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Original article

SPECIES-SPECIFIC FEATURES OF *AONCHOTECA BURSATA* AND *BARUSCAPILLARIA OBSIGNATA* (NEMATODA, CAPILLARIIDAE), INFECTING DOMESTIC CHICKENS *(GALLUS GALLUS DOM.)*

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Summary

Yevstafieva, V., V. Melnychuk, T. Bakhur, L. Nagorna, O. Nikiforova, I. Yatsenko & O. Reshetylo, 2022. Species-specific features of *Aonchoteca bursata* and *Baruscapillaria obsignata* (Nematoda, Capillariidae), infecting domestic chickens *(Gallus gallus dom.). Bulg. J. Vet. Med.,* **25**, No 3, 480–491.

Capillariids are a family of parasitic nematodes characterised by significant fluctuations of the taxonomic value of morphological features at the genus and species levels. Here, we present the results of a study identifying features of *Baruscapillaria obsignata* and *Aonchoteca bursata*, obtained from domestic chickens. Aside from the morphological differences in the studied capillariid species, significant differences in 14 metrical parameters of males and in 8 in females were found out. The comparative metrical analysis of eggs revealed significant differences in 4 parameters between *B. obsignata* and *A. bursata*. According to the conducted analysis, metrical comparative indices may enhance and increase the effectiveness of species identification based on the species-specific features of males and females.

Key words: *Aonchoteca bursata*, *Baruscapillaria obsignata*, identification, morphometric parameters, parasitic nematode

INTRODUCTION

One of the reasons that hinders the development of the poultry industry are helminthoses of the digestive tract; in particular, capilariidoses, which cause significant economic losses to farms. As a result of infestation, young birds retard their growth and development, their safety decreases, as well as the productivity and breeding value of poultry (Schou *et al.,* 2007; Baboolal *et al.,* 2012; Ola-Fadunsin *et al.,* 2019).

The results of ecological faunistic studies have shown the broad distribution of capillariids in wild and domestic birds in most countries of the world (Baruš & Sergeeva, 1989; De Rosa & Shivaprasad, 1999; D'Ávila *et al*., 2012; Yevstafieva *et al*., 2018).

Thus, four species of *Capillariidae*, namely *Capillaria retusa*, *C. columbae*, *C. bursata,* and *C. contorta*, were found in turkeys in Borno State, Nigeria. The prevalence of infection ranged from 17.2 to 34.0% (Benisheikh *et al*., 2020). In Turkey, five species of capillariids were identified in dissections of pheasants. The dominant nematode species in that study were *C. contorta* (64.7%), *C. bursata* (35.3%) and *C. caudinflata* (23.5%). *C. annulata* and *C. obsignata* were rarer, with 17.6% and 5.9% prevalence of infection, respectively (Gürler *et al*., 2012). Most researchers point out the dominance of *Baruscapillaria obsignata* (Syn.: *Capillaria obsignata*) in domestic birds, particularly in chickens. That wide distribution is explained by the life cycle of that parasitic nematode, which does not require an intermediate host. The parasite was observed in chickens in Africa, Vietnam, Germany, Philippines, and Japan (Schou *et al*., 2007; Mukaratirwa & Khumalo, 2010; Kaufmann *et al*., 2011).

The classification system of capillariids is extremely complex and frequently reviewed because of the slight expression of their morphological traits (Spratt, 2006). Moreover, the identification of those parasites is still problematic and currently the classification system is based mainly on the morphology of males (Moravec, 1982; Moravec & Justine, 2010). Other scientists recommend considering the specifics of biology, and the morphological features of capillariid egg, believed to be species-specific (Moravec *et al*., 1987; Traversa *et al*., 2011; Magi *et al*., 2012). This approach to the capillariid identification frequently results in inaccurate descriptions of morphology if only the female nematodes are obtained. There are suggestions of using the metrical indices of male and female capillariids in order to improve the species identification (Kajerová & Baruš, 2005; Dar *et al*., 2013).

Thus, we have conducted a comparative morphometric analysis of mature female and male capillariids of *Aonchoteca bursata* (Freitas et Almeida, 1934) and *Baruscapillaria obsignata* (Madsen, 1945) (Moravec, 1982) obtained from domestic chickens in order to collect more data on the species-specific features of those parasitic nematodes.

MATERIALS AND METHODS

The studied materials were collected from September 2019 to March 2020. The research protocol of the current study was approved by the Ethic Committee of the Poltava State Agrarian Academy (Approval number: 2020/03). Altogether, 205 specimens from domestic chickens (*Gallus gallus dom.*) from farms of Poltava,

Kharkiv, Kyiv and Sumy regions of Ukraine, i.e. from Central and Northeastern Ukraine, were investigated. Nematodes were collected during the helminthological dissection of the thin gut of dead or euthanised birds (Skrjabyn, 1928) at the Laboratory of Parasitology and Veterinary-Sanitary Expertise of the Department of Veterinary Medicine of Poltava State Agrarian Academy. Nematodes were identified according to the keys of Skrjabyn *et al*. (1957). The main indices of nematode abundance were the index of prevalence and infection rate. Morphology of 372 adult nematodes (103 male and 269 female specimens) of *B. obsignata* and 94 adult nematodes of *A. bursata* (32 males and 62 females) was analysed.

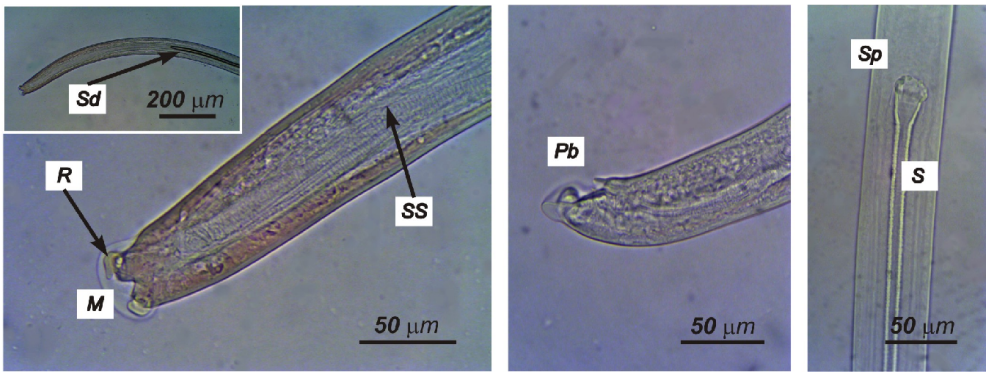
Morphometric parameters of capillariids were analysed using ImageJ for Windows® (version 2.00) in interactive mode using 5×, 10×, 40× objective and a 10× eyepiece. Image analyzer was calibrated with MikroMed object-micrometer. Photomicrographs were taken using a digital camera mounted on the MikroMed (China) microscope.

Statistical processing of the experimental data was carried out using MS Excel; mean value and standard deviation (SD) were calculated. Statistical significance of differences between mean values in studied groups was determined using one-factor analysis of variance by Fisher's criterion. Results were considered significant at $P<0.05$.

RESULTS

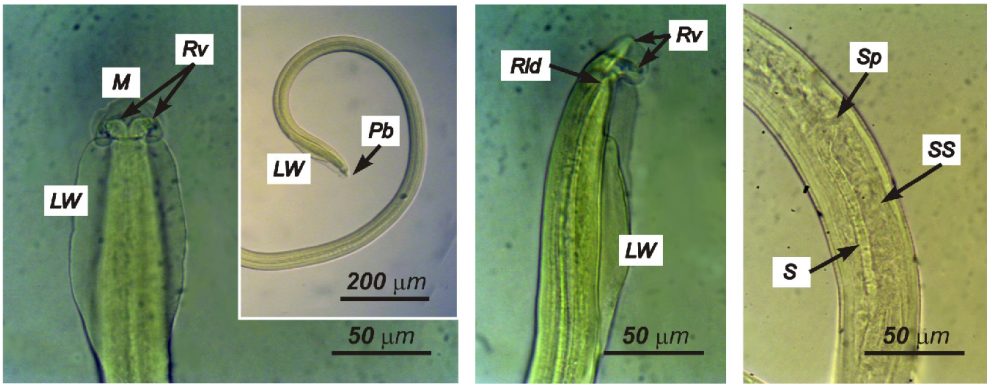
Baruscapillaria obsignata (Madsen, 1945) (Moravec, 1982) was found to be the dominant capillariid species in domestic chickens of Central and Northeastern Ukraine. Its prevalence was 52.6%, the infection rate was 1.2 specimens/host. The number of *Aonchoteca bursata* (Freitas et Almeida, 1934) (Moravec, 1982) nematodes was significantly lower, with 9.1% prevalence and 0.3 specimens/host.

Morphological study revealed differences in both female and male specimens of the studied capillariid species. Notably, the body of *B. obsignata* and *A. bursata* was typical for all capillariids: thin, filiform, translucent, with a thin head and thick tail body parts. The head part was without ornamentations and characteristic



A B C

Fig. 1. ♂ *Baruscapillaria obsignata*: A. caudal end, ventral side, B. caudal end, lateral side, C. area of proximal end of spicule; *Pb* – pseudobursa, *R* – ray of pseudobursa, *M* – membrane, *SS* – spicule sheath, *S* – spicule, *Sp* – proximal end of spicule, *Sd* – distal end of spicule.



A B C

Fig. 2. ♂ *Aonchoteca bursata*: A. caudal end ventral side, B. caudal end, lateral side, C. area of the proximal end of spicule; *Pb* – pseudobursa, *Rv* – ventral rays, *Rld* – dorsolateral rays, *M* – membrane, *SS* – spicule sheath, *S* – spicule, *Sp* – proximal end of spicule, *LW* – lateral wings.

morphological features. It was elongated, with two indistinct lips at the mouth.

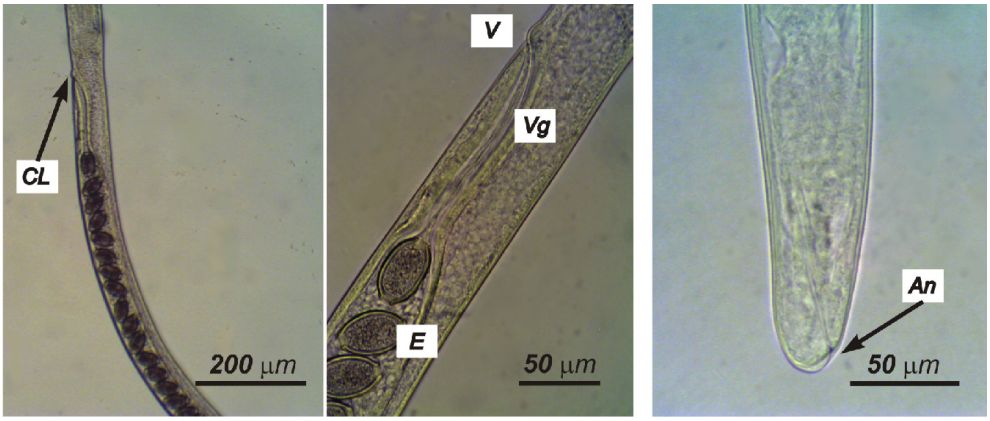
The main morphological differences between *B. obsignata* and *A. bursata* males were found in the structure of caudal end and spicule. In *B. obsignata* males, the caudal end was a pseudobursa, made up by two well-developed rounded rays and a membrane between them. The spicule was singular, covered by a smooth spicule sheath gathered in folds. The distal end of spicule was thinner and rounded. The proximal end of spicule was thicker, funnel shaped with a fringed end (Fig. 1).

The caudal end of *A. bursata* males was also a pseudobursa. However, it differed from that of *B. obsignata* by the presence of two lateral wings. There were also differences in the structure of the pseudobursa. It consisted of a membrane that was held on four rays, two of which dorsolateral, thick, straight, and aligned with the body nematode. The other two rays were ventral, thin, bent ventrally and ending on the pseudobursa's end. The spicule was singular, thin, with a sharp distal end and a rounded spoon-like proximal end. The spicule sheath was smooth and folded as in *B. obsignata* males (Fig. 2).

B. obsignata females had a slight cuticular lip in the vulva area. The vulva was located at the posterior part of oesophagus. The vagina was straight, without bends, with a strongly developed muscular layer. The uterus was made up by long loops filled with eggs. The eggs were morphologically typical of capillariids: barrel shaped, with slightly pressed in egg plugs on ends. The caudal end was slightly tapered, rounded. The anus was subterminal (Fig. 3).

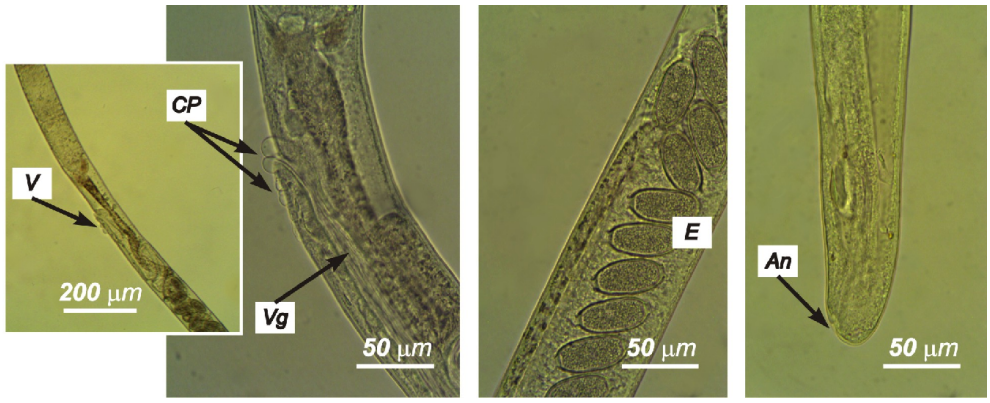
Morphologically, the vulva in *A. bursata* female was located posterior to the oesophagus. There were two large cuticular processes at the vulva opening, and two similar but much smaller cuticular processes posterior to these. The structure of the vagina was characteristic, with a well-developed S-shaped bend and a muscular wall. The uterus was filled with eggs; egg plugs were wide and prominent (Fig. 4).

Significant differences were found between the metric characters of female and



A B

Fig. 3. ♀ *Baruscapillaria obsignata*: A – vulva area, B – caudal end; *CL* – cuticular lip, *V* – vulva, *Vg* – vagina, *E* – eggs, *An* – anus.



A B C

Fig. 4. ♀ *Aonchoteca bursata*: A. vulva area, B. uterus with eggs, C. caudal end; *CP* – cuticular processes, *V* – vulva, *Vg* – vagina, *E* – eggs, *An* – anus.

male capillariids of *B. obsignata* and *A. bursata* species. Thus, males of the two species differed by 14 metric features. With respect to 8 of them, *A. bursata* males exceeded *B. obsignata*, and by the other 6 metric features, *A. bursata* males were smaller (Table 1).

A. bursata males were longer by 9.8% (11.17±1.02 mm, P<0.05) than those of *B. obsignata* (10.07±0.99 mm). The oesophagus was also longer in *A. bursata* by 24.1% (P<0.001). The body of *A. bursata* males at different levels (at the head and caudal ends, posterior part of esophagus) was significantly wider by 15.2–62.5% (P<0.001) compared to the respective values for *B. obsignata*. The *A. bursata* spicule was longer by 21.1% (1.61±0.05 μm, P<0.001) than that of *B. obsignata* (1.27±0.04 μm). The width of spicule of the studied species varied significantly at different levels. In *B. obsignata,* proximal and distal ends of the spicule were significantly wider (by 43.8 and 32.0%; P<0.001)

Table 1. Metric features of male *B. obsignata* and *A. bursata*, obtained from *Gallus gallus dom.* Data are presented as mean ± SD (n=10) and min–max range

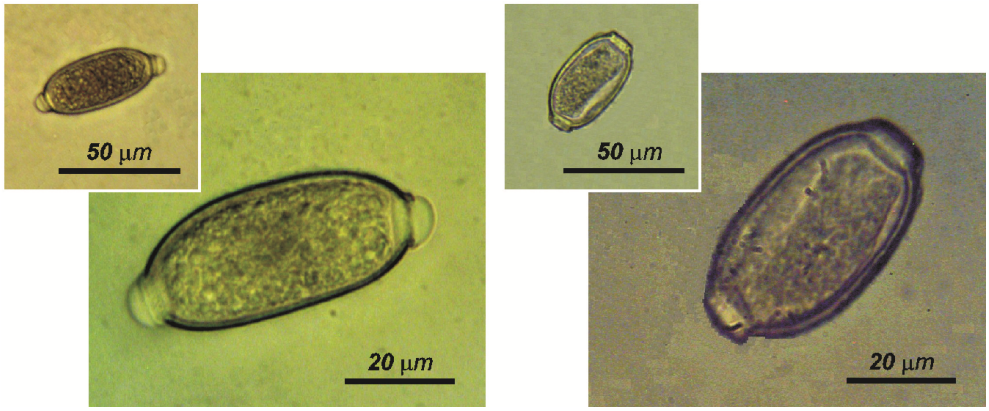
Features	*Baruscapillaria obsignata*		*Aonchoteca bursata*	
	mean±SD	Min–max	mean±SD	Min–max
Length of body, mm	10.07±0.99	8.59–11.62	11.17±1.02*	9.27–13.00
Length of oesophagus, mm	4.45±0.37	4.00–4.98	5.87±0.43***	5.18–6.37
Width of body (mm), at:				
– head end, µm	6.31±0.32	5.84–6.94	16.87±0.86***	15.29–18.22
– posterior part of oesophagus, µm	43.10±2.28	40.68–48.08	57.30±2.06***	56.60–61.02
– caudal end, µm	37.05±2.40	33.00–40.95	43.71±1.90***	40.38–46.85
Length of spicule, mm	1.27±0.04	1.20–1.34	1.61±0.05***	1.53–1.72
Width of spicule at:				
– proximal end, µm	20.57±1.44	18.45–22.69	11.54±0.69***	10.25–12.26
– the middle, µm	7.09±0.21	6.66–7.33	8.30±0.63***	7.36–9.12
– distal end, µm	5.80±0.31	5.18–6.11	3.94±0.29***	3.34–4.22
Width of spicule sheath at:				
– proximal end, µm	22.30±1.70	19.18–24.68	16.12±0.98***	14.28–17.11
– distal end, µm	9.23±0.80	8.01–10.39	10.52±1.71*	8.14–14.11
Length of left lateral wing, µm	–	–	123.57±2.80	120.08–127.22
Length of right lateral wing, µm	–	–	112.27±3.37	107.69–117.08
Width of left lateral wing, µm	–	–	16.42±1.08	14.64–18.22
Width of right lateral wing, µm	–	–	10.74±0.91	9.25–12.28
Length of pseudobursa, µm	20.23±1.37	18.63–22.67	10.21±0.74***	9.06–11.28
Width of pseudobursa, µm	33.48±1.90	30.84–36.55	22.59±1.53***	20.18–24.69
Width to length of pseudobursa ratio	1:1.67	1:1.36–1:1.96	1:2.22	1:1.84–1:2.56
Width of base of pseudobursa, µm	26.59±0.80	25.34–27.83	17.84±1.61***	15.70–20.04

* P<0.05; *** P<0.001 compared to respective values of *B. obsignata*.

Table 2. Metric characters of female *B. obsignata* and *A. bursata*, obtained from *Gallus gallus dom.* Data are presented as mean ± SD (n=10) and min–max range

Features	*Baruscapillaria obsignata*		*Aonchoteca bursata*	
	mean±SD	Min–max	mean±SD	Min–max
Length of body, mm	12.96±0.74	11.78–14.25	28.69±2.77***	25.29–32.20
Length of oesophagus, mm	5.71±0.36	5.22–6.21	8.39±0.62***	7.64–9.51
Width of body at:				
– head end, µm	8.98±0.41	8.33–9.68	22.19±2.12***	19.85–25.48
– posterior part of esophagus, µm	49.86±1.48	47.36–52.00	76.08±2.51***	71.16–79.85
– vulva, µm	48.77±1.58	45.60–51.13	65.43±1.58***	62.60–68.22
– anus, µm	29.16±0.95	27.08–30.18	29.09±2.53	25.66–34.50
Distance from head end to vulva, mm	5.80±0.36	5.31–6.30	8.44±0.62***	7.69–9.55
Distance from vulva to caudal end, mm	7.16±0.63	6.13–8.43	20.25±2.64***	16.69–23.73
Distance from posterior part of esophagus to vulva, µm	84.19±6.97	66.98–90.22	45.39±4.03***	39.75–51.22
Egg length, µm	50.25±1.35	48.22–52.33	59.14±1.41***	56.65–61.22
Egg width, µm	25.93±1.06	24.68–27.56	24.88±1.28	23.15–26.65
Eggshell thickness, µm	2.25±0.26	1.95–2.66	1.61±0.04***	1.53–1.68
Egg plug length, µm	4.56±0.57	3.55–5.19	3.85±0.77*	2.68–5.30
Egg plug width, µm	6.71±0.46	6.12–7.42	8.38±0.71***	7.26–9.22

* $P<0.05$; *** $P<0.001$ compared to respective values of *B. obsignata.*



A B

Fig. 5. Morphological features of eggs in *Aonchoteca bursata* (A) and *Baruscapillaria obsignata* (B).

yet narrower in the middle (by 14.5%, P<0.001) compared with *A. bursata*. Those measurements are explained by the specific spicule morphology in males of different species. The width of spicule sheath also varied at different areas: it was wider at its proximal end in *B. obsignata* (by 27.7%, P<0.001) and smaller at the distal end (by 12.2%, P<0.05). The pseudobursa of *B. obsignata* was longer (by 49.5%, P<0.001) and wider (by 32.5%, P<0.001) than that of *A. bursata*. Notably, the lateral wings at the caudal end of *A. bursata* males differed in size, the left wing being larger than the right one.

The comparison of metric characters of *B. obsignata* and *A. bursata* females revealed significant differences in eight of features. Seven of them were larger in *A. bursata* females; only one parameter was significantly larger in *B. obsignata* females (Table 2).

The body and the oesophagus of *A. bursata* females were significantly longer (by 54.8 and 31.9%, P<0.001) than those of *B. obsignata* females. *A. bursata* females were also wider (P<0.001) at head end (by 59.5%), posterior part of oesophagus (by 34.4%) and vulva (25.4%). Width of body at the anus area did not differ in the studied capillariids and ranged from 29.09±2.53 to 29.16±0.95 μm. The vulva location respective to the head and caudal ends, and to the posterior part of oesophagus was also an important trait. In *B. obsignata* females, the vulva was located almost in the middle of body, slightly nearer to the head end. In *A. bursata* females, the vulva was located in the anterior part of body. With respect to the oesophagus, the vulva of *A. bursata* females was closer by 46.0% (P<0.001) than that of *B. obsignata*.

The study of metric parameters of eggs revealed that 4 of studied metric features were statistically significantly different between eggs of *A. bursata* and *B. obsignata*. The eggs of *A. bursata* nematodes were longer (by 15.0%, P<0.001), with wider (by 19.9%, P<0.001) and shorter (by 15.5%, P<0.05) egg plugs, and had a thinner eggshell (by 28.4%, P<0.001). At the same time, egg width indices did not differ between the studied species. Metric differences were confirmed by morphological features of the studied species capillariids' eggs structure (Fig. 5A, 5B).

DISCUSSION

Capillariids are one of the most intricate group of parasitic nematodes for taxonomic identification, subject to constant revisions (Moravec, 1982; Moravec *et al*., 1987). Notably, capillariids can parasitise in many hosts, including humans (El-Dib *et al*., 2015; Ochi *et al*., 2017). Thus, a more detailed study of the identification traits of various capillariid species, with respect to the metric parameters and interspecies comparative analysis, results in additional data and a more exact identification.

In the present study, the morphometric features of capillariid species obtained from domestic chicken from Central and Northeastern Ukraine were analysed. *Baruscapillaria obsignata* (Madsen, 1945), Moravec, 1982 was the more prevalent of them, with prevalence rate of 52.6%, and infection rate of 1.2 specimens/host. The species *Aonchoteca bursata* (Freitas et Almeida) was more rarely found out, with 9.1% prevalence and 0.3 specimens/host infection rate. The domination of *B. obsignata* nematodes in domestic birds was noted by many authors in areas of various climatic conditions (Tanveer *et al*., 2015; Yevstafyeva *et al*., 2017). The distribution of this species is explained by its biological specifics and especially the ability to infect both wild, domestic and synanthropic birds which results in fast cross-infections of hosts and best possible preservation of parasitic nematodes (Schou *et al*., 2007; Kaufmann *et al*., 2011).

The comparative analysis of morphological characteristics of capillariids revealed that for species identification, the structure of caudal end of *B. obsignata* males is of interest. The caudal end of this species was a pseudobursa with two small rays and a membrane. Spicule structure, especially the funnel-shaped distal end of spicule was also important. Spicule sheath was smooth. In *B. obsignata* females, the specific morphological features were weakly developed. The trait of interest was the weak cuticular lip in the vulva area. In *A. bursata* males, species-specific morphological features were the presence of lateral wings in caudal end area, and four rays arranged in a certain way in pseudobursa. Spicule sheath was smooth as in *B. obsignata*. The distal end of spicule was slightly widened and spoon-shaped. In *A. bursata* females, the distinguishing feature was the presence of four cuticular processes of various shape, and the S-shaped bend of vagina.

These species-specific morphological traits have been noted for the studied species of capillariids (Moravec, 1982; Kajerová & Baruš, 2005; Gürler *et al.*, 2012). However, there are reports on the variability of morphological features to the absence of those in young worms, which is a problem in species identification (Skrjabin *et al*., 1957). Thus, a number of authors have suggested using also the metric parameters, which are likewise changeable at a species level in capillariids, obtained from birds of different species and from different climatic regions (Tamaru *et al*., 2015; Sakaguchi *et al*., 2020). Thus, the comparative analysis of the metric differences between males and females of *B. obsignata* and *A. bursata* capillariids, showed that males of the studied species are different by 14 parameters. The length of body and oesophagus; width of body at different areas of body; length of spicule and width of spicule in the middle; width of the distal end of spicule sheath) were larger in *A. bursata* than in *B. obsignata* males. Also, we suggest considering the metric parameters of width of the proximal and distal ends of spicule, and width of spicule

sheath. It is easy to distinguish *B. obsignata* from *A. bursata* by these parameters. Notably, the pseudobursa of *A. bursata* males was smaller than that of *B. obsignata* males, and had two lateral wings, the left one longer and wider than the right one.

The comparison of metric parameters of *B. obsignata* and *A. bursata* females revealed significant differences in 8 of studied traits. The length of body and oesophagus; width of body at different areas; distance from vulva to different parts of body were larger in *A. bursata* females, similarly to males. We also suggest taking into account the metric indices of vulva location in the identification of females. The metric assessment of eggs of the studied capillariid species revealed significant differences. The eggs of *A. bursata* were longer, with pressed in egg plugs and thinner egg shell compared to *B. obsignata* eggs. Many authors propose to take into account the morphological and metric characteristics of capillariid eggs in their species identification (Campbell & Little, 1991; Magi *et al*., 2012; Carvalho *et al*., 2019).

Thus, the results of the research will increase the efficiency of species identification of capillariids infecting domestic chickens.

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