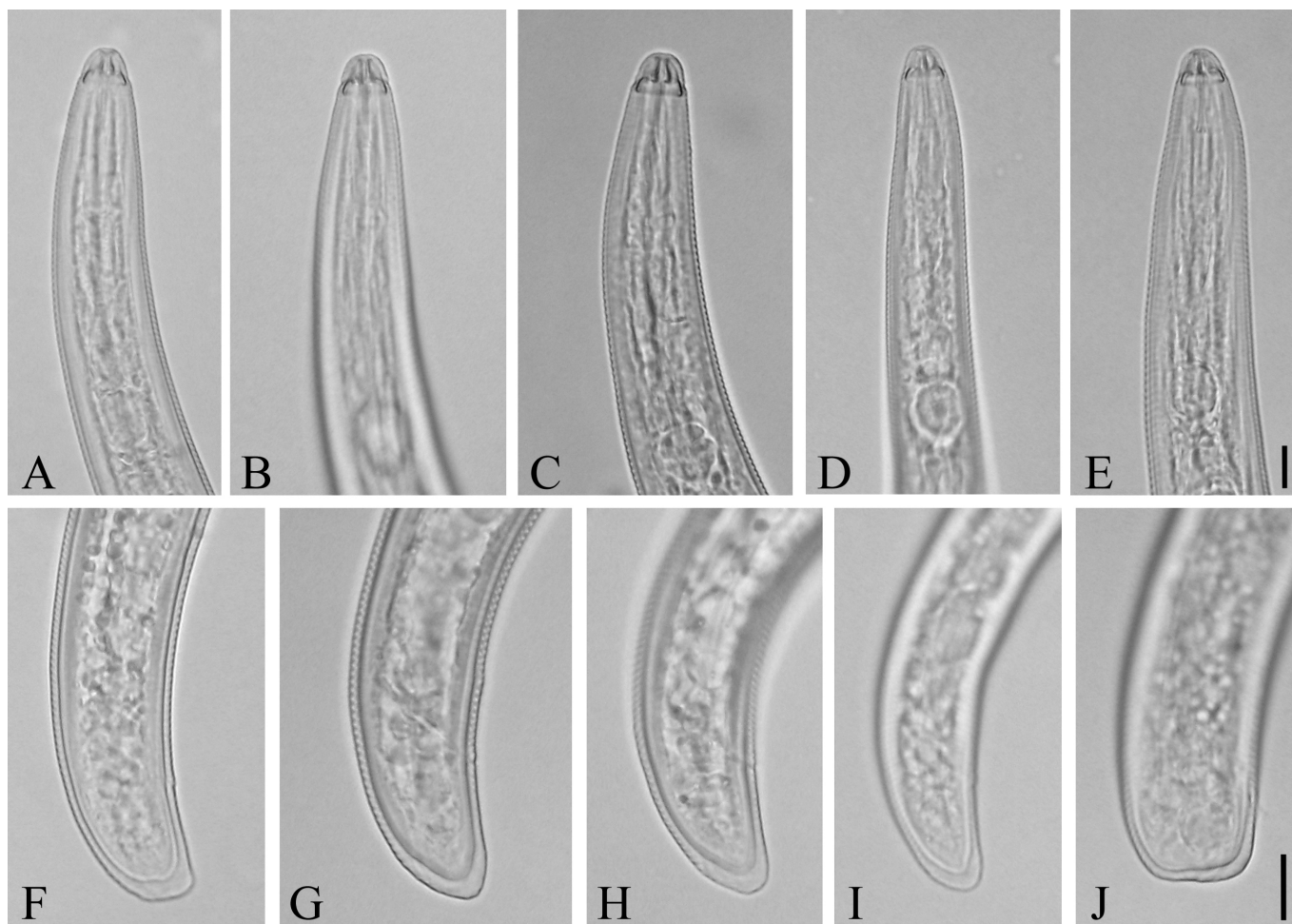
The species is ectoparasite and semi-endoparasite of the plants roots. Helminthes are inactive, localized near the root hairs. Polyphagous. Type host is unknown; the species was described from clay soil overgrown with grass [12]. Prefers moist humus-rich soils and plants with a well-developed fibrous root system. Recorded as a pest of trees and shrubs (including conifers), fruit and berry, cereal and vege-

table crops [7, 9]. One of the most serious pests of berry, pome and stone fruit crops in European part of Russia [9]. Feeding results in cortical lesions, necrotic streaks and rots on the roots.

In Donbass *R. fallorobustus* was registered in 2019 in rhizosphere of larch (*Larix* sp.). The average population density was significant – 304 specimens per 100  $\text{cm}^3$  of soil. The damage of the species on the growth and development of host plants 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 were recorded two species of *Rotylenchus* Filipjev, 1936 (Tylenchida: Hoplolaimidae): *R. buxophilus* Golden, 1956 was registered for the first time for Donbass and Ukraine, *R. fallorobustus* Sher, 1965 – for the first time for Donbass and Left-bank Ukraine. Both species are polyphagous root



**Fig. 3.** *Rotylenchus fallorobustus* Sher, 1965, females: A–E: anterior region; F–J: posterior region. Scale bar = 10  $\mu$ m  
**Рис. 3.** *Rotylenchus fallorobustus* Sher, 1965, самки: А–Е: головной конец; F–J: хвостовой конец. Шкала = 10 мкм

semi-endoparasites. *Rotylenchus buxophilus* was registered in 2020 in rhizosphere of common privet (*Ligustrum vulgare* L.), and *R. fallorobustus* – in 2019 in rhizosphere of larch (*Larix* sp.). The morphometrics of Donbass populations of both species generally are in agreement with literature data, but regional isolates has some differences.

1. Castillo P., Gómez-Barcina A. *Rotylenchus cazorlaensis* sp. n. and new record of *R. fallorobustus* Sher, 1965 (Nematoda: Tylenchida) from south-eastern Spain // *Nematologica*. 1987. Vol. 33, Iss. 4. P. 393–400.
2. Castillo P., Vovlas N. Bionomics and identification of the genus *Rotylenchus* (Nematoda: Hoplolaimidae). Leiden: Brill, 2005. 377 p.
3. Castillo P., Vovlas N., Gómez-Barcina A., Lambert F. The plant parasitic nematode *Rotylenchus* (a monograph) // *Nematologia Mediterranea*. 1993. Vol. 21 (supplement). 200 p.
4. Golden A.M. Taxonomy of the spiral nematodes (*Rotylenchus* and *Helicotylenchus*), and the developmental stages and host-parasite relationships of *R. buxophilus*, n. sp., attacking boxwood // *Bulletin of the Maryland Agricultural Experiment Station*. 1956. N 85. P. 1–28.
5. Hooper D.J. Handling, fixing, staining and mounting nematodes // *Laboratory methods for work with plant and soil nematodes*. Ed. J.F. Southey. London, 1986. P. 59–80.
6. Kozlovsky M.P. Phytonematods of terrestrial ecosystems of the Carpathian region. Lviv, 2009. 316 p.
7. Krall E.L. Root parasitic nematodes: Family Haplolaimidae. Leiden: Brill, 1990. 580 p.
8. Nguyen H.T., Trinh Q.P., Couvreur M., Singh P.R., Decraemer W., Bert W. Description of *Rotylenchus rhomboides* n. sp. and a Belgian population of *Rotylenchus buxophilus* (Tylenchomorpha:

- Hoplolaimidae) // Journal of Nematology. 2019. Vol. 51. P. 1–20.
9. Romanenko N.D., Tabolin S.B. On the issue of the study of epiphytotic situation in relation to fruit and berry trees in European part of RF // Russian Journal of Parasitology. 2014. Iss. 3. P. 130–136. [In Russian]
  10. Santos M.S.N.A., Abrantes I.M.O. Morphological characters and methods for preparing nematodes // Nematode Identification and Expert System Technology. Ed. R. Fortuner. New York: Plenum Press, 1988. P. 201–215.
  11. Seinhorst J.W. A rapid method for the transfer of nematodes from fixative to anhydrous glycerin // Nematologica. 1959. Vol. 4, Iss. 1. P. 67–69.
  12. Sher S.A. Revision of the Hoplolaiminae (Nematoda). V. *Rotylenchus* Filipjev, 1936 // Nematologica. 1965. Vol. 11, Iss. 2. P. 173–198.
  13. Siddiqi M.R. *Rotylenchus buxophilus* // C.I.H. Descriptions of plant-parasitic nematodes. 1974. Set 4, N 55. 4 p.
  14. Southey J.F. Laboratory methods for work with plant and soil nematodes. London: Her Majesty's Stationery Office, 1970. 148 p.
  15. Tabolin S.B., Migunova V.D., Akopyan K.V., Mkrtchyan R.S., Galstyan S.H. To the question of nematode fauna associated with rhizosphere of fruit and berry crops in Armenia and Nagorno-Kharabakh // Theory and practice of parasitic disease control. 2017. Vol. 18. P. 455–457. [In Russian]
  16. Wouts W.M., Sturhan D. Descriptions of *Rotylenchus* species from New Zealand, including *R. labiodiscus* sp. n. (Nematoda: Hoplolaimidae) // New Zealand Journal of Zoology. 1999. Vol. 26. P. 395–404.

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**К ИЗУЧЕНИЮ ФИТОПАРАЗИТИЧЕСКИХ НЕМАТОД ДОНБАССА:  
*ROTYLENCHUS BUXOPHILUS* GOLDEN, 1956 И  
*R. FALLOROBUSTUS* SHER, 1965 (TYLENCHIDA: HOPLOLAIMIDAE)**

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В результате изучения фитопаразитических нематод древесно-кустарниковых растений на территории Донецкого ботанического сада были зарегистрированы два вида рода *Rotylenchus* Filipjev, 1936 (Tylenchida: Hoplolaimidae): *R. buxophilus* Golden, 1956 отмечен впервые для Донбасса и Украины, *R. fallorobustus* Sher, 1965 – впервые для Донбасса и Левобережной Украины. Оба вида являются полифагами, корневыми семиэндопаразитами. *Rotylenchus buxophilus* был обнаружен в 2020 г. в ризосфере бирючины обыкновенной (*Ligustrum vulgare* L.); *R. fallorobustus* – в 2019 г. в ризосфере лиственницы (*Larix* sp.). В статье приведено описание, морфологические и морфометрические характеристики гельминтов из зарегистрированных популяций, сведения о трофических связях, а также краткие данные по распространению, биологии, экологии и вредности видов. Морфометрические показатели популяций из Донбасса в целом согласуются с литературными данными, но региональные изоляты имеют ряд отличий.

**Ключевые слова:** фитопаразитические нематоды, Донбасс, первое указание, *Rotylenchus*, морфометрия

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