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GREGARINE *STYLOCEPHALUS OBLONGATUS* (APICOMPLEXA, EUGREGARINIDA, GREGARINIDAE) FROM THE INTESTINE OF *OPATRUM SABULOSUM* (COLEOPTERA, TENEBRIONIDAE) IN THE RECLAMATION AREAS OF POKROVSKY MINING AND PROCESSING PLANT: THE FIRST REPORT AND MORPHOMETRIC VARIABILITY OF GAMONTS

S. S. Nazimov

Bogdan Khmelnitsky Melitopol State Pedagogical University,
Hetmanska St., 20, Melitopol, 72318 Ukraine
Naukovo Mischechka St., 59, Zaporizhzhia, Zaporizhzhia Region, 69000 Ukraine
E-mail: sergdnipro@gmail.com

S. S. Nazimov (https://orcid.org/0000-0001-6555-0482)

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Gregarine *Stylocephalus oblongatus* (Apicomplexa, Eugregarinida, Gregarinidae) from the Intestine of *Opatrum sabulosum* (Coleoptera, Tenebrionidae) in the Reclamation Areas of Pokrovsky Mining and Processing Plant: the First Report and Morphometric Variability of Gamonts. Nazimov, S. S. — The peculiarities of variability of forms and sizes of the gregarine *Stylocephalus oblongatus* (Hammerschmidt, 1838) Watson, 1916 in the intestines of the *Opatrum sabulosum* (Linnaeus, 1761) from the sites of reclamation of lands disturbed by mining industry in the south of the area of the Steppe Dnipro were investigated. This is a new record for the protozoan fauna of Ukraine. The prevalence of host infection was 7.44 %. To determine the limits of variability, a sample of 71 specimens was used. Gamonts of *S. oblongatus* were analysed by 15 linear characteristics and 18 indices of the ratio of these characteristics. For linear parameters, the average coefficient of variation was 33 %. The minimum values of the coefficient of variation were characteristic for the lengths of the gamont, protomerite and their ratio. The principal component analysis identified 4 principal components, the value of which was greater than 1. Together they explained 73 % of the variability. Cluster analysis identified 4 clusters that can be explained as 2 morphotypes and 2 morphosubtypes. The greatest contribution to the overall variability of *S. oblongatus* gamonts is made by the position of the cell nucleus of the gamont in relation to the septum, the width of the septum and protomerite and the distance from the posterior end of the deuteromerite to the axis of its maximum width. The observed morphological differences can be explained by different degrees of host infection. It is important to compare the obtained information on intraspecific variability of *S. oblongatus* with similar data from natural ecosystems, as well as from agrocenoses and urban populations of *O. sabulosum*.

Key words: *Opatrum sabulosum*, Tenebrionidae, *Stylocephalus oblongatus*, gregarines, Eugregarinida, Apicomplexa, parasitology, entomopathology, protistology, morphometry, morphometric characteristics.

Introduction

Rational use of natural resources and environmental protection are essential conditions for sustainable development of modern states. Studies carried out at the sites of JSC Pokrovsky mining and processing plant (on the outskirts of Pokrov) (Zhukov & Zadorozhnaya, 2016; Yorkina et al., 2018), where the stage of biological reclamation is underway, have proved the optimizing role of the invertebrate fauna in the processes of soil formation on technogenically disturbed lands (Kunah et al., 2019; Nazimov et al., 2019; Zhukov et al., 2021). *Opatrum sabulosum* (Linnaeus, 1761) from the family Tenebrionidae is one of the most abundant species of Coleoptera and at the same time of Insecta class in these areas.

O. sabulosum is an eutrophic xerophile and polyphytophagous (Kabanov, 1977; Parmenter & Macmahon, 1984). It prefers steppe ecosystems with sparse vegetation cover and agrocenoses (Whicker & Tracy, 1987; Grozea et al., 2021). This species is also a frequent inhabitant of settlements (Fattorini, 2011). The range of this species is quite extensive, extending from the south of France and Italy in the west (Carpaneto & Fattorini, 2001; Cepeda-Pizarro et al., 2005) to Kazakhstan in the east (Kabanov & Sedin, 1981). From north to south, *O. sabulosum* occurs from the southern part of Northern Europe to Italy, the southern Caucasus and the mountain ranges of Central Asia (Knor, 1975; Tanyeri et al., 2010). In Ukraine, it is most numerous in Zaporizhzhya, Mykolaiv, Kherson and Odesa regions (Chernej, 2005). The species is a pest of a wide range of crops, but the greatest damage is caused to dormant seedlings and seedlings of vegetable crops and tobacco in spring and June (Kabanov, 1977). At this time, from several tens to hundreds of individuals per 1 m^2 can be found. Significant damage is recorded on sunflower, corn, tomato, cucumber, cabbage, cabbage, soybean, onion, sugar beet, etc.. In the south, it also damages vineyards and tree plantations in well-lit forest belts (Chernej, 2005; Zymaroieva et al., 2021). The species is very dangerous in years of mass reproduction. At the same time, *O. sabulosum* to some extent regulates the amount of ruderal vegetation, as it actively feeds on weeds such as *Polygonum aviculare* L., *Chenopodium album* L. and *Convolvulus arvensis* L. (Rejnhardt, 1936; Rogers et al., 1988). The trophic spectrumof the species also includes plants with bitter sap and pubescent leaf blades (Brygadyrenko & Nazimov, 2015).

The dynamics of *O. sabulosum* populations abundance, like any other species (Koshelev et al., 2021; Bondarev et al., 2022), is affected by a complex of factors, including the influence of pathogens. Perhaps the most numerous group of darkling beetles parasites are gregarines. The subclass Gregarinasina (Apicomplexa) are relatively poorly studied protozoa. They are known to be intestinal pathogens that live in the digestive tract of invertebrates (Clopton, 1999). Little is known about the potential ability of gregarines to regulate host populations. To date, less than 1 % of Coleoptera have been tested for their presence (Sienkiewicz & Lipa, 2009). In Ukraine, the gregarine fauna of darkling beetles has not been studied yet. The gregarine *Stylocephalus oblongatus* (Hammerschmidt, 1838) Watson, 1916 was recorded in the gut of *O. sabulosum* (Geus, 1969). It is a common inhabitant of the intestines of darkling beetles of this genus both in Europe (Spain, France, Greece) and beyond (Israel, Sudan, Laos) (Corbel, 1971; Desportes & Schrével, 2013). The genus *Stylocephalus* includes 37 species, which are known mostly from the Palaearctic and are exclusively parasites of darkling beetles (Clopton, 2000). There is no information about *S. oblongatus* and other gergarines parasitizing *O. sabulosum* in Ukraine.

It was interesting to investigate the population of *O. sabulosum*, in the areas of reclamation of industrially disturbed lands, for their infection with *S. oblongatus*. Also, the aim was to investigate the species composition of gregarines living in the gut of *O. sabulosum* in these areas, and to determine the degree of infection of its population. Clopton states that for species identification of gregarines, the sample should be at least 30 individuals (Clopton, 2012). To date, the morphological variability of *S. oblongatus* is known only in the form of small samples that provide average or even random data. Therefore, it was of additional interest to analyse morphometric parameters in a sample at least twice as large as that reported by Geus (Geus, 1969) or proposed by Clopton (Clopton, 2012) to assess the overall variability of *S. oblongatus* and to determine the limits of normal variability of this species.

Material and Methods

Adults of *O. sabulosum* were collected from May to June 2016 in the areas of JSC "Pokrovsky mining and processing plant", which underwent the process of biological reclamation (namely, the following areas of Zaporizhzhya, Pokrovsky, Northern and Shevchenkivsky open pits) [28–30]. In total, 5 test plots (abbreviated as TP) were established with the following coordinates: TP No. 1 on the territory of Zaporizhzhya quarry (47.658407, 34.145254), TP No. 2 on the territory of Shevchenkivsky quarry (47.722191, 34.218112), TP No. 3 (47.704914, 34.190030), TP No. 4 (47.670440, 34.250973) and TP No. 5 (47.664610, 34.239682) on the territory of Northern quarry. The coordinates of the area were determined using the free web mapping service "Google Maps" from Google. Samples were collected using Barber traps without fixing liquids, as well as by manual collection. The traps were plastic containers with a volume of 0.5 l and a diameter of 75 mm. The soil cover of the sample plots was represented by southern heavy loamy chernozems, and the flora was dominated by hybrids of *Medicago sativa* L. and *Bromus inermis* Leyss. Collected insects were placed in 0.5 and 1 liter glass containers and transported to the laboratory.

The beetles were kept in 250 ml plastic cages with slightly moistened paper on the bottom, 10 individuals in each. During their stay in the laboratory they were not fed. The study of the contents of the digestive system of insects for the presence of intestinal parasites lasted 3 days. Intestines were removed and placed on a slide, where they were dissected in isotonic saline solution to avoid osmotic disruption of the morphological structure of gregarines. The obtained preparations were studied by direct light microscopy using magnifications of ×5, ×10 and ×40. Pathogen cells were photographed using a digital camera with a resolution of 5 megapixels. Measurements of the obtained digital photos were performed using the TpsDig2 1.1 software package.

The species of gregarine was identified according to Geus (Geus, 1969) and Clopton (Clopton, 2012). The indices proposed by Clopton allowed to identify the gregarine species with high accuracy.

To analyse morphometric characteristics, 71 mature gamonts *S. oblongatus* were measured. The measurements were carried out according to the methods proposed by Clopton (Clopton, 2006). This technique generally corresponds to Filipponi's vision of the anatomy of the gregarines of the genus *Stylocephalus* (Filipponi, 1952; Desportes & Schrével, 2013). The following 15 linear characteristics were measured (fig. 1) (1 general, 6 for protomerite and 8 for deuteromerite): total length of the gamont — TL; length of the protomerite — PL; width of the septum between the protomerite and deuteromere — PDSW; width of the protomerite along the equatorial axis — PWE; maximum width of the protomerite — PWM; distance from the anterior end of the protomerite to the axis of its maximum width — PLAM; distance from the posterior end of the protomerite to the axis of its maximum width — PLPM; length of the deuteromerite — DL; distance from the septum between protomerite and deuteromerite to the nucleus — NDS; width of the cell nucleus — NW; length of the cell nucleus — NL; width of deuteromerite along the equatorial axis — DWE; maximum width of deuteromerite — DWM; distance from the front end of deuteromerite to the axis of its maximum width — DLAM; distance from the rear end of deuteromerite to the axis of its maximum width — DLPM. The ratios of the following morphometric parameters were also determined: PL/PWE, PL/PWM, PL/PDSW, PLAM/PL, PLAM/PLPM, PWM/PWE, DL/DWE, DL/DWM, DLAM/DL, DLAM/DLPM, DWM/DWE, NL/NW, DL/PL, DWM/PWM, TL/DL, TL/PL, NDS/NL and DL/NDS. A total of 33 linear characteristics were measured. Below is a schematic graphic representation of the set of morphometric characteristics of *S. oblongatus* gamont, which is a modification of the image developed by Clopton for gregarines of the genus *Xiphocephalus* (Clopton, 2006).

The results were analysed by standard methods of variation statistics. Mean (X), minimum (Min) and maximum (Max) values, standard deviation (± SD), median (Median), coefficient of variation (CV), range of variation (D), skewness (As) and kurtosis (Ex) were calculated.

A principal component analysis (PCA) was performed to extract the first principal component (PC 1), which is an indicator of the body size (quantitative index), as well as other principal components that have the greatest influence on the variability of gregarine cells (Mirzoeva & Zhukov, 2021). In further data analysis, size

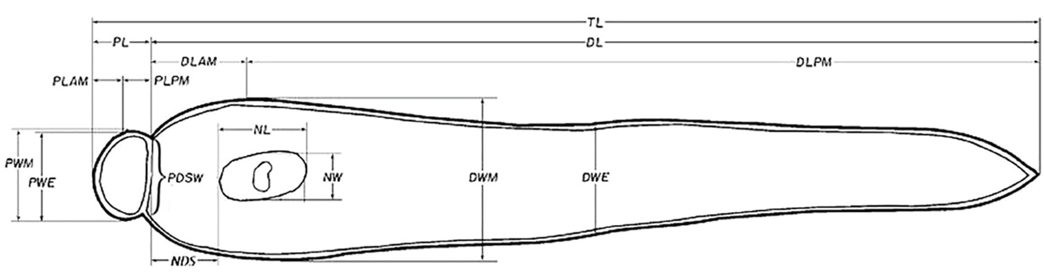

Fig. 1. Measured morphometric characteristics of the *Stylocephalus oblongatus* gamont (picture by Clopton, 2006): TL — total length of the gamont; PL — length of the protomerite; PDSW — width of the septum between the protomerite and deuteromerite; PWE — width of the protomerite along the equatorial axis; PWM — maximum width of the protomerite; PLAM — distance from the anterior end of the protomerite to the axis of its maximum width; PLPM — distance from the posterior end of the protomerite to the axis of its maximum width; DL — length of the deuteromerite; NDS — distance from the partition between protomerite and deuteromerite to the nucleus; NW — width of the cell nucleus; NL — length of the cell nucleus; DWE — width of deuteromerite along the equatorial axis; DWM — maximum width of deuteromerite; DLAM — distance from the front end of deuteromerite to the axis of its maximum width; DLPM — distance from the rear end of deuteromerite to the axis of its maximum width.

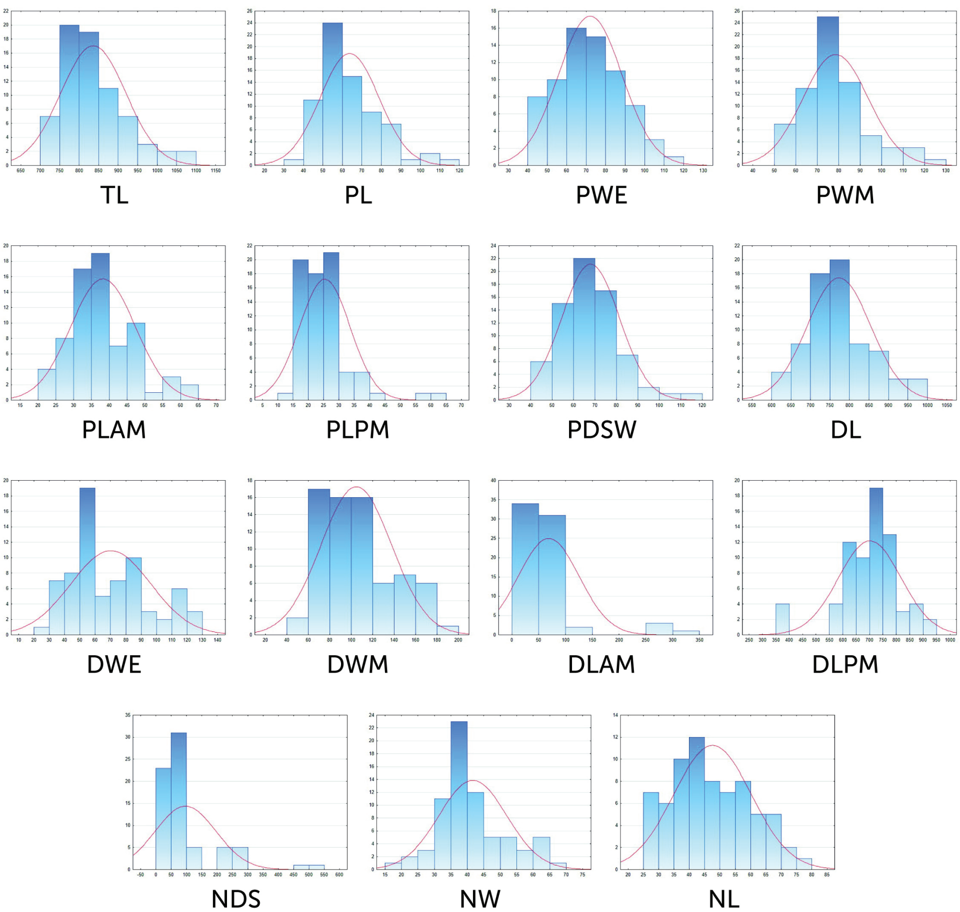

Fig. 2. Histograms of morphometric variability of *Stylocephalus oblongatus* gamonts. The red line indicates the histogram, which is formed by the hypothetical normal distributions.

variability was separated from shape variability. This was done by regressing the scores on the first principal component, which represents size (Budakova et al., 2021). The residuals of the regression models were used in cluster analysis (Zhukov et al., 2019).

Meaningful interpretation of the obtained clusters and determination of the weight of morphometric parameters in their differentiation was performed using discriminant analysis (Yorkina et al., 2020).

Statistical analysis of the data was performed using Statistica 13.3 software package from StatSoft.

Results

Only one gregarine species, *S. oblongatus*, was found in the digestive system of *O. sabulosum* (fig. 5). The degree of infection was low, only 9 individuals out of 121 beetles were infected (infection prevalence 7.44 %). A total of 143 gregarines were found: 72 trophozoites and 71 gamonts. Analysis of histograms of morphometric characters of *S. oblongatus* showed that they are characterised by normal distribution (fig. 2).

At the same time, significant variability of the characters was observed (table 1). The coefficient of variation for most linear parameters averaged 32.4 %. It was the highest for the distance from the septum between protomerite and deuteromerite (septum) to the core (NDS, 102.7 %), and for the distance from the front end of deuteromerite to the axis of its maximum width (DLAM, 83.3 %). The lowest values of the coefficient of variation were noted for the length of deuteromerite (DL, 10.5 %) and the total length of the gamont (TL, 10.8 %). As for the variability of linear characteristics of the core, they varied within the average values (NW, 24.6 % and NL 26.5 %).

Most of the morphometric parameters of hamonts were characterised by significant asymmetry and kurtosis. A slight negative asymmetry was observed for the distance from the posterior end of the deuteromerite to the axis of its maximum width (DLPM). The highest values of positive skewness were noted for the distance from the partition between protomerite and deuteromerite to the core (NDS), the distance from the anterior end of deuteromerite to the axis of its maximum width (DLAM) and the distance from the posterior end of protomerite to the axis of its maximum width (PLPM). Negative kurtosis was typical for the linear parameters NL, PWE, PWM and DWE. The highest value of positive kurtosis was the distance from the front end of deuteromerite to the axis of its maximum width (DLAM). Also relatively high kurtosis values were observed for PLPM and NDS.

Table 1. Descriptive statistics of gamonts morphometric traits in *Stylocephalus oblongatus*

Traits	X ± SD	Mediane	CV	Min	Max	D	Ex	As
TL	770.0 ± 83.2	821.2	10.8	703.2	1096.2	392.9	0.9	0.9
PL	63.4 ± 15.0	59.9	23.7	37.1	111.3	74.1	1.3	1.1
PDSW	67.7 ± 13.4	65.6	19.8	44.2	111.6	67.4	1.1	0.8
PWE	71.8 ± 16.3	72.2	22.7	41.9	113.3	71.5	–0.5	0.3
PWM	78.3 ± 15.2	77.2	19.4	51.9	121.5	69.7	0.6	0.7
PLAM	38.1 ± 9.0	36.7	23.6	22.3	64.1	41.8	0.3	0.7
PLPM	25.2 ± 8.2	24.4	32.6	13.7	62.8	49.1	7.7	2.3
DL	771.9 ± 81.3	763.4	10.5	623.1	989.1	366.0	0.3	0.7
NDS	96.0 ± 98.6	58.6	102.7	18.6	503.8	485.2	5.4	2.3
NW	41.5 ± 10.2	38.2	24.6	19.8	66.0	46.2	0.0	0.7
NL	47.4 ± 12.6	45.1	26.5	28.2	75.6	47.4	–0.9	0.3
DWE	69.9 ± 26.0	60.4	37.2	27.5	127.1	99.6	–0.6	0.6
DWM	104.3±32.8	100.4	31.5	42.9	180.3	137.4	–0.3	0.6
DLAM	68.1 ± 56.8	51.7	83.3	32.9	305.2	272.3	11.2	3.4
DLPM	698.5 ± 116.5	709.0	16.7	350.3	934.6	584.3	2.4	-1.0
PL/PWE	0.9 ± 0.2	0.9	24.0	0.5	1.6	1.1	1.2	1.1
PL/PWM	0.8 ± 0.2	0.8	22.5	0.5	1.5	1.0	3.3	1.6
PL/PDSW	1.0 ± 0.2	0.9	21.4	0.5	1.7	1.2	2.0	0.9
PLAM/PL	0.6 ± 0.1	0.6	9.7	0.4	0.7	0.3	0.5	-0.5
PLAM/PLPM	1.6 ± 0.4	1.5	22.4	0.8	2.3	1.6	–0.4	0.1
PWM/PWE	1.1 ± 0.1	1.1	10.2	0.9	1.4	0.6	1.1	0.9
DL/DWE	12.7 ± 5.3	12.6	41.8	6.0	30.0	24.0	1.2	1.2
DL/DWM	8.1 ± 2.6	7.4	32.4	4.4	15.9	11.4	–0.2	0.6
DLAM/DL	0.1 ± 0.1	0.1	86.8	0.0	0.4	0.4	11.2	3.4
DLAM/DLPM	0.1 ± 0.2	0.1	143.5	0.0	0.8	0.8	13.0	3.8
DWM/DWE	1.6 ± 0.4	1.5	24.6	0.8	2.8	2.0	0.9	1.0
NL/NW	1.2 ± 0.4	1.2	36.8	0.6	2.9	.3	1.9	1.3
DL/PL	12.8 ± 3.2	12.2	25.3	7.3	22.1	14.8	0.1	0.6
DWM/PWM	1.3 ± 0.3	1.3	21.1	0.8	2.0	1.2	0.9	0.8
TL/DL	1.1 ± 0.0	1.1	2.0	1.0	1.1	0.1	–0.1	0.4
TL/PL	13.8 ± 3.2	13.2	23.5	8.3	23.1	14.8	0.1	0.6
NDS/NL	1.9 ± 1.7	1.4	88.1	0.4	7.8	7.4	3.2	2.0
DL/NDS	14.4 ± 9.3	12.6	64.3	1.7	37.7	36.0	0.1	0.8

Table 2. Results of a Principal component analysis of variation of morphological traits and indices of the *Stylocephalus oblongatus* gamonts

Variable	Factor 1	Factor 2	Factor 3	Factor 4
TL	–	–	0.77	0.38
PL	–0.86	–0.40	–	–
PDSW	–0.66	0.41	0.49	–
PWE	–0.71	0.45	0.40	–
PWM	–0.68	0.40	0.44	–0.24
PLAM	–0.74	–0.40	0.28	–0.35
PLPM	–0.77	–0.29	–	0.45
DL	–	0.28	0.75	0.38
NDS	–0.41	–	0.31	0.43
NW	–0.37	0.36	0.39	–0.36
NL	–0.54	–0.29	0.29	–
DWE	–0.80	0.44	–	–
DWM	–0.75	0.56	–	–
DLAM	–0.31	0.59	–0.58	–
DLPM	0.25	–	0.91	–
PL/PWE	–	–0.87	–	–
PL/PWM	–0.33	–0.81	–	–
PL/PDSW	–0.32	–0.84	–0.26	–
PLAM/PL	–	–	–	–0.82
PLAM/PLPM	–	–	0.26	–0.76
PWM/PWE	0.30	–0.27	–	–0.48
DL/DWE	0.80	–	–	–
DL/DWM	0.72	–0.43	–	0.24
DLAM/DL	–0.31	0.55	–0.65	–
DLAM/DLPM	–0.27	0.58	–0.63	–
DWM/DWE	0.39	–	–	–
NL/NW	–	–0.54	–	–
DL/PL	0.82	0.44	–	–
DWM/PWM	–0.49	0.49	–0.31	–
TL/DL	–0.80	–0.51	–	–
TL/PL	0.83	0.44	–	–
NDS/NL	–0.26	–	0.28	0.37
DL/NDS	0.40	–	–	–

For the indices of linear parameters of gamonts, the average coefficient of variation was 38.9 %. Its values were highest for DLAM/DLPM (143.5 %), NDS/NL (88.1 %) and DLAM/DL indices. The lowest mentioned coefficient was for the ratio of the total length of the gamont to the length of deuteromerite (TL/DL, 2 %). Next to the values of TL, DL and PLAM/PL index, this is one of the most stable linear parameters of the gamont in our study.

A slight negative asymmetry was noted for the ratio of the distance from the front end of the protomerite to the axis of its maximum width, to the total length of the protomerite. The highest positive skewness was observed for DLAM/DL and DLAM/DLPM indices. A slight negative kurtosis was typical for the ratio of the total length of the gamont to the length of deuteromerite, as well as for the DL/DWM and PLAM/PLPM indices. High values of positive kurtosis were observed for DLAM/DL and DLAM/DLPM indices.

The principal component analysis (PCA) (table 2) of the morphometric characters of *S. oblongatus* revealed 4 components with values greater than one.

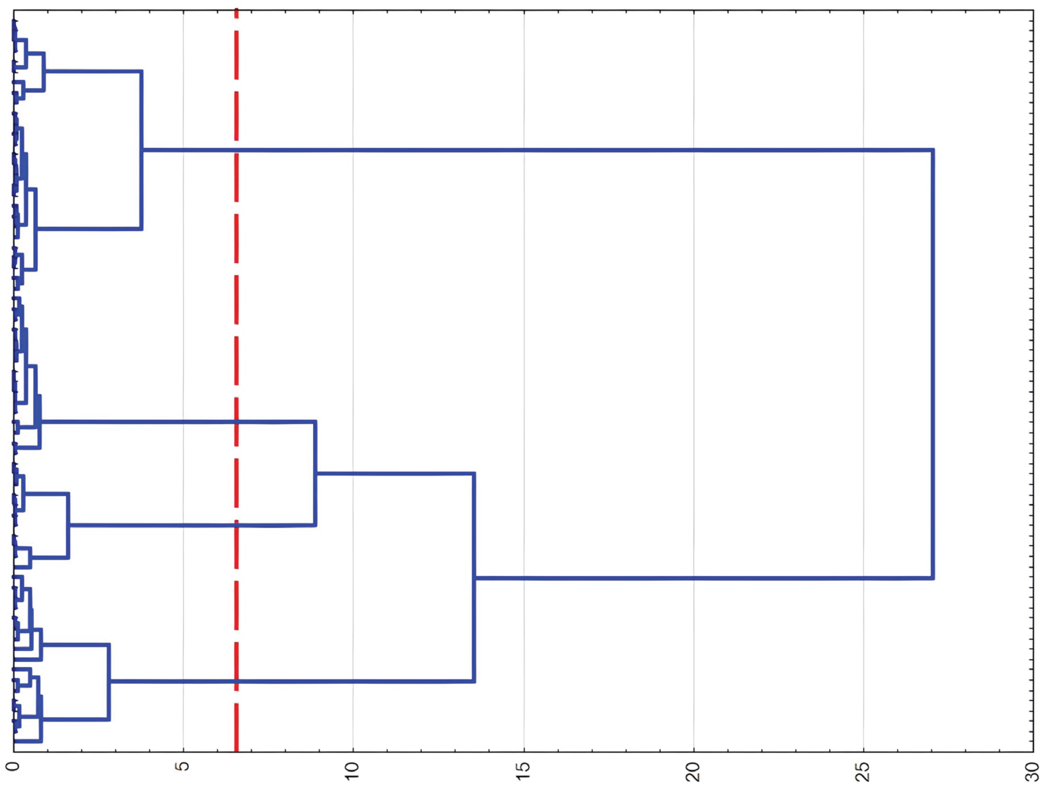

Fig. 3. Cluster analysis of *Stylocephalus oblongatus* gamonts. The linkage distance is 1— *r*, where *r* is a Pearson correlation coefficient, the amalgamation rule is Ward's method. The red line is the level of splitting the set into 4 clusters.

These latent variables are multivariate factors that explain 73 of the variation. Factor 1 (PC1), which explains 29.2 % of the variability, correlates with almost all variables and reflects the size of the studied objects (gamonts). Factor 2 (PC2, determining 19.6 % of the variability) had the highest correlation with DWM and DLAM, as well as with the ratios of DLAM to DL and DLPM. It is also characterised by the highest inverse correlation with the ratio of PL to PWE, PWM and PDSW. Thus, this factor correlates with the maximum width of deuteromerite and the distance from its anterior end to the axis of maximum width. At the same time, a high negative correlation was observed for the ratio of protomerite length to its width along the equatorial axis and to the width of the partition between the proto- and deuteromerite.

Thus, this factor determines to a greater extent the shape of the cell (width of deuterite, ratio of maximum width to length). Factor 3 (PC3, determines 14.6 % of variability) was most correlated with DLPM, as well as with TL and DL. At the same time, it was characterised by a high inverse correlation with DLAM, as well as with the ratio of DLAM to DL and DLPM. Thus, this factor is most correlated with the distance from the posterior end of deuteromerite to the axis of its maximum width, as well as with the total length. The factor also had a high negative correlation with the distance from the anterior end of deuteromerite to the axis of its maximum width, as well as with the ratio of this value to the total length of deuteromerite and the distance from the posterior end of deuteromerite to the axis of its maximum width. Thus, this factor also determines the shape of the cell, namely, it affects the ratio of the axis of maximum width of deuteromerite to its length. Factor 4 (PC4, determines 9.4 % of variability) was most correlated with PLPM and NDS. At the same time it is characterised by high inverse correlation for PLAM to PL and PLPM ratios. Thus, the factor correlates most of all with the distance from the posterior end of protomerite to the axis of its maximum width, as well as with the distance from the partition between proto- and deuteromerite to the nucleus. At the same time, there is a negative correlation of the ratio of the distance from the anterior end of the protomerite to the axis of its maximum width, to the total length of the protomerite and to the distance from the posterior end of the protomerite to the axis of its maximum width. Thus, this factor determines the location of the nucleus in relation to the septum, and at the same time determines the shape of deuteromerite by influencing the location of its ”thickest place”.

The residuals of the principal component analysis are morphometric characteristics from which size was extracted. Cluster analysis of these residuals allowed to divide the gregarine gamonts into four clusters (fig. 3).

To interpret these clusters, discriminant analysis was performed with the residuals of regression models as predictors (table 3). The results of the analysis show that the obtained clusters are well differentiated using canonical variables (fig. 4).

Table 3. Factor structure matrix (the standardised coefficients of canonical variables at $p < 0.05$ are presented)

Traits	PC1	PC2	PC3
TL	0.52	0.70	–
PL	–	0.29	–
PDSW	–	0.56	–
PWE	–	0.34	0.29
PWM	–	0.39	–
PLAM	–	0.28	–
PLPM	–	–	–
DL	0.53	0.69	–
NDS	0.85	–0.32	–
NW	–	0.30	–
NL	0.28	0.33	0.33
DWE	–	–	–
DWM	–	–	–
DLAM	–	–	–0.28
DLPM	0.52	0.60	–
PL/PWE	–	–	–0.34
PL/PWM	–	–	–0.25
PL/PDSW	–	-0.24	–0.26
PLAM/PL	–	–	–
PLAM/PLPM	–	–	–
PWM/PWE	–	–	–0.28
DL/DWE	0.26	–	-0.27
DL/DWM	–	–	–
DLAM/DL	–	–0.26	–0.27
DLAM/DLPM	–	–	–0.26
DWM/DWE	–	–	–
NL/NW	–	–	–
DL/PL	0.39	0.24	–
DWM/PWM	–	–	–
TL/DL	–0.34	–	–
TL/PL	0.39	0.24	–
NDS/NL	0.82	–0.41	–
DL/NDS	–0.49	0.27	–

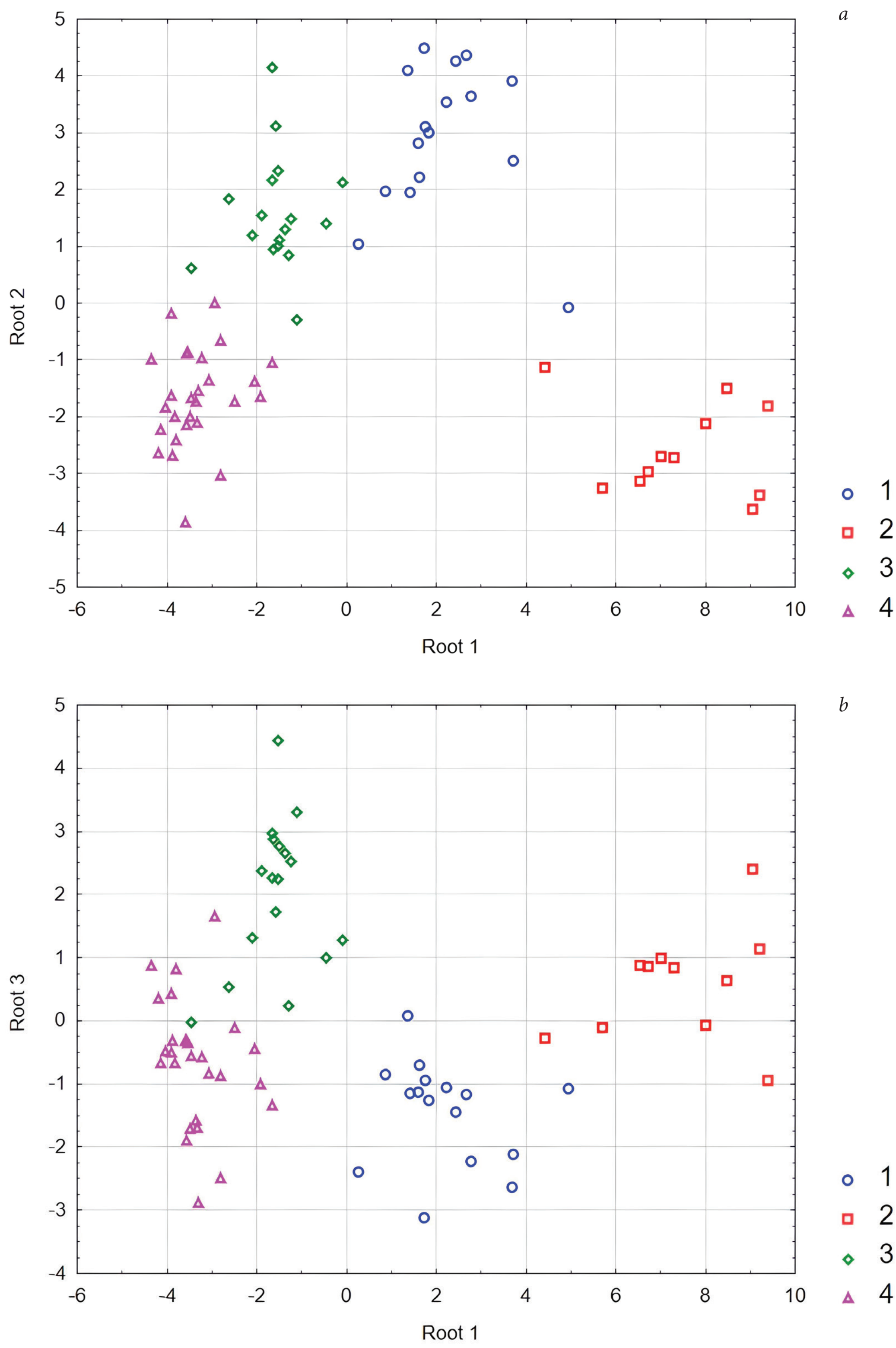

Fig. 4. Discriminant analysis results of *Stylocephalus oblongatus* gamonts by morphometric traits. 1, 2, 3, 4 are clusters. *a* — for roots 1 and 2; *b* — for roots 1 and 3.

The canonical variable 1 shows the highest sensitivity to the total length of the gamont, the length of the deuteromerite, the distance from the septum to the nucleus, and the distance from the posterior end of the deuteromerite to the axis of its maximum width, as well as the index of the ratio of the distance from the septum to the nucleus to the length of the nucleus. Accordingly, cluster 2 is characterised by the location of the cell nucleus closer to the middle of the deuteromerite, in contrast to clusters 1, 3 and 4.

Canonical variable 2, in addition to sensitivity to the total length of the gamont, the length of the deuteromerite, and the distance from the posterior end of the deuteromerite to the axis of its maximum width, shows sensitivity to the width of the septum between protomerite and deuteromerite.

Canonical variable 3 is most sensitive to the width of protomerite along the equatorial axis, the length of the nucleus and the index of the ratio of the total length of protomerite to the axis of its maximum width. Thus, cluster 3 differs from the others by a slightly larger width of protomerite (on average by 14 %), as well as by a larger width of the septum between protomerite and deuteromerite. At the same time, clusters 1 and 4 are generally quite similar, except that gregarines from cluster 1 are larger.

Thus, two morphotypes (cluster 2 and all other clusters together), and 2 morpho-subtypes (cluster 3 and clusters 1 and 4 together) were identified among the *S. oblongatus* gamonts.

Discussion

This is the first finding of *S. oblongatus* in the Steppe Podniprovia and Ukraine in general. In the literature, the issue of parasitism of gregarines, both in darkling beetles (Clopton, 2000; Clopton, 2006) and specifically in *O. sabulosum* (Geus, 1969) was raised. Unfortunately, for the last few decades such data are absent and authors mostly refer to older studies. A pleasant exception is the work of Richard Clopton, which mentions the genus *Stylocephalus*, representatives of which, to date, have been recorded exclusively in the darkling beetles (Clopton, 2006; Clopton, 2012).

The purpose of such studies is to name the average values of linear parameters and describe the centroid (point of intersection of medians) of the population of a certain species (Clopton, 2012). Our data showed that most of the linear parameters of *S. oblongatus* are marked by significant variability, high asymmetry and kurtosis, and form several clearly distinguished morphological groups. This situation is observed even for large samples (more than 70 specimens, against the minimum of 30 specimens proposed by Clopton (Clopton, 2006). Also, it should be noted that gamonts of *S. oblongatus* specimens from the Pokrovsky GOK reclamation sites are characterised by smaller average and maximum width of deuteromerite than in previously described samples (Geus, 1969). In the case of all other parameters there is a significant similarity of linear characteristics. Perhaps this is due to the fact that gregarine cells do not grow gradually, but first in length, and only then begin to increase in diameter. To date, the gregarine fauna has been well described for many insect taxa, primarily dragonflies (Canales-Lazcano et al., 2005; Locklin & Vodopich, 2010), orthopterans (Harry, 1970; Zuk, 1987 a, b), ground beetles (Sienkiewicz & Lipa, 2009) and darkling beetles (Clopton, 2006). The morphology of gregarines is best described for marine and oceanic fauna, in particular ascidians. Gregarines parasitizing these animals are characterised by considerable intraspecific variability (Rueckert et al., 2011). Gregarines *Clitellocephalus ophoni* from the intestine of *Harpalus rufipes* collected in Poland are on average

Fig. 5. Gamonts of *Stylocephalus oblongatus* from the intestine of *Opatrum sabulosum*.

shorter and wider than individuals of the same species from France (Sienkiewicz & Lipa, 2009). Also, there is evidence that the size of the gregarine *Leidyana subramanii*, depending on the host, can vary by 5 times (Hussain et al., 2013).

It would be interesting to compare our results with similar data from natural ecosystems. The influence of heavy metals and pesticides on the intraspecific variability of gregarines has not been studied. To do this, it is necessary to compare the morphometric parameters of gregarines from natural ecosystems, agrocenoses and urban cenoses. The question of the relationship between the intraspecific variability of gregarine species and the degree of host infection remains open.

Gregarines are common parasites of most arthropods, but their impact on the host is not fully understood. It is believed that their pathological impact is not high, but there is information that under certain conditions gregarines can cause significant physiological disorders in the host (Wolz et al., 2022). This is another important issue that requires further research. As mentioned earlier, *O. sabulosum* is a widespread pest of many crops (Chernej, 2005), and therefore the study of the effect of *S. oblongatus* on the host is interesting from the point of view of considering it as a biological control agent (Sienkiewicz & Lipa, 2009).

Conclusion

S. oblongatus was the only gregarine species parasitizing in the intestine of *O. sabulosum* in the reclamation areas of Pokrovske mining and processing plant. A low degree of gregarine infection of the *O. sabulosum* population was observed. The infection prevalence was 7.44 %. During the research, only gamonts and trophozoites of *S. oblongatus* were detected. This apicomplexan species was registered in Ukraine for the first time.

The coefficient of variation of morphometric characters in the studied gamonts was, on average, 32.4 % for linear characteristics and 38.9 % for their ratios. The coefficient of variation was the lowest for the total length of the gamont (10.8 %), deuteromerite (10.5 %)

and their ratio (2 %). In all cases, the diameter of deuteromerite varied significantly more than its length.

Morphometric characteristics and indices allowed to distinguish two morphotypes and two more morpho-subtypes of *S. oblongatus*. Each of these groups is characterised by specific features of shape and size. To the greatest extent, the differences are due to the variability in the location of the cell nucleus, the distance from the posterior end of the deuteromerite to the axis of its maximum width, the width of the protomerite and the width of the septum between the protomerite and deuteromerite. The adaptive significance of the variability in these traits is unclear. The degree of host infection may be the leading factor determining variability.

In addition to expanding the list of protozoan species of Ukraine, the work provides an opportunity to assess the limits of intraspecific variability of *S. oblongatus* in the intestine of *O. sabulosum*, as well as the degree of infection of the host population, in area of the Steppe Dnipro, which have undergone significant anthropogenic impact, and to establish priorities for further research.

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