Arch Biol Sci. 2016;68(3):587-601 DOI:10.2298/ABS150923049B

MORPHOMETRIC VARIABILITY OF *CLITELLOCEPHALUS OPHONI* (EUGREGARINIDA, GREGARINIDAE) IN THE INTESTINES OF *HARPALUS RUFIPES* (COLEOPTERA, CARABIDAE)

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Received: September 23, 2015; **Revised**: November 30, 2015; **Accepted**: December 3, 2015; **Published online**: May 10, 2016

Abstract: *Clitellocephalus ophoni* (Tuzet and Ormieres, 1956) Clopton, 2002, is one of the parasites of a common ground beetle species, *Harpalus rufipes* (De Geer, 1774), inhabiting practically the entire temperate zone of Eurasia. Photographs of 177 gamonts and 74 syzygies of *C. ophoni* from specimens of three populations of *H. rufipes* collected from the countryside near Dnipropetrovsk (Ukraine), were analyzed according to 15 linear characteristics and 18 indices for gamonts and 6 indices for syzygies. The coefficient of variation (CV) for the majority of linear parameters for gamonts ranged between 28.2-71.3%. Out of 15 linear parameters, only 6 showed a normal distribution; the others showed a statistically significant asymmetry (the maximum indicators on the histogram of distribution were inclined to the area of minimum value). The distribution of all 18 morphological indices diverged from the norm. Two maximum indicators were registered in the disposition of the widest point of the protomerite and deutomerite. The minimum CV was registered for the ratio of gamont length to the length of its deutomerite. The size ratios of the primite and satellite in the syzygies were more constant than the morphological indices for the gamonts. The sex of the host does not affect the length of the protomerite and deutomerite, but does affect their width, the disposition of the nucleus and the widest point of the deutomerite. The length of the protomerite and deutomerite relative to the length of the gamont is better described through linear functions, while their width has a nonlinear dynamic and is better described through parabolic function. The ratio of morphometric indices to total length of *C. ophoni* gamonts is also better described through nonlinear functions. The data obtained on the morphological variability of *C. ophoni* needs to be compared with the results for artificially infected individuals of other *Harpalus* species.

Key words: *Clitellocephalus*; *Harpalus rufipes*; gregarine; morphometric characteristics; ratio of morphometric indices

INTRODUCTION

Harpalus rufipes (De Geer, 1774) has a very wide range, inhabiting practically the entire temperate zone of Eurasia [1], and as an introduced species, North America [2]. The species is numerically dominant among ground beetles, especially in anthropogenically disturbed and agricultural ecosystems, on account of its strong propensity to migration, the variability of its life cycle and the fact that it reproduces mostly in autumn [3-6]. The results of numerous studies of the dietary range of this species of ground beetle [7-11] attest that it causes significant damage, primarily to grain crops (wheat, rye, millet, barley, oats, rice, sorghum, corn, buckwheat) and, to a lesser extent, to leguminous plants (peas, haricot, soy-beans, beans), industrial crops (beet, potatoes, sunflower, pea-nut, mustard, rape, chufa sedge, tanacetum, plantain) and fodder crops (Sudan grass, timothy-grass, vetch, lupine, clover, sainfoin) [12,13]. However, the damage caused by this species to agricultural crops is commonly exaggerated because besides consuming the seeds and sprouts of agricultural plants, *H. rufipes* also destroys the seeds of weeds and consumes numerous pests of agricultural crops [14].

The wide distribution and wide dietary range of *H. rufipes* make it a convenient object for the study of the variability of the parasitic fauna of its intestine. The results of our previous laboratory research show that the food preferences of individual ground beetles can diverge significantly [15]. Therefore, the living conditions for their intestinal parasites must also differ. In different ecosystems, and also among individuals of different sex, different conditions can develop in the intestine, which one would expect to be reflected in the absolute sizes and proportions of the parasites' bodies. Thus it is of interest to study the morphological variability of gregarines as the most numerous group of intestinal parasites of *H. rufipes*.

According to the data of Geus [16] *Actinocephalus echinatus* Wellmer 1911, *Gregarina amarae* (Hammerschmidt 1839) Frantzius 1848 and *Gregarina ophoni* Tuzet and Ormieres 1956, inhabit the intestine of *H. rufipes*.

Two gregarine parasites of *H. rufipes* are known for the territory of Poland: *G. amarae* and *Clitellocephalus ophoni* (Tuzet and Ormieres 1956) Clopton 2002 [17,18]. Previous studies of the morphology and distribution of *C. ophoni* are summarized in some works [19,20]. The authors present average data and the range of variability for the morphometric characteristics of *C. ophoni* in Poland and USA. In addition, Sienkiewicz and Lipa [20] compare the average values for morphometric characteristics with the data and first description of the species from France [21] and Ecuador [22]. A general picture of the distribution of this gregarine species is presented in the monograph of Desportes and Schrével [23]. We are not aware of any other data in the literature on the morphological variability of this species of gregarine. Gregarines of ground beetles have been virtually unstudied in Ukraine [24], and to date no one has made any mention of *C. ophoni*.

Discussing mistakes in identification of gregarine species, Clopton [25] formulated the general rule that any morphometric study of gregarine species' boundaries must recognize and address six principles: (i) delineating species boundaries and describing species is fundamentally a population-level endeavor; (ii) sample size must be sufficient to accurately reflect the population centroid and variation of morphometric characters and allow discrimination of underlying categorical shape characters; (iii) careful study of developmental and life cycle stage variation is required in order to correctly identify life cycle stages and select mature, representative specimens for morphometric analysis; (iv) descriptions must consider, and account for, sexual dimorphism in mature gamonts; (v) morphometric and categorical shape data must be taken from uniformly prepared specimens with minimal artifacts due to fixation or physiology; (vi) any attempt to delimit gregarine species must be comparative and must consider variation in a large character set over multiple life cycle stages.

Clopton [25] remarks: "For each life cycle stage used in a description, the sample size should include at minimum 30-45 individuals so that developmental outliers can be recognized and excluded from the description of normal species variation". Considering that the morphological variability of *C. ophoni*, like that of the majority of gregarine species has been studied only for small samples, it is interesting to analyze a larger number of gregarines than the minimum suggested by Clopton [25].

Thus the objective of this article was (i) to assess the overall variability of *C. ophoni* in the countryside near Dnipropetrovsk, (ii) to define the variability of the gamont and syzygy forms of *C. ophoni* relative to their length, and (iii) to identify the morphological characteristics that are the most affected by variability relative to the gregarines' size and abundance in particular host specimens, relative to the sex of the host and the ecosystem from which the host was collected.

MATERIALS AND METHODS

Location and gregarine collection method

H. rufipes specimens were collected manually from May to September 2014 from the litter and soil surface of windbreak plantations of *Robinia pseudoacacia* L., *Fraxinus excelsior* L., *Quercus robur* L., *Acer tataricum* L. and *A. negundo* L. near Dnipropetrovsk (Central

Ukraine). Specimens were also collected manually from plant litter under different canopy conditions. The ground beetles were placed in separate containers without food and were checked over a period of 24-48 h to identify the extent of gregarine infection of their intestines. The intestines were removed from the beetles, placed on a microscopic slide in physiological solution, and sliced into 10-12 cross sections with a scalpel. Three hundred and sixty three *H. rufipes* specimens were used in this study and were obtained at the following localities: (i) ecosystem 1: near Doslidnoe village (48.380891°N, 35.035367°E); 5 specimens out of 188 were infected; (ii) in the immediate vicinity of Dnipropetrovsk Airport (48.359749°N, 35.068351°E); 3 specimens out of 55 were infected; (iii) in the immediate vicinity of the town of Pridneprovsk (48.420543°N, 35.132973°E); 4 specimens out of 120 were infected. Thus, the extent of infection of *H. rufipes* by the gregarine *C. ophoni* on the outskirts of Dnipropetrovsk (middle index for all regions) was 3.3%.

Photographs of the gregarines were taken through a microscope by digital camera with a resolution of 5 megapixels. Observations were made using a microscope with ×5, ×10 and ×40 plan apochromatic objectives. Measurements were made by digital photographs in the software package TpsDig 2.17 (2013, Rohlf F.J., Ecology & Evolution, SONY at Stony Brook). Images of 177 gamonts and 74 syzygies were analyzed. The gregarines were identified according to Geus [16] and Clopton and Nolte [19].

Morphometrical methods

Fifteen linear characteristics, 18 indices of gamonts and 6 indices of syzygies were measured (Figs. 1 and 2, Table 1). The measurements were made in accordance with standard measurements for this family of gregarines [19,26,27]. The measured characteristics were as follows: DL – length of the deutomerite; DLAM – distance from the protomerite-deutomerite septum to the deutomerite axis of maximum width; DLPM – distance from the posterior end of the deutomerite to the deutomerite axis of maximum width; DWE – width of the deutomerite at the equatorial axis; DWM – maximum width of the deutomerite; NDS – distance from the nucleus to the protomerite-deutomerite septum; NL, length of the nucleus; NW, width of the nucleus; PDSW – width of the protomerite-deutomerite septum; PL – length of the protomerite; PLAM – distance from the anterior end of the protomerite to the protomerite axis of maximum width; PLPM – distance from the protomerite-deutomerite septum to the protomerite axis of maximum width; PTL – total length of the primite; PWE – width of the protomerite at the equatorial axis; PWM – maximum width of the protomerite; STL – total length of the satellite.

For gamonts as well as for syzygies (for primite and satellite), the following indices were selected: PL/PWE, PL/PWM, PL/PDSW, PLAM/PL, PLAM/PLPM, PWM/PWE, DL/DWE, DL/DWM, DLAM/DL, DLAM/DLPM, DWM/DWE, PTL/PL, DL/PL, DWM/PWM, PTL/DL, NL/NW, NDS/NL and DL/NDS. Apart from the abovementioned indices, the following ratios were also selected for syzygies: PTL/STL, PPL/SPL, PPWM/SPWM, PDL/SDL, PDWM/SDWM and PDWE/SDWE (Fig. 3, Table 1).

Statistical procedures

Primary processing of measurement results was performed in MS Excel software package. Further statistical processing of the data was performed using Statistica software package (version 8, StatSoft, USA). In the text and tables the following parameters are presented: $x \pm SD$, minimum and maximum, excess, asymmetry, coefficient of variation, range of characteristic, minimum and maximum values. Because in most cases the distribution of linear characteristics and indices was different from the norm, the median was also measured (Table 1). Differences between samples were considered significant at $P<0.05$ (when evaluated with the use of MANOVA) (Tables 2 and 3). The ratios of morphometric indices to total length of *C. ophoni* gamonts (Figs. 4 and 5) are described through linear and parabolic relationships; the model considered as optimum was that with a minimum value of R^2.

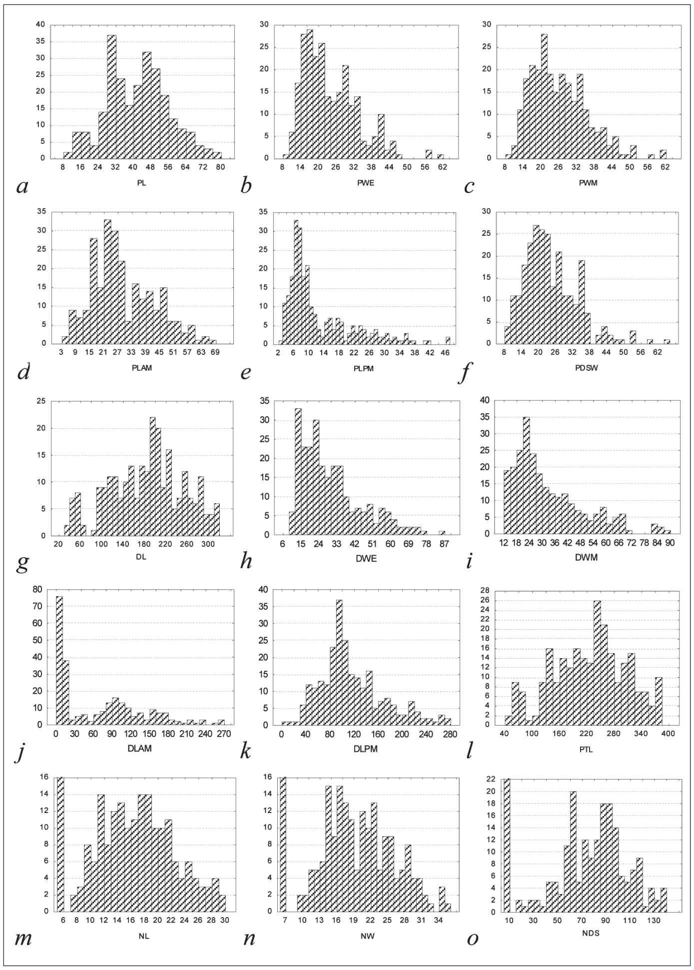

Fig. 1. Morphometric characteristics of *C. ophoni* gamonts. **a** – PL, length of the protomerite; **b** – PWE, width of the protomerite at the equatorial axis; **c** – PWM, maximum width of the protomerite; **d** – PLAM, distance from the anterior end of the protomerite to the protomerite axis of maximum width; **e** – PLPM, distance from the protomerite-deutomerite septum to the protomerite axis of maximum width; **f** – PDSW, width of the protomerite-deutomerite septum; **g** – DL, length of the deutomerite; **h** – DWE, width of the deutomerite at the equatorial axis; **i** – DWM, maximum width of the deutomerite; **j** – DLAM, distance from the protomerite-deutomerite septum to the deutomerite axis of maximum width; **k** – DLPM, distance from the posterior end of the deutomerite to the deutomerite axis of maximum width; **l** – PTL, total length of the gamonts; **m** – NL, length of the nucleus; **n** – NW, width of the nucleus; **o** – NDS, distance from the nucleus to the protomerite-deutomerite septum; on the X-axis – value of characteristic in μm; on the Y-axis – number of specimens (n = 177).

RESULTS

Variability in sizes of *C. ophoni* gamonts and syzygies

C. ophoni gamonts vary significantly according to linear parameters (Table 1). The coefficient of variation for most linear characteristics of the gamonts ranges from 28.2-71.3%. Maximum variability is typical for the distance from the protomerite-deutomerite septum to the deutomerite axis of maximum width (DLAM, 98.9%). For some individuals (45.4%), the widest point on the deutomerite was close to the septum (3-20 μm from the septum), between the protomerite and the deutomerite. With other gamonts (48.2%), the widest point was situated 60-265 μm from the distal part of the deutomerite (Fig. 1j).

Table 1. Variability of morphometric characteristics and indices of *C. ophoni* gamonts and syzygies.

Stage of life cycle	Characteristic	x±SD	Mediane	CV	Min – Max	D	Ex	As
gamonts	PTL	226.4±80.9	235.0	35.7	48.3 – 380.8	332.5	–0.550	–0.206
	PL	41.7±14.1	43.0	33.9	9.3 – 78.1	68.9	–0.362	0.138
	PWE	24.0±9.3	21.4	38.7	9.6 – 60.1	50.6	0.980***	0.993***
	PWM	26.3±9.6	24.6	36.4	9.6 – 60.6	51.0	0.724	0.853***
	PLAM	29.3±13.4	26.2	45.9	4.5 – 66.3	61.9	–0.305	0.563***
	PLPM	12.8±9.1	9.1	71.3	2.9 – 46.6	43.7	1.562***	1.453***
	PDSW	24.3±9.4	22.3	38.8	9.2 – 64.4	55.2	1.731***	1.072***
	DL	184.6±68.7	191.0	37.2	37.5 – 317.3	279.8	–0.603	–0.172
	DWE	30.1±15.4	25.8	51.3	11.4 – 85.8	74.4	0.625	1.074***
	DWM	32.9±16.6	27.7	50.5	12.1 – 88.0	75.9	0.864**	1.175***
	DLAM	68.8±68.1	44.8	98.9	2.6 – 264.7	262.2	–0.207	0.858***
	DLPM	115.9±53.7	102.7	46.4	4.1 – 279.1	274.9	0.466	0.849***
	NL	17.3±5.1	17.1	29.7	7.6 – 29.7	22.0	–0.530	0.328
	NW	20.4±5.8	20.1	28.2	9.7 – 35.6	25.9	–0.511	0.403
	NDS	82.3±25.3	84.7	30.8	16.7 – 138.1	121.4	–0.193	–0.117
	PL/PWE	1.83±0.57	1.78	31.3	0.50 – 3.66	3.15	0.291	0.511***
	PL/PWM	1.66±0.48	1.57	29.0	0.50 – 2.94	2.44	–0.164	0.397**
	PL/PDSW	1.81±0.53	1.73	29.1	0.51 – 3.30	2.79	0.155	0.385**
	PLAM/PL	0.69±0.18	0.79	25.9	0.23 – 0.93	0.70	–1.323***	–0.494***
	PLAM/PLPM	3.53±2.60	3.53	73.8	0.31 – 12.06	11.75	0.226	0.807***
	PWM/PWE	1.10±0.10	1.09	9.3	0.94 – 1.58	0.65	1.756***	1.197***
	DL/DWE	6.56±1.76	6.54	26.8	2.84 – 11.42	8.58	–0.104	0.337*
	DL/DWM	5.97±1.61	5.95	26.9	2.50 – 10.67	8.17	–0.240	0.259*
	DLAM/DL	0.32±0.26	0.22	81.8	0.03 – 0.91	0.88	–1.367***	0.358*
	DLAM/DLPM	0.86±1.24	0.28	144.0	0.03 – 9.83	9.80	14.890***	3.212***
	DWM/DWE	1.11±0.11	1.07	9.9	1.00 – 1.75	0.75	4.541***	1.658***
	PTL/PL	5.43±1.12	5.24	20.6	3.53 – 10.49	6.96	5.851***	2.059***
	DL/PL	4.43±1.11	4.22	25.2	2.53 – 9.46	6.93	5.920***	2.079***
	DWM/PWM	1.21±0.23	1.15	18.6	0.76 – 2.56	1.79	5.260***	1.723***
	PTL/DL	1.24±0.05	1.24	3.9	1.11 – 1.40	0.29	1.236***	0.047
	NL/NW	0.88±0.25	0.85	28.4	0.44 – 1.73	1.29	0.476	0.715**
	NDS/NL	5.14±2.34	4.67	45.6	1.20 – 17.38	16.18	4.929***	1.737***
	DL/NDS	2.79±1.38	2.42	49.7	1.53 – 16.50	14.97	55.38***	6.176***
syzygies	PTL/STL	1.16±0.16	1.13	13.4	0.87 – 1.84	0.97	5.804***	1.931
	PPL/SPL	1.78±0.38	1.74	21.6	0.94 – 2.93	1.99	1.153***	0.723**
	PPWM/SPWM	1.00±0.16	0.97	16.3	0.65 – 1.51	0.86	0.610	0.558*
	PDL/SDL	1.08±0.15	1.06	14.2	0.80 – 1.75	0.95	5.107***	1.783***
	PDWM/SDWM	1.05±0.14	1.05	13.7	0.76 – 1.66	0.90	3.193***	1.014***
	PDWE/SDWE	1.07±0.12	1.07	11.6	0.74 – 1.51	0.77	1.493***	0.234

x – mean value, SD – standard deviation, Ex – excess, As – asymmetry, CV – coefficient of variation (%), D – range of characteristic or index variation, Min – Max – minimum and maximum values; for As and Ex * – $P<0.05$, ** – $P<0.01$, *** – $P<0.001$; for morphometric characteristics and indices of gamonts $n = 251$, for syzygies $n = 74$, for NL, NW, NDS, NL/NW, NDS/NL, DL/NDS $n = 177$.

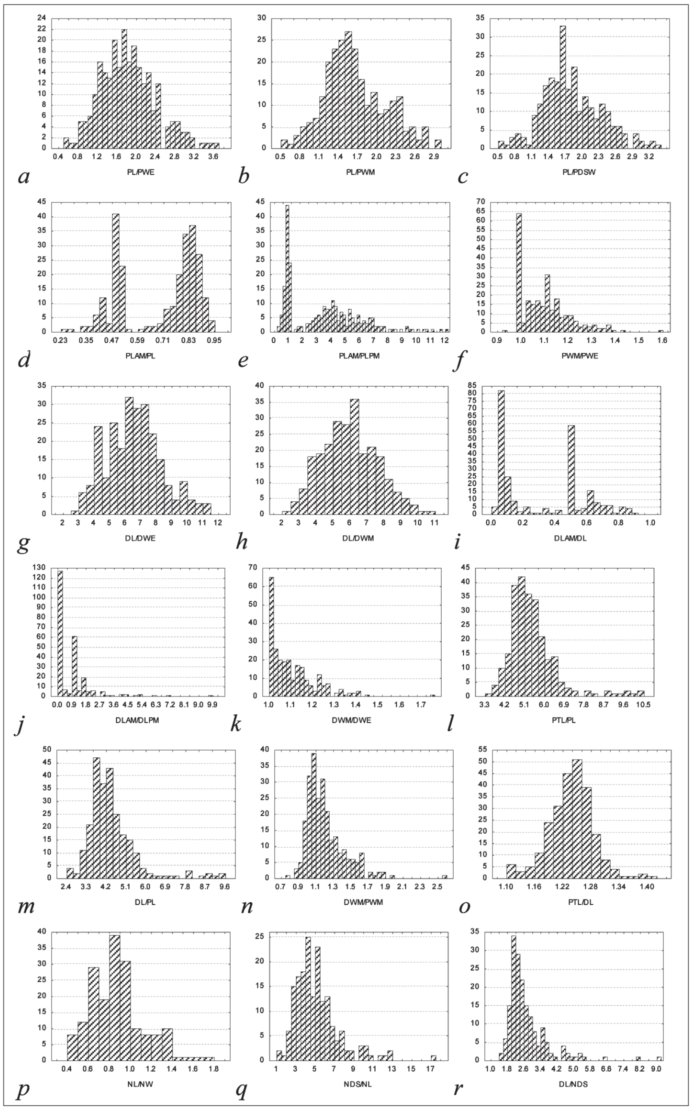

Fig. 2. Morphometric indices of *C. ophoni* gamonts: **a** – ratio of PL, length of the protomerite, to PWE, width of the protomerite at the equatorial axis; **b** – ratio of PL, length of the protomerite to PWM, maximum width of the protomerite; **c** – ratio of PL, length of the protomerite to PDSW, width of the protomerite-deutomerite septum; **d** – ratio of PLAM, distance from the anterior end of the protomerite to the protomerite axis of maximum width to PL, length of the protomerite; **e** – ratio of PLAM, distance from the anterior end of the protomerite to the protomerite axis of maximum width to PLPM, distance from the protomerite-deutomerite septum to the protomerite axis of maximum width; **f** – ratio of PWM, maximum width of the protomerite to PWE, width of the protomerite at the equatorial axis; **g** – ratio of DL, length of the deutomerite to DWE, width of the deutomerite at the equatorial axis; **h** – ratio of DL, length of the deutomerite, to DWM, maximum width of the deutomerite; **i** – ratio of DLAM, distance from the protomerite-deutomerite septum to the deutomerite axis of maximum width to DL, length of the deutomerite; **j** – ratio of DLAM, distance from the protomerite-deutomerite septum to the deutomerite axis of maximum width to DLPM, distance from the posterior end of the deutomerite to the deutomerite axis of maximum width; **k** – ratio of DWM, maximum width of the deutomerite to DWE, width of the deutomerite at the equatorial axis; **l** – ratio of PTL, total length of the gamonts to PL, length of the protomerite; **m** – ratio of DL, length of the deutomerite to PL, length of the protomerite; **n** – ratio of DWM, maximum width of the deutomerite to PWM, maximum width of the protomerite; **o** – ratio of PTL, total length of the gamonts to DL, length of the deutomerite; **p** – ratio of NL, length of the nucleus to NW, width of the nucleus; **q** – ratio of NDS, distance from the nucleus to the protomerite-deutomerite septum to NL, length of the nucleus; **r** – ratio of DL, length of the deutomerite to NDS, the distance from the nucleus to the protomerite-deutomerite septum; on the X-axis – index value; on the Y-axis – number of specimens (n = 177).

Out of the 15 linear characteristics studied, a normal distribution (there were no statistically significant deviations from 0 for Ex and As) was characteristic only for PTL, PL, DL, NL, NW and NDS (Table 1, Fig. 1). In other words, the sizes of the nuclei (its length and width) and also its distribution relative to the septum are stable, with a symmetrical distribution corresponding to average values. This is also characteristic for the length of the gamont and for the length of its protomerite and deutomerite.

For the distribution of the remainder of the linear parameters of the gamonts (PWE, PWM, PLAM, PLPM, PDSW, DWE, DWM, DLAM, DLPM), a statistically significant positive asymmetry was characteristic ($P<0.001$), i.e. the maxima on the histogram are distributed to the left, in the zone of minimal values (Table 1, Fig. 1). A statistically significant positive excess ($P<0.001$ for PWE, PLPM, PDSW and $P<0.01$ for DWM) is characteristic for the width of the protomerite and deutomerite, i.e. the diameter of the protomerite and deutomerite of all studied individuals varies to a lesser extent compared to their length.

The morphometric indices of gamonts used for identifying the gregarines have an average variation coefficient of 37.8% (for the abovementioned linear parameters the CV was on average 44.9%). Distribution of all 18 morphometric indices deviated from the norm (Table 1, Fig. 2). The indices that deviated the least from the norm were DL/DWE and DL/DWM (they had a statistically significant positive asymmetry, $P<0.05$). This is due to the gradual increase in deutomerite size, when its diameter is comparatively stable as the gamonts increase in size.

A statistically significant ($P<0.001$) negative excess was found for two indices: PLAM/PL and DLAM/DL (Table 1, Fig. 2 d, i). This indicates that the widest point in the protomerite and deutomerite may be situated randomly, often shifting in a forward or backward direction. The PLAM/PL index is also characterized by a negative asymmetry, which indicates the dominance of two morphotypes with maximum values on this index of 0.47 and 0.83, respectively: in one gamont morphotype, the maximum width of the protomerite is in front of the middle of the protomerite; in the other it is shifted towards the back edge (Fig. 2 d). This bimodal distribution is registered also for the DLAM/DL (Fig. 2 i) index: two maximums were noted in the disposition of the widest point of the deutomerite – at 10% and 50% of the length of the deutomerite.

Statistically significant ($P<0.001$) high positive values of both asymmetry and excess were expressed in the distributions of the indices PWM/PWE, DLAM/DLPM, DWM/DWE, PTL/PL, DL/PL, DWM/PWM, NDS/NL and DL/NDS (Table 1, Fig. 2).

The minimum CV was expressed for the index PTL/DL (3.9%). This indicates that for all the individuals studied, the length of the protomerite and deutomerite varies in proportion to the increase in size of the gamont (Table 1, Fig. 2 o): gamont length is 1.24 ± 0.05-fold greater than the length of its deutomerite. We point out once again that this is the most constant of all the morphometric indicators mentioned in this article.

The ratios of primite and satellite sizes in the syzygies are more constant than the gamonts' morphometric indices (Table 1, Fig. 3): the CV is in the range of 11.6-21.6%. For four out of six morphometric indices of syzygies, a statistically significant positive asymmetry was observed, and for five out six indices a statistically significant positive excess (Table 1).

The minimal CV for indices of syzygies was characteristic for the indices PDWE/SDWE (11.6%) and PDWM/SDWM (13.7%): the primite was correspondingly 6.7% and 5.4% greater in diameter than the satellite (Table 1, Fig. 3 e, f). The overall length of the primite is on average 15.8% greater than the length of the satellite (PTL/STL, Fig. 3 a). The length of the protomerite of the primite is 77.9% greater than that of the satellite (PPL/SPL, Fig. 3 b). The maximum width of the protomerite does not differ between primate and satellite (PPWM/SPWM = 1.001, Fig. 3 c). The length of the primite deutomerite is 8.4% greater than the length of the satellite's deutomerite (PDL/SDL, Fig. 3 d).

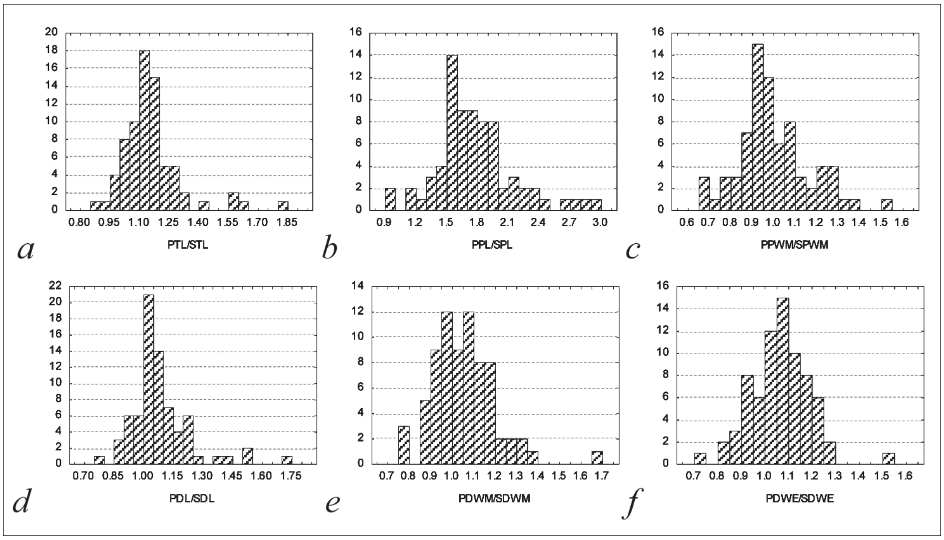

Fig. 3. Morphometric indices of *C. ophoni* syzygies: **a** – ratio of PTL, total length of the primite to STL, total length of the satellite; **b** – ratio of PPL, length of the primite protomerite to SPL, length of the satellite protomerite; **c** – ratio of PPWM, maximum width of the primite protomerite to SPWM, maximum width of the satellite protomerite; **d** – ratio of PDL, length of the primite deutomerite to SDL, length of the satellite deutomerite; **e** – ratio of PDWM, maximum width of the primite deutomerite to SDWM, maximum width of the satellite deutomerite; **f** – ratio of PDWE, width of the primite deutomerite at the equatorial axis to SDWE, width of the satellite deutomerite at the equatorial axis; on the X-axis – index value; on the Y-axis – number of specimens (n = 74).

The influence of intensity of infection, host sex, ecosystem and size of gamont on the morphometric characteristics and indices of *C. ophoni*

The results of multivariate analysis of variance (MANOVA) on the morphometric characteristics of *C. ophoni* gamonts (Table 2) indicate that the intensity of infection affects only the length of the protomerite and deutomerite (PL and DL), and does not have a statistically significant effect upon the maximum width of the protomerite (PWM), maximum width of the deutomerite (DWM), width of the protomerite-deutomerite septum (PDSW) and distance from nucleus to protomerite-deutomerite septum (NDS). Out of the morphometric indices, the intensity of infection significantly affects PL/PWM, NL/NW, PTL/PL and DLAM/DL, but not DL/DWM, DWM/PWM, PWM/PWE and DWM/DWE (Table 3). Thus massive infection is probably connected with a beetle's ingestion of a large number of mature oocysts in the course of a single feeding episode, which leads to retardation of the linear growth of primites and satellites. At the same time, the diameters (maximum and equatorial) of the protomerites and deutomerites, and also the distance from nucleus to septum between the protomerites and deutomerites, are not dependent upon the number of gamonts in a host's intestines.

While PL and DL depended on the intensity of infection, they were not affected by the sex of the host, which does however affect PWM, DWM, PDSW and NDS (Table 2). The sex of a *H. rufipes* host depended on PL/PWM, DL/DWM, PWM/PWE and DWM/DWE in *C. ophoni*, and did not affect NL/NW, DWM/PWM, PTL/PL and DLAM/DL (Table 3). In other words, the sex of a ground beetle determines a gamont's diameter, but not the length of its body parts.

Similarly, the ecosystem from which the *H. rufipes* specimens were collected did not affect PL or DL, but depended on PWM, DWM, PDSW and NDS in *C. ophoni* (Table 2). The ecosystem determines LP/PWM, DL/DWM, NL/NW, PTL/PL, DLAM/DL and DWM/DWE in *C. ophoni*, and does not depend on PWM/PWE (Table 3).

Table 2. MANOVA results of morphometric characteristics of *C. ophoni* gamonts.

Characteristic	Factor	Beta±SE	B±SE	$t_{(172)}$	P
PL	Eco	−0.08±0.05	−1.68±0.92	−1.82	0.070
	Sex	−0.01±0.04	−0.38±1.23	−0.31	0.759
	TL	0.93±0.04	0.16±0.01	23.52	$<1.0*10^{-16}$
	II	0.19±0.04	3.06±0.70	4.37	$2.2*10^{-5}$
	Eco * Sex * TL * II	–	37.53±125.67	0.30	0.766
DL	Eco	0.02±0.01	1.64±0.92	1.78	0.076
	Sex	0.002±0.008	0.24±1.23	0.20	0.844
	TL	0.99±0.01	0.84±0.01	122.45	$<1.0*10^{-16}$
	II	−0.04±0.01	−2.89±0.70	−4.12	$5.9*10^{-5}$
	Eco * Sex * TL * II	–	−24.19±125.64	−0.19	0.848
PWM	Eco	−0.22±0.05	−3.14±0.71	−4.41	$1.8*10^{-5}$
	Sex	0.23±0.04	4.96±0.95	5.20	$5.5*10^{-7}$
	TL	0.67±0.04	0.08±0.01	15.20	$<1.0*10^{-16}$
	II	−0.09±0.05	−0.99±0.54	−1.82	0.070
	Eco * Sex * TL * II	–	−492.51±97.13	−5.07	$1.0*10^{-6}$
DWM	Eco	−0.22±0.05	−5.02±1.16	−4.31	$2.8*10^{-5}$
	Sex	0.20±0.05	6.76±1.56	4.33	$2.5*10^{-5}$
	TL	0.70±0.05	0.13±0.01	15.50	$<1.0*10^{-16}$
	II	−0.04±0.05	−0.70±0.89	−0.79	0.433
	Eco * Sex * TL * II	–	−680.57±158.90	−4.28	$3.1*10^{-5}$
PDSW	Eco	−0.16±0.05	−2.41±0.77	−3.12	$2.1*10^{-3}$
	Sex	0.21±0.05	4.55±1.03	4.42	$1.8*10^{-5}$
	TL	0.68±0.05	0.08±0.01	14.47	$<1.0*10^{-16}$
	II	−0.09±0.05	−0.96±0.59	−1.64	0.102
	Eco * Sex * TL * II	–	−453.99±104.97	−4.32	$2.6*10^{-5}$
NDS	Eco	−0.30±0.06	−18.88±3.51	−5.37	$2.5*10^{-7}$
	Sex	0.22±0.05	21.34±4.70	4.54	$1.1*10^{-5}$
	TL	0.63±0.05	0.33±0.03	12.77	$<1.0*10^{-16}$
	II	−0.03±0.05	−1.32±2.67	−0.50	0.620
	Eco * Sex * TL * II	–	−2168±479	−4.53	$1.1*10^{-5}$

Names of characteristics are given in section Materials and Methods; Eco – ecosystem; sex – sex of *H. rufipes*; TL – total length of gamonts; II – intensity of infection; Eco * Sex * TL * II – intercept; n = 177

The general length of *C. ophoni* gamonts correlates with their age: the duration of a host's (*H. rufipes*) infection with oocysts of the parasite (*C. ophoni*). Gamont length significantly affects all 6 studied morphometric parameters (Table 2) and 6 out of 8 (except NL/NW and DWM/PWM) morphological indices (Table 3).

The connection between gamont age (length) and variability of linear sizes and morphometric indices can be most clearly shown graphically. The length of the protomerite (Fig. 4 a) and deutomerite (Fig 4 c) is better described through linear functions. At the same time, gamont width (Fig. 4 b, d) has a nonlinear dynamic and is most correctly described using a parabolic function.

The ratio of morphometric indices to total length of *C. ophoni* gamonts is also best described through nonlinear functions. If the dynamics of changes in morphological indices is described by an equation for a parabolic curve, then before x^2 the coefficient has a positive value (the convex part of the parabola turns downward) for the ratio of maximum width of the deutomerite to width of deutomerite at equatorial axis (Fig. 4 c), the ratio of maximum width of deutomerite to maximum width of protomerite (Fig. 4 d) and the

Table 3. MANOVA results of morphometric indices of *C. ophoni* gamonts. Note: see Table 2; n = 177

Characteristic	Factor	*Beta±SE*	*B±SE*	$t_{(172)}$	*P*
PL/PWM	Eco	0.026±0.09	0.18±0.06	2.95	$3.6*10^{-3}$
	Sex	−0.23±0.07	−0.25±0.08	−3.01	$3.0*10^{-3}$
	TL	0.36±0.08	0.0020±0.0005	4.69	$5.5*10^{-6}$
	II	0.24±0.08	0.13±0.05	2.83	$5.1*10^{-3}$
	Eco * Sex * TL * II	–	25.74±8.34	3.09	$2.3*10^{-3}$
DL/DWM	Eco	0.49±0.08	1.12±0.20	5.73	$4.4*10^{-8}$
	Sex	−0.20±0.07	−0.71±0.26	−2.73	$6.9*10^{-3}$
	TL	0.41±0.07	0.008±0.001	5.62	$7.5*10^{-8}$
	II	0.04±0.08	0.08±0.15	0.52	0.598
	Eco * Sex * TL * II	–	75.47±26.59	2.84	$5.1*10^{-3}$
NL/NW	Eco	0.45±0.10	0.15±0.03	4.40	$2.5*10^{-5}$
	Sex	0.01±0.09	0.01±0.05	0.11	0.913
	TL	0.02±0.09	0.00±0.00	0.21	0.835
	II	−0.36±0.09	−0.11±0.03	−3.97	$1.3*10^{-4}$
	Eco * Sex * TL * II	–	0.40±4.85	0.08	0.935
DWM/PWM	Eco	−0.13±0.07	−0.03±0.02	−1.71	0.089
	Sex	0.03±0.07	0.01±0.03	0.44	0.664
	TL	0.56±0.07	0.0012±0.0002	8.19	$5.7*10^{-14}$
	II	0.04±0.08	0.01±0.02	0.50	0.618
	Eco * Sex * TL * II	–	−0.27±2.75	−0.10	0.922
PTL/PL	Eco	0.17±0.08	0.29±0.15	2.02	0.045
	Sex	0.03±0.07	0.08±0.19	0.42	0.676
	TL	0.38±0.07	0.006±0.001	5.31	$3.3*10^{-7}$
	II	−0.33±0.08	−0.45±0.11	−4.10	$6.4*10^{-5}$
	Eco * Sex * TL * II	–	−3.26±19.79	−0.16	0.869
PWM/PWE	Eco	0.11±0.09	0.02±0.01	1.14	0.258
	Sex	−0.28±0.08	−0.06±0.02	−3.39	$8.7*10^{-4}$
	TL	0.19±0.08	0.0002±0.0001	2.30	0.023
	II	0.09±0.09	0.01±0.01	1.05	0.295
	Eco * Sex * TL * II	–	7.42±1.89	3.94	$1.2*10^{-4}$
DLAM/DL	Eco	−0.28±0.08	−0.09±0.03	−3.67	$3.2*10^{-4}$
	Sex	−0.11±0.07	−0.06±0.03	−1.62	0.108
	TL	0.60±0.07	0.0017±0.002	9.20	$1.2*10^{-16}$
	II	0.23±0.07	0.06±0.02	3.24	$1.4*10^{-3}$
	Eco * Sex * TL * II	–	5.52±3.50	1.58	0.116
DWM/DWE	Eco	0.20±0.09	0.03±0.02	2.15	0.033
	Sex	−0.24±0.08	−0.06±0.02	−2.99	$3.2*10^{-3}$
	TL	−0.15±0.08	−0.0002±0.0001	−1.81	0.071
	II	−0.13±0.09	−0.02±0.01	−1.52	0.129
	Eco * Sex * TL * II	–	7.46±2.10	3.56	$4.8*10^{-4}$

Note: see Table 2.

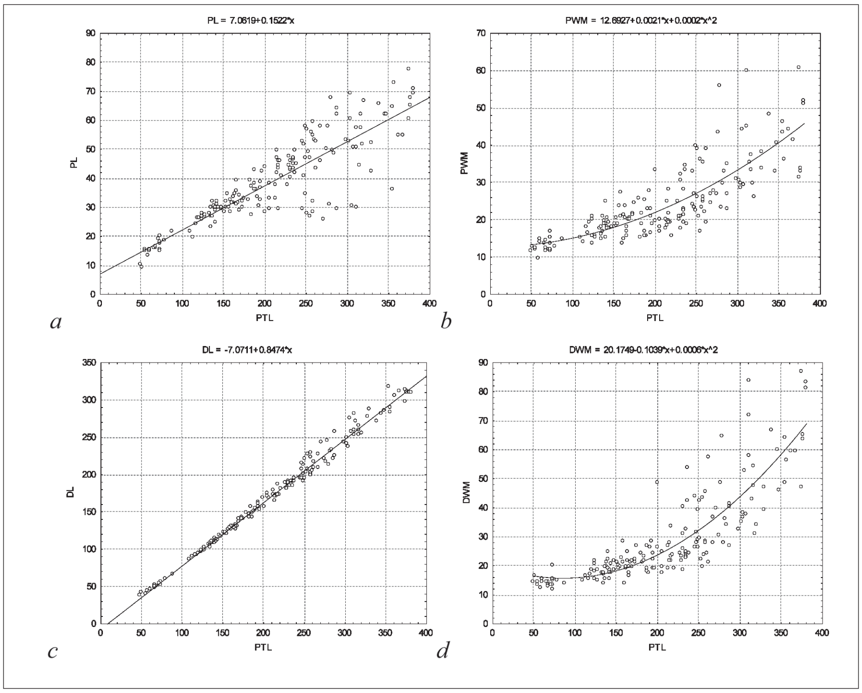

Fig. 4. Ratio of morphometric characteristics to total length of *C. ophoni* gamonts: **a** – PL, length of protomerite; **b** – PWM, maximum width of protomerite; **c** – DL, length of deutomerite; **d** – DWM, maximum width of deutomerite; on X-axis – PTL, total length of gamonts in µm, on Y-axis – value of characteristic in µm (n = 177).

ratio of length of the nucleus to the width of nucleus (Fig. 4 f). Before x^2, the coefficient has a negative value (the convex part of the parabola turns upwards) for the ratio of length of protomerite to maximum width of protomerite (Fig. 4 a), ratio of length of deutomerite to maximum width of deutomerite (Fig. 4 b) and ratio of total length of gamont to length of protomerite (Fig. 4 e).

DISCUSSION

Our evaluation of the variability of gamonts and syzygies of *C. ophoni* shows that the distribution of most of the parameters and morphological indices deviates from the norm, even when the selection exceeds 170 individuals. It is worth recalling that Clopton [25] points out that "the sample size should include at minimum 30-45 individuals so that developmental outliers can be recognized and excluded from the description of normal species variation". Perhaps asymmetry and excess is typical for gamonts and syzygies of *C. ophoni*, and is not manifested so clearly in other species. With *C. ophoni*, instead of isomorphic increase in size (an equal tempo of growth in length and width), which is typical, for example, for many gregarines of the *Stenophora* genus [28], pronounced growth in length and retarded growth in width are characteristic.

The MANOVA results obtained for morphometric parameters and indices also require further comparison with indicators for other gregarine species. It would be interesting to conduct laboratory experiments and to examine a deliberate infection of *H. rufipes* specimens, subjected to different diets, and to perform morphometric analyses of *C. ophoni* gregarines infecting *H. rufipes* collected in different types of ecosystems (from shores of water bodies, different types of forests, steppe ecosystems, agricultural environments). As with populations of other living organisms, for gregarines "the objective is to describe the centroid and

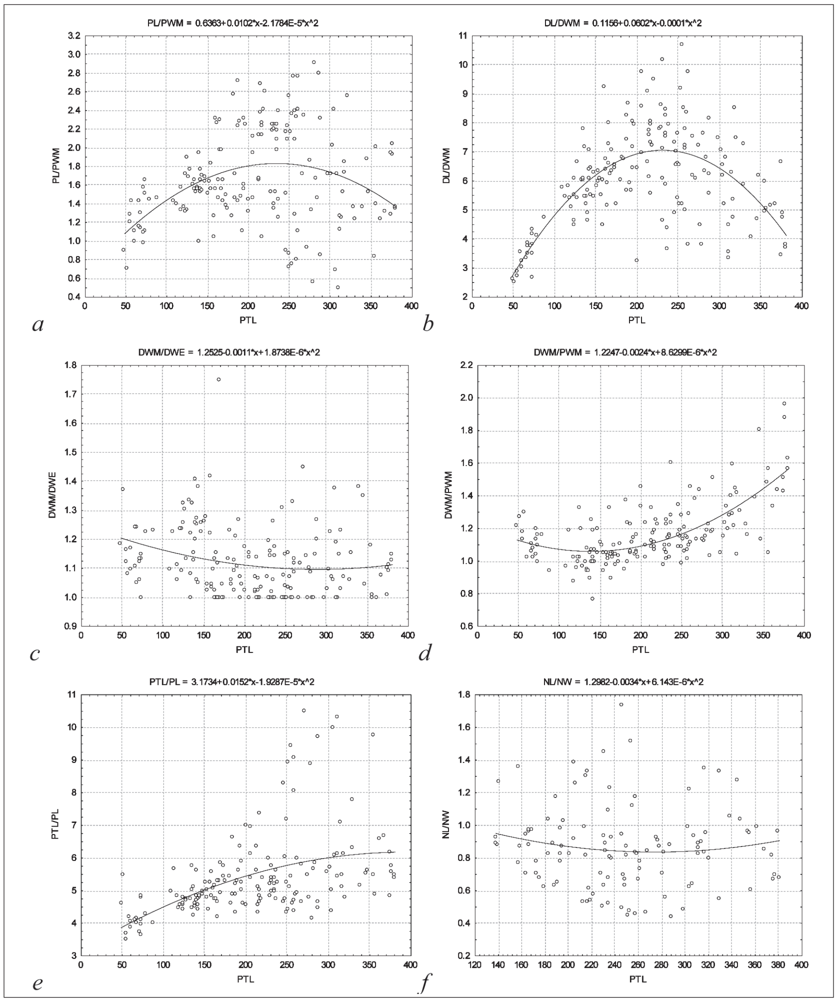

Fig. 5. Ratio of morphometric indices to the total length of *C. ophoni* gamonts: **a** – ratio of PL, length of the protomerite to PWM, maximum width of the protomerite; **b** – ratio of DL, length of the deutomerite to DWM, maximum width of the deutomerite; **c** – ratio of DWM, maximum width of the deutomerite to DWE, width of the deutomerite at the equatorial axis; **d** – ratio of DWM, maximum width of the deutomerite to PWM, maximum width of the protomerite; **e** – ratio of PTL, total length of gamont to PL, length of the protomerite; **f** – ratio of NL, length of the nucleus to NW, width of the nucleus; on the X-axis – PTL, total length of the gamonts in μm; on the Y-axis – index value (n = 177).

normal variation of a population or metapopulation and not to describe an individual" [25].

The influence of gregarines' influence on their hosts has been comparatively well studied for certain species of parasites of dragonflies [29-32], crickets [33,34], desert locust [35], beetles of the Dermestidae [36] and Tenebrionidae families [37], social wasps [38] and pseudoscorpions [39].

There are known studies on the morphological features of ascidian [40], sea cucumber [41] and polychaete [42] gregarines, in which are highlighted the different morphotypes of the parasite cells within one species. The form of different morphotype cells varies considerably. The nucleus of trophozoites with a bulbous head-like region is situated in the anterior part of the cell. The nucleus of trophozoites with a long posterior tail-like region is situated closer to the middle of the cell [40].

Intraspecific variability of gregarines depends on the host species. The various trophic preferences of different grasshopper species significantly affect gregarine morphology. The size of *Leidyana subramanii* in the gut of *Eyprepocnemis alacris alacris* is 5 times greater than the sizes of the individuals of the same species, which are separated from *Poekilocerus pictus* [43].

The influence of pesticides on ground beetles and their gregarine parasites is worthy of separate research and, in this case, *C. ophoni* and *H. rufipes* are convenient objects. Nonetheless, the effect of agricultural pesticides has been assessed only for the morphology of certain ground beetle species [44], and its effect upon gregarine morphology has remained uninvestigated. This is another promising direction of study for *C. ophoni* and *H. rufipes* [10].

The data obtained on the morphological variability of *C. ophoni* needs to be compared with the results for artificially infected individuals of other *Harpalus* species. The results of our unpublished research show that *C. ophoni* was not found among the 5 other species of that genus that inhabit the ecosystems described in this article. Perhaps the reason for this is that the number of studied individuals was quite low, although Sienkiewicz and Lipa [17,18,20] also do not mention Carabidae species as hosts of *C. ophoni*. An analysis of the morphological variability of *C. ophoni* when introduced to other *Harpalus* host species in laboratory experiments would contribute to an understanding of the adaptive radiation [45] of *Clitellocephalus* species, and possibly deepen our understanding of the adaptive mechanism of the given species of gregarine to different ground-beetle host species.

CONCLUSIONS

The coefficient of variation for the majority of linear characteristics of *C. ophoni* gamonts ranged between 28.2 and 71.3%. With 45.4% of the sample, the widest point on the deutomerite was located near the septum (3-20 μm from the septum) and for 48.2% of gamonts, the nucleus was shifted 60-265 μm towards the distal part of the deutomerite. Only 6 of the 15 linear characteristics showed normal distribution; the distribution of the remaining linear characteristics of the gamonts showed a significant positive asymmetry, i.e. the maximums on the histogram of distribution were located in the zone of minimum values. A significant positive excess was characteristic for the width of the protomerite and deutomerite, while for all the individuals studied, the diameter of the protomerite and deutomerite varied considerably less than the length. The distribution of all 18 morphometric indices deviated from the norm. A double-peaked distribution was characteristic for the indices PLAM/PL and DLAM/DL; two maximums were noted in the distribution of the widest points in the protomerite and deutomerite. The lowest CV was expressed for the ratio of gamont length to the length of its deutomerite (3.9%). The ratio of the sizes of the primite and satellite in the syzygies was more constant than the morphometrical indices of the gamonts. The intensity of infection of *H. rufipes* by gregarines influenced only the length of the protomerite and deutomerite and did not show a significant influence on their width or location of the nucleus. On the other hand, the sex of the host did not influence the length of the protomerite and deutomerite, though it did influence their widths, the location of the nucleus and the widest point on the deutomerite. The relationship between the length of the protomerite and the deutomerite and the length of the gamont are best described by linear functions, while their width has a nonlinear function and is most correctly described by parabolic function. The ratio of the morphometric indices to total length of *C. ophoni* gamonts is also best described through nonlinear functions.

Acknowledgments: We are grateful to Dr Sergey V. Lukyantsev (Omsk State University, the Russian Federation) for his valuable help and consultation to this research.

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