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Measurement of the Exterior of Bees: Comparison of Methods †

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Abstract: A comparison of the two measurement methods using nonparametric criteria has made it possible to establish that the measurement results of some exterior features obtained through one method are significantly different from the results obtained using another measurement method. Measurement using Altami Studio 3.4.0 allows getting results with less trait variability between repeated measurements of one operator and between operators compared to MBS-9. With the help of the analysis of variance, it was established that two factors influence the measurement results: the measurement method and the operator performing the measurement, as well as the interaction of these two factors. Repeated measurements using the software will allow identifying operators prone to less variability of results, as well as controlling the accuracy of measurements.

Keywords: exterior; honey bee; measurement method; repeatability; reproducibility

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1. Introduction

The founder of the scientific approach to the study of body parts of bees is Kozhevnikov [1]. He used a microscope, an ocular micrometer, and a drawing machine. Kozhevnikov's student and his follower Alpatov used an eyepiece micrometer for measurements, which was calibrated employing a micrometer lens [2]. A 6x or 10x magnifier with divisions [3], a projector, and a ruler were used abroad to measure exterior features [3,4]. Not so long ago various systems in which measurements are taken on a computer have come into use [5–7]. When choosing optics for measurements, one must take into account its application and quality. It is known, for example, that microscope objectives are divided into groups according to the degree of perfection of aberration correction [8]. Alpatov, Tjunin [9] pointed out the importance of spherical aberration of the microscope objective. The optical system of a flatbed scanner is also capable of distortion. Therefore, in the case of using it as a source for obtaining a digital image, it is necessary to determine in advance the location of the object. For example, one can select its central part [10] and/or test an existing device to select areas for the optimal placing of micro specimens [11].

After obtaining a digital image of an object, it is measured in software designed for working with images with sufficient accuracy [12] and the required functions. Before starting work, the program is calibrated according to the image of the object obtained under the same conditions as the images of body parts of bees. Calibration of the software or device is the basis for obtaining reliable results in studies related to dimensions, and its procedure depends on the measurement method used, and the choice of the method is not of great importance [12].

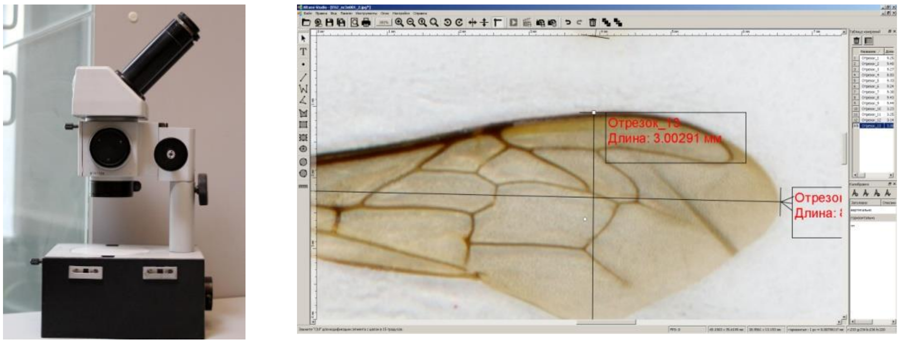
There are some scientific publications in which the authors compare methods for measuring the length of segments of the "media" vein: "A" (YZ) and "B" (XY) on the basis of which the "cubital index" is calculated. So, Lomaev and colleagues compared the measurement of these signs with the MBS-9 stereo microscope with automatic measurement in the program they developed after receiving the image from the scanner [13,14].

We faced the following tasks:

- to determine which of the two methods will give the smallest coefficient of variation between measurements made by one operator and between operators;
- to determine the repeatability and reproducibility of the methods used [15];
- to determine the reliability of the influence of two factors: the measurement method and the operators who performed measurements, as well as their interaction on the measurement results;
- to determine the advantages of one method over another.

2. Materials and Methods

The following hardware and software were used: MBS-9 stereoscopic microscope (Figure 1a) supplied with an eyepiece with dioptre adjustment with a snap-in scale (the scale division value is 0.1 mm); flatbed scanner Epson Perfection V600 Photo (with a resolution of 3200 dpi in 24-bit colour mode); Altami Studio 3.4.0® software (has a certificate of conformity) were used to measure images of objects (Figure 1b); the Altami Studio 3.4.0® was calibrated before the measurement.



(**a**) (**b**)

Figure 1. (**a**) The microscope MBS-9; (**b**) Working window of the Altami Studio® program.

Biometric processing was performed: in MS Office Excel® software using the "Data Analysis" package—"Descriptive Statistics"; the coefficients of variation were additionally calculated [16]; analysis of repeatability and reproducibility, calculation of U (Mann-Whitney) and T (Wilcoxon) criteria were performed using Statistica 13.0® software. All measurements were performed according to the modified and supplemented method which had been worked out by Alpatov [17].

Four slides were prepared with body parts of 30 bees from one colony placed on them. Then four operators measured the sample, every three times using MBS-9. Further, the specimens were placed in turn on the scanner glass in a certain place [11] and their digital image was obtained. Then the same four operators measured them three times using Altami Studio 3.4.0®. The linear measurements made in the scale divisions were then converted to millimetres dividing the wing length by 10, and the rest of the measured data of the exterior features by 20.

3. Results

For each of the three dimensions of every operator taken separately, averages (*M*) were obtained. Further, these averages were compared using non-parametric criteria, the results are presented in Table 1.

Table 1. Indication of differences between two measurement methods using non-parametric criteria Mann-Whitney (*U*) and Wilcoxon (*T*) ($n_{MBS\text{-}9} = n_{Altami\ Studio} = 12$).

The Measured Characteristic	Measurement Method	*M* ± *m*	*U*	*T*
L_x [1], mm	MBS-9	6.79 ± 0.031	24.0 [2]	0.0 [4]
	Altami Studio	6.71 ± 0.020		
L_{kr}, mm	MBS-9	9.25 ± 0.067	64.0	25.0
	Altami Studio	9.30 ± 0.006		
W_{kr}, mm	MBS-9	3.22 ± 0.017	52.5	32.5
	Altami Studio	3.21 ± 0.013		
L_{XY}, mm	MBS-9	0.52 ± 0.005	66.0	38.5
	Altami Studio	0.53 ± 0.006		
L_{YZ}, mm	MBS-9	0.26 ± 0.001	19.0 [2]	4.5 [4]
	Altami Studio	0.28 ± 0.009		
L_t, mm	MBS-9	2.27 ± 0.011	28.0 [1]	12.0 [2]
	Altami Studio	2.25 ± 0.009		
W_t, mm	MBS-9	4.85 ± 0.010	1.0 [2]	0.0 [4]
	Altami Studio	4.80 ± 0.006		
L_{chl}, mm	MBS-9	2.19 ± 0.009	4.5 [2]	1.0 [4]
	Altami Studio	2.15 ± 0.004		
W_{chl}, mm	MBS-9	1.20 ± 0.011	38.5	19.5
	Altami Studio	1.18 ± 0.007		

[1]—MBS-9—microscope biological stereoscopic; L_x—the length of the proboscis, mm; L_{kr}—length of the right forewing, mm; W_{kr}—width of the right forewing, mm; L_{XY}—the length of the segment "XY" of the "media" vein on the right forewing, mm; L_{YZ}—the length of the segment "YZ" of the "media" vein on the right forewing, mm; L_t—length of the third tergite (longitudinal distance), mm; Wt—the conventional (transverse) width of the third tergite, mm; L_{chl}—length of the first segment of the right hind tarsus, mm; W_{chl}—width of the first segment of the right hind tarsus, mm.

Two opposite non-directional hypotheses were tested: H_0 (or zero) M_1 does not differ from M_2 and H_1 (alternative or experimental) M_1 differs from M_2. The obtained empirical values of the criteria were compared with the critical values found in the tables (according to Gravetter, Wallnau [18]), and denoted by numbers from 1 to 4 (superscript). As our hypothesis is non-directional, critical values were taken for two-sided distribution [19]. At the same time, one adhered to the following rule [20]: if the empirical value of U or T is equal to or less than the critical value of the same criterion indicated in the table for $p < 0.05$, then H_0 is rejected, but H_1 cannot yet be accepted with confidence; if the empirical value of U or T is equal to or less than the critical value of the same criterion indicated in the table for $p < 0.01$, then H_0 is rejected and H_1 is accepted. For the Mann-Whitney criterion (U), two levels of significance were determined (1—$p < 0.05$, 2—$p < 0.01$), and for the Wilcoxon criterion (T) four levels of significance were determined (1—$p < 0.1$, 2—$p < 0.05$, 3—$p < 0.02$, 4—$p < 0.01$). The coefficients of variation were calculated: between the averages of three measurements of each of the four operators and between the operators by each method.

A variance analysis of the repeatability and reproducibility of the process of measuring the exterior characteristics of honey bees was carried out. Its results are presented in Table 2, as well as the final graphs of the repeatability and reproducibility of the measurement process for each measured exterior sign (Figure 2A,B).

Table 2. Components of the measurement process variance (S^2) (as a percentage of the total variability) and the confidence index of the influence * (F) of factors.

Statistical Indicator	L_x	L_{kr}	W_{kr}	L_{XY}	L_{YZ}	L_t	W_t	L_{chl}	W_{chl}
$S^2_{repeatability}$	9.4	16.3	16.2	34.7	4.7	57.8	17.0	14.3	8.3
$S^2_{reproducibility}$	43.1	8.7	0.0	0.0	8.0	30.6	0.0	12.0	0.0
$F_{reproducibility}$	29.0 [3]	18.0 [3]	11.4 [3]	2.9	39.9 [3]	3.4 [1]	0.3	7.4 [2]	11.5 [3]
$S^2_{\text{the interaction operator-method}}$	1.3	75.0	83.8	65.3	43.3	0.0	18.3	6.5	88.6
$F_{\text{the interaction operator-method}}$	1.4	14.8 [3]	16.5 [3]	6.7 [2]	29.4 [3]	0.3	4.2 [1]	2.4	32.8 [3]
$S^2_{\text{between methods}}$	46.2	0.0	0.0	0.0	43.9	11.6	64.7	67.2	3.1
$F_{\text{between methods}}$	60.6 [3]	6.5 [1]	8.4 [1]	1.0	144.4 [3]	2.7	49.9 [3]	58.9 [3]	37.3 [3]

*—determined by the Table IX [20].

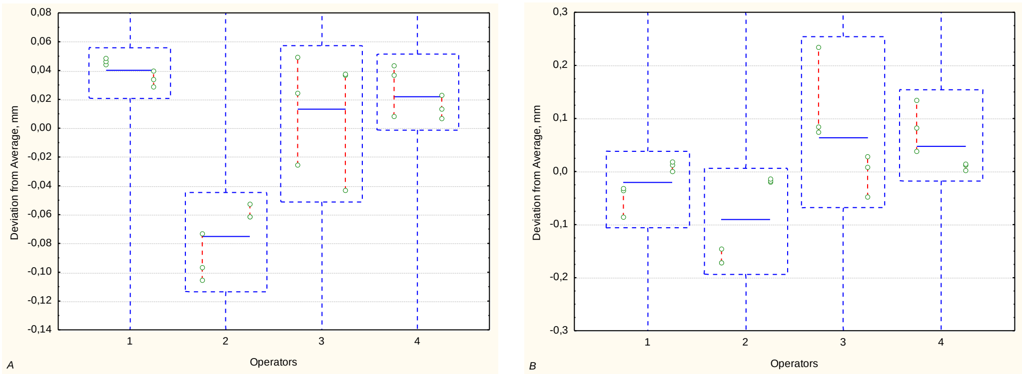


Figure 2. Final graphs of measurement of repeatability and reproducibility: (**A**)—L_x, (**B**)—L_{kr}.

Explanations for Figure 2A,B. Three points connected by a vertical dashed line are the deviations of measurements from the average value of the measurement for the corresponding method, the line on the left indicates the measurement performed on MBS-9, and the right line indicates the measurement carried out on the computer using Altami Studio 3.4.0® software. The length of these lines determines the measurement limit by the same method, the longer they are, the greater the limit and the more inaccuracy is. Measurements taken by one operator are enclosed in a dotted frame. The average deviation of measurements for each operator is represented by a horizontal solid line in each frame. Frame height is an indicator of measurement variability.

4. Discussion

The parallel use of the Mann-Whitney (U) test for independent samples and Wilcoxon (T) test for dependent (Table 1) is justified by the fact that the measurement results obtained by different methods can, on the one hand, be considered independent, and on the other hand, they can be considered dependent on each other because they have been received by the same operators. The use of the U criterion in dependent samples, while considering them as independent, is justified in cases where the connections between groups are weak and the differences between them are strong [21]. Since the critical values for criterion U are given for two significance levels, in the given investigation its power turned out to be equal to the power of criterion T. There is a slight difference in the average values of the measured attributes obtained by the two measurement methods, which amounts to a few hundredths of a millimetre.

Analysis of the coefficient of variation (C_V) has shown that:

(1) comparing the measurements received by one operator, C_Vs were mainly less when measuring on a computer using Altami Studio 3.4.0® than when measuring on MBS-9;

(2) *Cv*s between the average measurements of all operators using Altami Studio 3.4.0® were also mostly less than when measuring on MBS-9;

In our opinion, the differences revealed between measurements using nonparametric criteria may result from lower coefficients of variation of the measurement method using Altami Studio 3.4.0 software compared to measurements using MBS-9.

According to Table 2, the measurement process is significantly influenced by the measurement method, the operator, and operator-method interaction. The influence of gradations of the "Operator" factor turned out to be statistically significant in seven out of nine cases, with five cases being at the third significance level. The results obtained indicate that the "human" factor has a significant effect when measuring exterior features. The influence of the gradations of the factor "Method" turned out to be statistically significant in seven cases as well, and there are five cases found at the third level of significance, which can be explained by the utility of this or that method when measuring a particular attribute. The interaction between the gradations of the factors "Operator" and "Method" turned out to be statistically significant in six cases, with four cases being at the third level of significance which is explained by a combination of the "human" factor and the utility of a measurement method or the tendency of certain operators to carry out measurements more accurately using one or another method. The variability of the measurement result of the exterior sign "Proboscis Length" is significantly affected by the measurement method and the operator using it. This, first of all, may happen because the proboscis of bees is often bent, that is why its measurement on MBS-9 is approximated, whereas the measurement using software allows observing it with maximum accuracy. When measuring the length of an object using MBS-9, the measurement cut-off point often falls between the graduations of the scale. So, when counting the sections, they are rounded off to the nearest whole number of sections and, accordingly, the smaller the measurement object, the more this rounding will influence the result. The measurement carried out using software eliminates the necessity for rounding and the possibility of displacement of a specimen during the measurement process.

Analyzing the obtained graphs (Figure 2A,B), we can conclude that the minimum deviation from the average value by the measurement method in the majority of measurement results was given by operators 1 and 4, which was indicated in smaller sizes of their frames compared to operators 2 and 3. Accordingly, Operators 1 and 4 are more preferable to participate in important measurements.

5. Conclusions

Measurement in software has a number of advantages over measurement on MBS-9:

- the ability to measure the curved proboscis correctly;
- scanned body parts of bees are conveniently stored in electronic form, exchanged by e-mail with other researchers, as well as used for training employees within the organization to obtain identical results;
- less labor and time costs;
- while measuring in the program does not require any rounding;
- the operators who took part in the experiment agreed that it is more convenient to measure on a computer than on MBS-9.

The disadvantage of Altami Studio 3.4.0® is the inability to determine the angle or sign (+, −, 0) of discoidal displacement. The use of software and repeated measurements carried out with it will allow identifying operators prone to less variability of results, as well as control the accuracy of measurements. The use of digital images of objects will allow more effective training in the measurement methodology and will make it possible to exchange them via e-mail for cross-validations. Such validations are recommended not only between laboratories involved in morphometry but also between experienced and inexperienced operators [12].

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