

## Comparative morphology and biology of nematodes of genus *Heterakis* (Nematoda, Heterakidae), parasites of the domestic goose (*Anser anser*) in Ukraine

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The genus *Heterakis* Dujardin, 1845 is one of the most widely distributed genera of nematode parasites of domestic and wild birds. Geographically and ecologically predominant species include *Heterakis gallinarum* Schrank, 1788 and *Heterakis dispar* Schrank, 1790. Prevalence and occurrence of these nematodes in the domestic goose (*Anser anser* dom.) depend on their biological and morphological specifics which ensure the highest viability, fast growth and maximum fertility in the host. This study presents the abundance and species composition of nematodes of the genus *Heterakis* in the domestic goose in Ukraine. Comparative study of morphological and metrical characteristics of *H. gallinarum* and *H. dispar* adult females and males is presented. Additional morphometric characteristics are proposed for easier identification of the two species. Stages and periods of embryonic development of *H. gallinarum* and *H. dispar* nematodes obtained from domestic geese are established according to morphological and metrical characters. Of the two species, *H. dispar* is prevalent in domestic geese from Poltava, Kharkiv and Kyiv regions. Abundance index of this species is 9.8 specimens, and intensity of infection index is up to 62 specimens. *H. gallinarum* is rarer, its abundance index is 1.2 specimens and maximum intensity of infection is 30 specimens. Species-specific morphological differences are more distinct in male nematodes in the size and structure of the spicules, lateral wing-like protrusions of pseudobursas, numbers and position of tail papillae. Additional metrical characteristics differ between *H. gallinarum* and *H. dispar* nematodes, allowing us to identify not only adult specimens but also eggs. Embryonic development of both species occurs in four morphologically distinct stages: protoplast, blastomere cleavage, formation of first and second stage larvae. Eggs of *H. gallinarum* nematodes become infectious in eight days at 27 °C, eggs of *H. dispar* in four days, their viability in laboratory culture is  $84.3 \pm 0.58$  and  $91.3 \pm 1.53\%$  respectively.

**Keywords:** heterakiasis, *Heterakis gallinarum*, *Heterakis dispar*; identification characters; embryogenesis; metrical parameters

### Introduction

Nematodes are one of the leading groups of animals on Earth. Their expected diversity may well equal a million species, and most known species can parasitize in humans, animals and plants (Blaxter et al., 1998; Castagnone-Sereno & Danchin, 2014). Parasitic nematodes are essential components of biocoenoses; they utilize a lot of energy in ecosystems and can regulate the abundance of their hosts (Bongers & Ferris, 1999; Lok, 2016; Viney, 2017). Nematodes are the most widely distributed parasites of domestic and wild animals, and the genus *Heterakis* Dujardin, 1845 includes especially common and prevalent parasite species. Representatives of the genus have been found in domestic chickens in West and South Africa (Phiri et al., 2007; Ogbaje et al., 2012), Great Britain (Pennycook & Steel, 2001) and Germany (Kaufmann et al., 2011; Wongrak et al., 2014), in *Colinus virginianus*, *Branta bernicla nigricans*, *Chen caerulescens caerulescens*, *Chen rossii* and *Anser albifrons* in USA (Moore & Simberloff, 1990; Shutler et al., 2012; Amundson et al., 2016; Boone et al., 2017), in wild migratory birds and domestic waterfowl in South Asia (Hoque et al., 2014), and in birds of the families Psittacidae, Caccatiidae, Phasianidae and Anatidae in Spain (Cordón et al., 2009). In Ukraine, nematodes of the genus

*Heterakis* have been found in domestic birds (chickens, turkeys and geese) with prevalence ranging 2.9–100.0% (Bogach & Taranenko, 2003; Zaikina & Marshalkina, 2015).

*Heterakis gallinarum* Schrank, 1788 is a typical and most common species in domestic and wild terrestrial birds (*Gallus gallus* dom., *Numida meleagris*, *Meleagris gallopavo*, *Coturnix coturnix*) representative of the genus (Tompkins et al., 2000; Brener et al., 2006; Sherwin et al., 2013; Al-Moussawi, 2016). In waterfowl (*Anser anser* dom., *Anas platyrhynchos* dom.), *Heterakis dispar* Schrank, 1790 is the most characteristic and common species (Gicik & Arslan, 2003; Fedynich et al., 2005).

It was however proved that terrestrial birds can be infected with *H. dispar*, and waterfowl with *H. gallinarum*. Hence, these parasites can adapt to uncharacteristic hosts, especially in areas where different bird species are kept or found together (Avcioglu et al., 2008; Hoque et al., 2011; Wang et al., 2012; Kornaš et al., 2015). There are a few reports of these parasitic species being found together in waterfowl (Harradine, 1982; Dube et al., 2010; Nagwa et al., 2013).

Adult *H. gallinarum* and *H. dispar* nematodes are differentiated by morphological specifics of the tail ends in males, namely the shape and length of spicules and the number of tail papillae. Other morphological

characters are very similar, especially in females which can be considered identical (Abou Znada, 1993; Rahman & Manap, 2014; Sheikh et al., 2016). There are also diagnostically important metrical species characters, such as length and width of body, size of bulbous and esophagus. In males, other characters include size of preanal sucker, distance from anus to tail end and various parameters describing the position of tail papillae in relation to each other and to other body parts. Metrical characters of females include size of eggs in the vulva and position of the vulva. At the same time, data on metrical characters by different authors for one parasite species from different avian species vary (Avcioglu et al., 2008; Park & Shin, 2010; Tanveer et al., 2015).

Helminth abundance in general and that of *H. gallinarum* and *H. dispar* in particular in waterfowl populations depends on specifics of parasite biology and their maximum adaptation to the widest variety of hosts. Life cycles of the two parasite species are direct. Embryogenesis of *H. gallinarum* is researched relatively better, while development of *H. dispar* eggs is a subject of few studies (Clapham, 1933; Roberts, 1937; Madsen, 1950; Skrjabin et al., 1961).

Notably, nematodes of the genus *Heterakis* as other parasitic organisms are constantly evolving, which is a process associated with morphological, metrical and biological changes. Thus, morphology and biology of *Heterakis* nematodes should be studied in different species from varying climatic and geographic conditions. Also, new approaches should be implemented in studies of identifying characters in different taxa of the genus. The purpose of this work was to study the fauna, abundance, morphological and biological species-specific characters of nematodes of the genus *Heterakis* which parasitize domestic geese (*Anser anser dom.*) in Ukraine.

## Materials and methods

The studies were conducted in 2016–2017 at the Laboratory of Parasitology and Veterinary-Sanitary Expertise of the Department of Veterinary Medicine of Poltava State Agrarian Academy. Material for species identification, abundance and parasitological analysis of nematodes of the genus *Heterakis* was collected during helminthological investigation of the intestine of 463 domestic geese (Skrjabin, 1928) from Poltava, Kharkiv and Kyiv regions. Abundance, occurrence and intensity of infection were used to quantify numbers of *Heterakis* nematodes. The roundworms were identified according to Skrjabin et al. (1961). In morphological analysis, 568 specimens of the species *H. gallinarum* (173 males and 395 females) and 4,541 specimens of the species *H. dispar* (3,077 males and 1,464 females) were used.

Biological specifics of embryogenesis of *H. gallinarum* and *H. dispar* in culture were studied on eggs collected from the gonads of female nematodes. The eggs were cultured to the infectious stage with the second stage larvae in a Petri dish for 10 days in a thermostat at 27 °C. The cultures were examined daily under a light microscope. Embryonic stage of development and morphological characteristics were studied. Eggs that stopped developing or were destroyed were also noted. Each experiment was performed in triplicate.

To measure metrical characteristics of adult and embryonic nematodes of the genus *Heterakis*, ImageJ for Windows® (version 2.00) software was used in interactive mode using 5×, 10×, 40× objective and 10× photo eyepiece. To calibrate the image analyzer, the ruled scale of an ocular micrometer was coincided with the scale of stage micrometer included in MikroMed microscope kit. Microphotographs were taken using a digital camera of MikroMed (China) microscope.

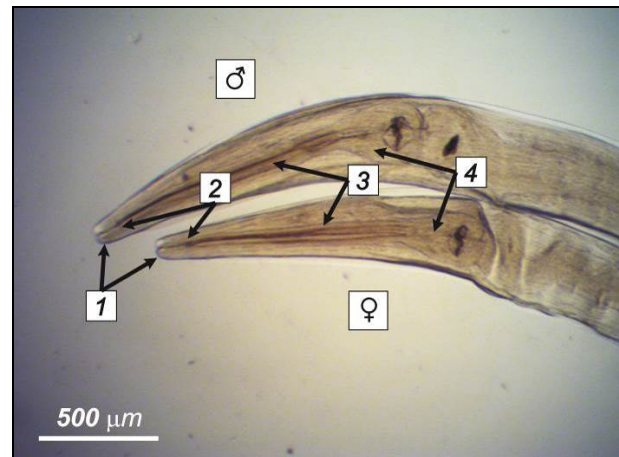
Standard deviation (SD) and average values (x) were calculated. Significance of difference between average values in studied groups of nematodes was established using one-way analysis of variance and F-test for P < 0.05 confidence level.

## Results

The genus *Heterakis* in domestic geese from Ukraine is represented by two species, *H. gallinarum* and *H. dispar*. The latter is more common, its prevalence in studied birds was 68.4%. The species *H. gallinarum* was less common, found in 16.4% of studied birds.

Abundance of both nematode species also varied. Index of Abundance for *H. dispar* was up to 9.8 specimens, intensity of infection was 14.3 specimens in average (ranging from 1 to 62 specimens). The abundance of *H. gallinarum* in geese was much lower, 1.2 specimens with average intensity of infection 7.4 specimens (ranging from 1 to 30 specimens). Helminthological investigation revealed adult male and female nematodes of the genus. Females were almost twice as common as males in both species. Their ratio ranged from 2.1 : 1 (for *H. dispar*) to 2.2 : 1 (for *H. gallinarum*).

Comparison of morphological characters of *H. gallinarum* and *H. dispar* from domestic geese revealed differentiating characteristics that are more distinct in males. The two species are in general visually identical. The mouth at the head ends of both female and male nematodes of either species is surrounded by three indistinct lips. The esophagus consists of three parts: short pharynx, cylindrical middle part and bulbous. The head end can be slightly curved (Fig. 1).



**Fig. 1.** Head end of nematodes of the genus *Heterakis*, isolated from geese: 1 – lips, 2 – pharynx, 3 – cylindrical part of esophagus, 4 – bulbous

Females of both species were visually identical. The tail end is elongated, gradually tapered. The anal opening is at the posterior part of body (Fig. 2d). The vulva of *H. gallinarum* female is located in the middle of the body. There are three bends in the vagina after the vulva, angled posteriorly, anteriorly and once again posteriorly (Fig. 2b). The vulva of the *H. dispar* female is located in the posterior part of body. Another characteristic feature is presence of small wart-like cuticular protrusions in the vulval area. The vagina has bends that are less pronounced than in *H. gallinarum* females (Fig. 2a). In both species, the vagina is connected to two opposing uteri. The uteri contain oval eggs with distinct two-contour shells (Fig. 2c).

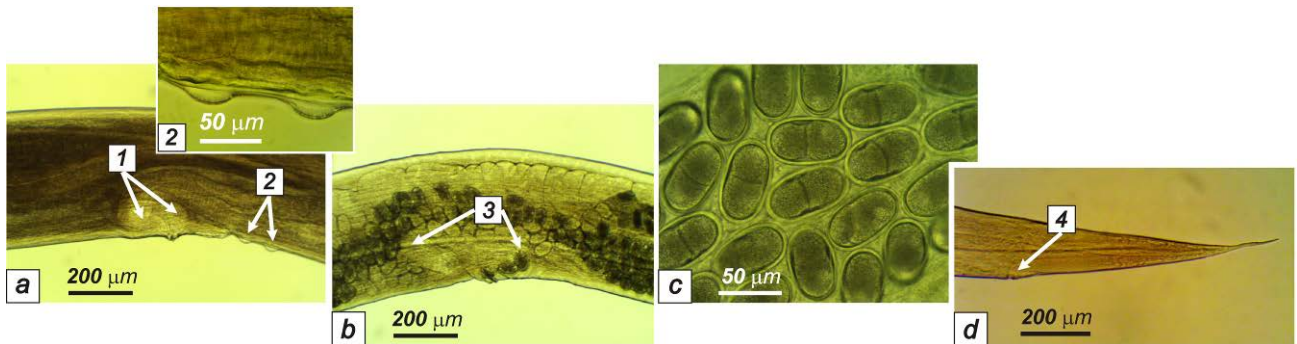
The main species characters of the studied males were specifics of spicule size and structure, and numbers and position of tail papillae. Male *H. gallinarum* have stylet-like tail end and pseudobursa with well-developed lateral “wings” that smoothly taper. The left spicule is significantly longer than the right, and has a tapered sharp end. The right short spicule is larger, has wing-like protrusions, its distal end is partly coarse, gradually tapered to a pointy hook. Gubernaculum is absent (Fig. 3).

Twelve pairs of tail papillae are located at the tail end (four postanal pairs, six adanal pairs, two preanal pairs) and one medial unpaired prelocaal papilla. The preanal sucker is easily seen, round, well-developed, surrounded by a chitinized ring (Fig. 4).

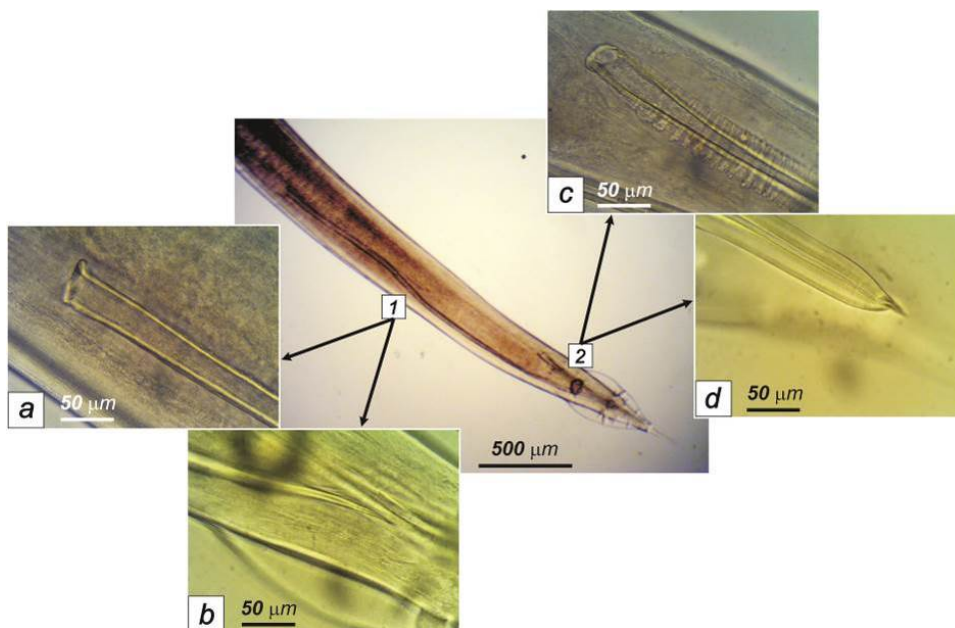
The tail pseudobursa of *H. dispar* males also consists of lateral wings. However, they are smaller than in *H. gallinarum* and have wavy edges that follow the shape of the tail papillae, which support the wings. The tail ends into a stylet-like protrusion. Two spicules are visually equal. The proximal ends of both spicules are wide and blunt. Distally, spicules taper to sharp ends. The gubernaculum is absent. Males of *H. gallinarum* similarly to *H. dispar* males have round preanal suckers with a well-developed chitinized ring and an unpaired medial papilla. Distinctively, *H. dispar* males have 13 pairs of tail papillae: four preanal pairs (two pairs of stalked papillae at the sides of the sucker, and two

lateral pairs of papillae supporting the anterior part of the pseudobursa), four adanal pairs of papillae (two pairs of unstalked papillae at the sides of the anus and two lateral pairs of large papillae that support the middle of the pseudobursa), five postanal pairs of papillae (one pair of large papillae lateral to the anus; one pair of averagely sized papillae directly behind the former; three pairs of small papillae grouped together at the

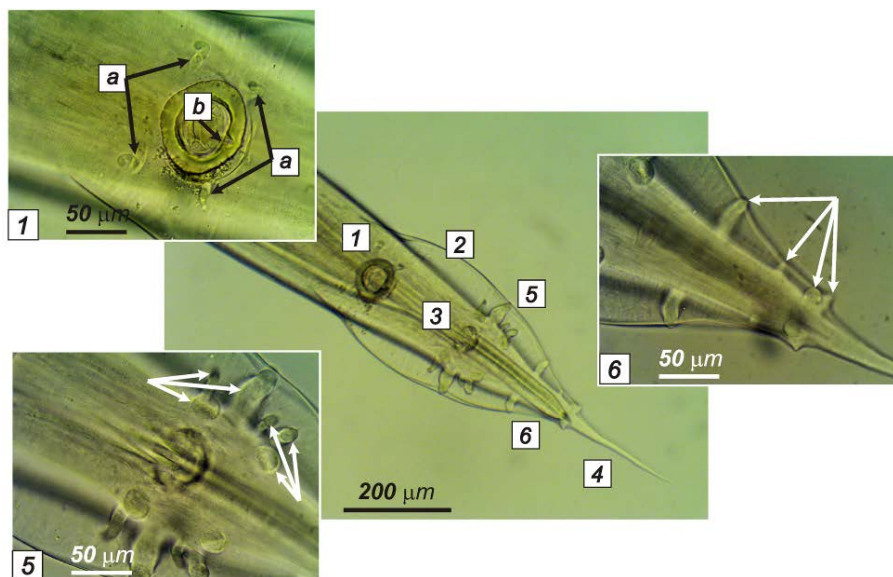
divide between the tail end and tail protrusion) (Fig. 5). Hence, the metrical parameters of adult females and males of *H. gallinarum* and *H. dispar* are significantly different, which is very important in species identification. For males of these species, 20 morphological parameters were found, 19 of which are significantly different and can be considered as taxonomic characters (Table 1).



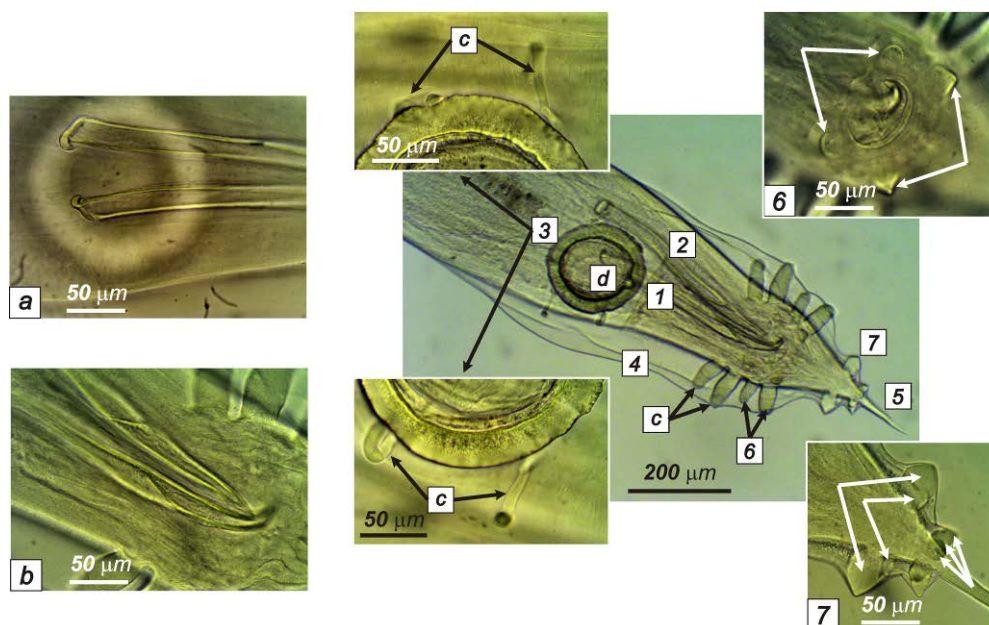
**Fig. 2.** ♀ *Heterakis* from geese: *a* – *H. dispar* vulval area, 1 – vaginal bends, 2 – cuticular protrusions; *b* – *H. gallinarum* vulval area, 3 – vaginal bends; *c* – eggs in uterus; *d* – tail end, 4 – anus



**Fig. 3.** Spicule structure in ♂ *H. gallinarum*: 1 – left spicule, *a* – proximal end, *b* – distal end; 2 – right spicule, *c* – proximal end, *d* – distal end



**Fig. 4.** Tail end of ♂ *H. gallinarum*: 1 – preanal sucker; *a* – position of two preanal pairs of papillae; *b* – medial unpaired papilla; 2 – wings of pseudobursa; 3 – anus; 4 – stylet-like protrusion; 5 – position of six pairs of adanal papillae; 6 – position of four pairs of postanal papillae



**Fig. 5.** Tail end ♂ *H. dispar*: 1 – left spicule; 2 – right spicule; a – proximal end of spicule, b – distal end of spicule; 3 – preanal sucker, c – position of four preanal pairs of papillae, d – medial unpaired papilla; 4 – lateral wing of pseudobursa; 5 – stylet-like protrusion; 6 – position of four adanal pairs of papillae; 7 – position of five postanal pairs of papillae

**Table 1**  
Morphometric characters of ♂ *H. dispar* and *H. gallinarum* (n = 20)

Characters	<i>H. dispar</i>			<i>H. gallinarum</i>		
	x ± SD	Min	Max	x ± SD	Min	Max
Length of body, mm	13.36 ± 0.98	11.58	15.67	8.26 ± 1.14*	6.12	10.25
Width of body:						
– at bulbus, mm	0.41 ± 0.03	0.37	0.46	0.27 ± 0.03*	0.23	0.32
– in the middle, mm	0.39 ± 0.02	0.36	0.42	0.31 ± 0.03*	0.27	0.38
– at base of wings of pseudobursa, mm	0.31 ± 0.02	0.28	0.34	0.22 ± 0.02*	0.19	0.25
Width of pseudobursa wings at:						
– preanal sucker, mm	0.32 ± 0.02	0.29	0.35	0.24 ± 0.01*	0.21	0.26
– adanal papillae, mm	0.30 ± 0.02	0.27	0.33	0.21 ± 0.01*	0.19	0.24
– first pair of postanal papillae, mm	0.12 ± 0.01	0.10	0.14	0.12 ± 0.02	0.10	0.15
– the maximum width of first pair of postanal papillae, mm	0.34 ± 0.02	0.31	0.38	0.28 ± 0.02*	0.25	0.31
– division between tail end and tail protrusion, mm	0.07 ± 0.01	0.06	0.08	0.06 ± 0.01*	0.05	0.07
Sucker diameter, μm	149.58 ± 5.03	140.88	158.69	76.00 ± 3.95*	69.98	82.15
Length of medial unpaired papilla, μm	13.69 ± 1.69	11.05	16.01	11.23 ± 0.80*	9.67	12.19
Width of medial unpaired papilla, μm	12.01 ± 1.54	9.42	14.39	5.64 ± 0.83*	4.12	6.98
Distance from preanal sucker to tail end, mm	0.57 ± 0.06	0.44	0.67	0.63 ± 0.02*	0.59	0.67
Length of left spicule, mm	0.39 ± 0.02	0.37	0.42	2.27 ± 0.23*	1.92	2.85
Width of left spicule:						
– at proximal end, μm	29.33 ± 1.01	27.15	30.85	43.64 ± 2.77*	38.14	47.22
– in the middle, μm	25.42 ± 1.03	23.61	27.44	21.94 ± 2.10*	19.54	26.33
Length of right spicule, mm	0.40 ± 0.01	0.38	0.42	0.66 ± 0.07*	0.55	0.77
Width of right spicule:						
– at proximal end, μm	30.30 ± 1.34	28.16	32.45	25.04 ± 1.76*	22.45	28.15
– in the middle, μm	27.40 ± 1.29	22.66	27.33	13.77 ± 1.27*	12.00	16.33
Left to right spicule length ratio	0.99 : 1	0.88 : 1	1.11 : 1	3.47 : 1*	2.82 : 1	4.75 : 1

Note: \* – P < 0.05 compared to values for *H. dispar*.

According to the metrical data, the values of most parameters (73.6%) in *H. gallinarum* males are significantly lower than in *H. dispar*. For example, *H. gallinarum* males are shorter (by 38.2%, P < 0.05) and narrower in different parts of body (by 20.5–34.1%). The wings of the pseudobursa in *H. gallinarum* males are narrower by 14.2–30.0% (P < 0.05) than in *H. dispar*. However, in other areas of the pseudobursa, width parameters are not significantly different for these two species. For example, width of wings of the pseudobursa measured at the level of first postanal papillae is similar in *H. gallinarum* and *H. dispar* (0.12 ± 0.02 and 0.12 ± 0.01 mm, relatively). Other characteristic metrical differences in *H. gallinarum* are the two times smaller preanal sucker (P < 0.05) and smaller medial unpaired papilla (17.9–53.0%, P < 0.05). In this species, the preanal sucker is located farther from the tail end (by 9.5%, P < 0.05) than in *H. dispar*. Due to morphological differences, males of the two species differ in length of the left spicule,

which is confirmed by measurements. In *H. gallinarum* average length of the left spicule was 2.27 ± 0.23 mm, which is 5.8 times longer (P < 0.05) than in *H. dispar* (0.39 ± 0.02 mm). Males of the different species are also significantly different by the following parameters: width of left spicule, size of right spicule. The proximal end of the left spicule in *H. gallinarum* is wider (by 32.7%, P < 0.05) and its middle is, conversely, narrower (by 13.6%, P < 0.05) compared with *H. dispar*. The right spicules for the two species are visually equal but are statistically different by measurements. The right spicule of male *H. gallinarum* is longer by 39.3% (P < 0.05) and narrower by 17.3–49.7% (P < 0.05) than that of *H. dispar*. Respectively the ratios of lengths of right to left spicule are 3.47 : 1 in *H. gallinarum* and 0.99 : 1 in *H. dispar* and are significantly different (P < 0.05). Females of *H. gallinarum* and *H. dispar* are significantly different by 14 morphological parameters which can make their species identification easier (Table 2).

**Table 2**Morphometric characters of ♀ *H. dispar* and *H. gallinarum* (n = 20)

Characters	<i>H. dispar</i>			<i>H. gallinarum</i>		
	x ± SD	Min	Max	x ± SD	Min	Max
Length of body, mm	16.43 ± 1.80	13.18	19.25	9.41 ± 0.89*	7.60	11.08
Width of body:						
– at bulbus, mm	0.51 ± 0.06	0.39	0.62	0.34 ± 0.03*	0.28	0.40
– in the middle, mm	0.58 ± 0.04	0.52	0.65	0.37 ± 0.03*	0.32	0.42
– at vulval area, mm	0.42 ± 0.02	0.38	0.46	0.35 ± 0.03*	0.30	0.41
– at anus level, mm	0.19 ± 0.01	0.18	0.21	0.13 ± 0.02*	0.10	0.16
Distance:						
– from vulva to the last cuticular protrusion, µm	0.38 ± 0.04	0.32	0.48	–	–	–
– from anus to tail end, mm	0.87 ± 0.04	0.78	0.92	1.17 ± 0.05*	1.09	1.28
– from head end to vulva, mm	9.50 ± 0.92	8.19	11.12	4.35 ± 0.37*	3.19	4.98
– from vulva to tail end, mm	6.93 ± 0.94	4.94	8.25	5.06 ± 0.68*	3.63	6.52
– from vulva to anus, mm	8.63 ± 0.92	7.27	10.28	3.18 ± 0.37*	2.07	3.86
Egg length, µm	68.34 ± 1.52	65.73	71.25	66.58 ± 1.57*	63.55	69.33
Egg width, µm	46.24 ± 1.17	44.25	47.98	39.01 ± 0.48*	38.18	39.85
Eggshell width, µm	1.84 ± 0.07	1.73	1.96	1.59 ± 0.16*	1.22	1.82
Area of inner egg surface, µm <sup>2</sup>	1798 ± 57	1713	1910	1728 ± 56*	1614	1874

Note: \* – P < 0.05 compared to values for *H. dispar*.

Values of metrical indices of *H. gallinarum* females are lower than those of *H. dispar*, similarly to males. The differences are even more statistically significant in females (100%) than in males (73.6%). Females of *H. gallinarum* are shorter (by 1.7 times, P < 0.05) and consistently narrower (by 1.2–1.6 times, P < 0.05) compared to *H. dispar* females. Another characteristic difference is the position of the vulva relative to the tail and head ends. In *H. gallinarum* females, the vulva is positioned almost at the middle of the body, and in *H. dispar* females it is positioned nearer to the tail end. The latter species is also characterized by cuticular protrusions near the vulva. Hence we suggest the distance between the vulva and the last cuticular protrusion (0.38 ± 0.04 µm) as a metrical index. The distance between vulva and anus in *H. gallinarum* females was shorter by 2.7 times (P < 0.05) than in *H. dispar* females. Visually, eggs of both nematode species were very alike, however their measurements were statistically different (P < 0.05). All studied egg parameters in *H. gallinarum* nematodes were lower compared to *H. dispar*. Eggs from gonads of *H. gallinarum* females were shorter (by 2.6%) and narrower (by 15.6%), their eggshell was thinner (by 13.6%), and inner egg surface area was lower (by 3.8%) than in eggs from uteri of *H. dispar* females.

The four stages of embryogenesis of *H. gallinarum* and *H. dispar* at 27 °C in laboratory culture are protoplast (Fig. 6a), blastomere cleavage (Fig. 6b), L1 formation (Fig. 6c), and L2 formation (infectious egg) (Fig. 6d).

(Fig. 6b), formation of first stage larvae, L1 (Fig. 6c), and second stage larvae, L2 (infectious egg) (Fig. 6d). Infectious eggs developed in *H. gallinarum* in eight days, in *H. dispar* in four days. Viability of *H. dispar* nematode eggs during embryogenesis was 91.3 ± 1.5% and was higher than in *H. gallinarum* (84.3 ± 0.6%) (Fig. 7).

All of isolated egg cultures (100%) were at the protoplast stage. Though the later embryonic development was similar in both species, it varied quantitatively. On the second day culture, 72.7 ± 2.5% eggs of *H. gallinarum* were at the blastomere cleavage stage while 55.0 ± 1.7% eggs of *H. dispar* contained L1. Also in the second day culture, 23.7 ± 2.1% of eggs of the latter species contained L2 and were therefore infectious. Development of *H. gallinarum* was longer in comparison: at the fourth day of culture, 55.0 ± 1.7% of eggs contained L1. Later, in the sixth to eighth day cultures, numbers of infectious eggs of *H. gallinarum* increased from 52.7 ± 1.5 to 84.3 ± 0.6%. The development of 15.7 ± 0.6% of *H. gallinarum* eggs and 8.7 ± 1.5% of *H. dispar* eggs was not successful and they gradually decayed. Morphologically this manifested as broken egg shells, resorption of embryos, air bubbles under egg shells.

Metrical parameters of eggs also changed during embryogenesis. However these changes are very different in *H. dispar* and *H. gallinarum* which can also be used in species identification (Table 3).

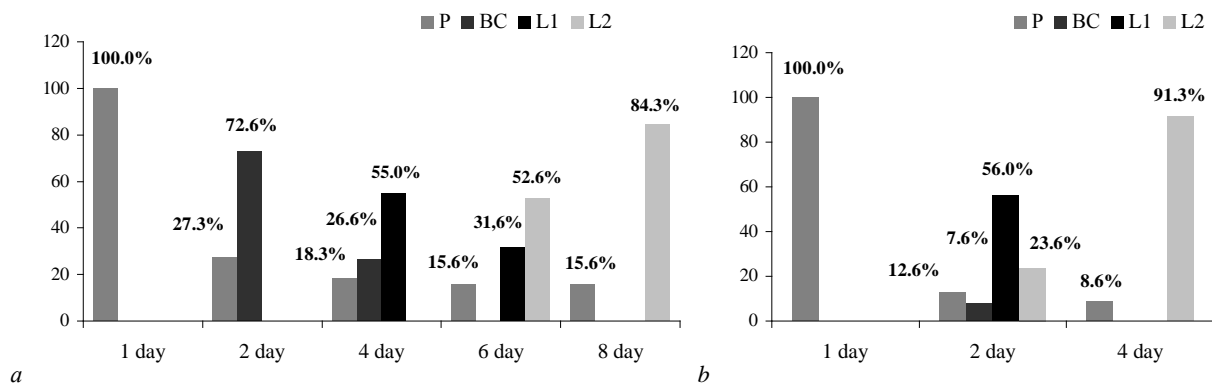


**Fig. 6.** Embryonic stages of development of *Heterakis* nematodes from domestic geese: a – protoplast, b – blastomere cleavage, c – L1 formation, d – L2 formation (infectious egg)

**Table 3**Morphometric parameters of developing *Heterakis gallinarum* and *H. dispar* eggs in experimental culture (x ± SD, n = 20)

Characters	<i>Heterakis dispar</i>		<i>Heterakis gallinarum</i>	
	Protoplast stage	L2 stage	Protoplast stage	L2 stage
Length, µm	68.29 ± 1.46	69.37 ± 1.84*	66.70 ± 1.99	64.93 ± 1.78*
Width, µm	46.10 ± 1.65	39.02 ± 1.47*	38.59 ± 0.87	39.44 ± 0.92*
Eggshell thickness, µm	1.85 ± 0.09	1.71 ± 0.10*	1.60 ± 0.16	1.59 ± 0.14
Area of inner egg surface, µm <sup>2</sup>	1786 ± 79	1695 ± 65*	1711 ± 67	1739 ± 75

Note: \* – P < 0.05 compared to values of eggs at protoplast stage.



**Fig. 7.** Embryonic stages of development of *Heterakis gallinarum* (a) and *H. dispar* (b) in experimental culture *in vitro*: P – protoplast, BC – blastomere cleavage, L1 – formation of first stage larva, L2 – formation of second stage larva (n = 100)

Eggs of *H. dispar* cultured from protoplast stage to L2 lengthened (by 1.6%,  $P < 0.05$ ), became narrower (by 15.4%,  $P < 0.05$ ), and lost 7.6% of their eggshell thickness ( $P < 0.05$ ) and 5.1% of inner egg surface area ( $P < 0.05$ ). Length and width of *H. gallinarum* eggs changed during embryonic development, L2 eggs were shorter (by 2.7%,  $P < 0.05$ ) and wider (by 2.2%,  $P < 0.05$ ) compared to protoplast stage, but their eggshell thickness and inner egg surface area did not change. Such changes are connected to different biological species specifics, and possibly to specifics of viability and ability to adapt to nonspecific hosts.

## Discussion

There are two species of the genus *Heterakis* parasitizing in domestic geese from Poltava, Kharkiv and Kyiv regions of Ukraine. One of them is a specific parasite of geese, *H. dispar*, another, *H. gallinarum*, is mostly adapted to parasitizing in terrestrial birds. The species *H. gallinarum* was found in domestic geese for the first time in Ukraine. According to many studies, the latter species is widely distributed in domestic, synanthropic and wild terrestrial birds all over the world (Menezes et al., 2001; Malatji et al., 2016; Wuthijaree et al., 2017). In our opinion, *H. gallinarum* adapted to parasitizing geese that were cross-infected when kept together with chickens and turkeys. Density indices also indicate that *H. dispar* nematodes are better adapted to parasitizing domestic geese. The maximum occurrence index of *H. dispar* was 68.4%, maximum abundance was 9.8, maximum intensity of infection was 62 specimens per host. Density of *H. gallinarum* nematodes in domestic geese was significantly lower and was 16.4%, 1.2 and 7.4 specimens per host, respectively. There are many reports of specificity of *H. dispar* to waterfowl and *H. gallinarum* to terrestrial birds (Skrjabin et al., 1961; Fedynich et al., 2005; Sherwin et al., 2013). However there are several studies showing adaptation of both species to waterfowl and terrestrial birds, especially if hosts are kept or found together (Wang et al., 2012; Nagwa et al., 2013; Komaš et al., 2015), in accordance to our results.

Comparative analysis of morphological and metrical characteristics of adult male and female *H. gallinarum* and *H. dispar* nematodes resulted in new data on their morphometric structure that can facilitate the species identification. Morphologically, male *Heterakis* nematodes are more distinct. Males of *H. dispar* have two almost equal spicules and 13 specifically positioned pairs of tail papillae. The lateral wings of their pseudobursas have wavy edges. The left spicules of *H. gallinarum* males are visually longer than the right spicules, there are only 12 pairs of tail papillae, and the lateral wings of the pseudobursa are symmetrically arched. Microscopy study of the structure and position of tail papillae in males of those species shows significant differences. In *H. dispar* there are four preanal, four adanal, and five postanal pairs of papillae. In males of *H. gallinarum* there are two preanal, six adanal, and four postanal pairs of papillae. The position of one unpaired medial papilla is same in both species. These distinct characters in males of both species are confirmed in long-term morphological studies of *H. gallinarum* nematodes obtained mostly from domestic terrestrial birds

and *H. dispar* nematodes obtained mostly from waterfowl (Abou Znada, 1993; Rahman & Manap, 2014; Sheikh et al., 2016). Females are more morphologically similar. We found additional species specific characters of female nematodes, namely cuticular protrusions near the vulva in *H. dispar*, and shape and position of the vaginal bends. In *H. dispar* females, the vaginal bends are shorter and intersecting. In *H. gallinarum* females they are significantly longer and easily seen in one plane. These morphological characters can be used if male nematodes are absent in a sample, which is a common situation.

Comparison of metrical parameters of adult *H. gallinarum* and *H. dispar* nematodes resulted in 19 parameters in males and 14 in females by which the species statistically differ ( $P < 0.05$ ). By most measurements (73.6%) of male and by all measurements (100%) of females *H. gallinarum* nematodes are smaller than *H. dispar* specimens. However, our metrical data on *H. gallinarum* obtained from domestic geese differ from those for specimens obtained from domestic terrestrial poultry. For our specimens, average length of body was  $8.26 \pm 1.14$  mm for *H. gallinarum* males, and  $9.41 \pm 0.89$  mm for females; width of body ranged from  $0.22 \pm 0.02$  to  $0.31 \pm 0.03$  mm in males and  $0.13 \pm 0.02$  to  $0.37 \pm 0.03$  mm in females. According to other studies, length and width of body can vary from 5.0 to 10.0 mm and 0.2 to 0.3 mm in males, and from 6.0 to 13.2 mm and 0.2 to 0.4 mm in females of the species (Abou Znada, 1993; Al-Moussawi, 2016; Sheikh et al., 2016). Measurements of *Heterakis* nematodes obtained from different birds reportedly can vary significantly due to their adaptive variability to more or less suitable hosts (Madsen, 1950). Only a few studies deal with metrical characteristics of *H. dispar*. According to those, the length and width of males range from 10 to 15 mm and from 0.36 to 0.38 mm, and from 15 to 17 mm and 0.45 to 0.47 mm in females, respectively (Madsen, 1950; Skrjabin et al., 1961; Avcioglu et al., 2008). In our study, average length of body was  $13.36 \pm 0.98$  mm and width of body  $0.31 \pm 0.02 - 0.41 \pm 0.03$  mm in male *H. dispar* specimens, and for females the respective values were  $16.43 \pm 1.80$  mm (length of body) and from  $0.19 \pm 0.01$  to  $0.58 \pm 0.04$  mm (width of body at different areas).

Laboratory cultures revealed that eggs of *H. gallinarum* and *H. dispar* have very similar embryonic development. According to morphological characters, there are four stages of embryonic development: protoplast, blastomere cleavage, formation of L1 and L2 (infectious egg). The egg at the protoplast stage was filled with amorphous mass. At the stage of blastomere cleavage, the protoplast was actively cleaving into round blastomeres. At L1 stage, a mobile though grainy and nebulous larva of indistinct shape was observed. At L2 stage, the larva was mobile, distinctly structured, with graininess in the middle. These data are in line with the published morphological data on egg development of *Heterakis*, however the stages of embryonic development had not been described before. Comparative study of embryogenesis of *H. gallinarum* and *H. dispar* revealed that eggs from gonads of *H. dispar* females become infectious at 27 °C in four days with  $91.3 \pm 1.5\%$  viability. Eggs from gonads of *H. gallinarum* females developed at the same temperature to infectious stage longer, in eight days with  $84.3 \pm 0.6\%$  viability. Reports on the duration of development of infectious eggs in *H. gallinarum* and *H. dispar* vary significantly.

According to one study, *H. gallinarum* eggs at 33 °C become infectious at the fifth day of embryogenesis and at 27 °C at the seventh day (Roberts, 1937). At 25 to 30°C, embryogenesis of *H. gallinarum* in the external environment is reported to last from 12 to 17 days (Skryabin et al., 1961). The biology of *H. dispar* is insufficiently studied, there are but a few reports according to which infectious eggs develop in five to six days at 20–24 °C (Hildenblat, 1956). We also found that metrical parameters of *H. gallinarum* and *H. dispar* eggs during embryogenesis were significantly different. The development of *H. dispar* eggs obtained from gonads was associated with more changes. They slightly (by 1.6%) lengthened, became narrower (by 15.4%), the eggshell became thinner by 7.6%, inner egg surface area decreased by 5.1%. In our opinion, these changes sustain the high viability of developing eggs. Metrical characteristics of *H. gallinarum* eggs did not change greatly during embryonic development. The eggs became shorter (by 2.7%) and wider (by 2.2%). Our data on the duration of development of infectious eggs and their viability can be used in species identification by eggs, if adult helminths are not observed in coprological surveys.

## Conclusion

In Ukraine, there are two species of the genus *Heterakis* which are adapted to parasitizing domestic goose (*Anser anser* dom.), *H. gallinarum* and *H. dispar*. The latter species is more prevalent (occurrence and abundance indices were 68.4% and 9.8 specimens, respectively). Males of *H. gallinarum* and *H. dispar* can be differentiated by morphological characters including length and structure of spicules, number and position of tail papillae. Morphological characters used in species identification of females are the shape and position of the bends of the vagina, and in *H. dispar*, presence of cuticular protrusions near the vulva. Nineteen statistically different metrical parameters in males and 14 in females are proposed to assist in differentiation between *H. gallinarum* and *H. dispar* species.

There are four stages in embryonic development of *H. dispar* and *H. gallinarum* nematodes: protoplast, blastomere cleavage, L1 and L2 formation. The stages are morphologically distinct. Eggs of *H. dispar* become infectious in fourth day culture at 27 °C, and are highly viable (91.3%). Embryonic development of *H. gallinarum* eggs occurs in eight days, and the average egg viability is 84.3%. The two species differ by changes in egg metrical parameters during embryonic development. In *H. dispar*, significant changes are observed in length and width of eggs, eggshell thickness and inner egg surface area, while in *H. gallinarum* they are found only in the length and width of eggs. These data add to our understanding of the biological specifics of nematodes of these two species parasitizing in the domestic goose.

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