biology and life sciences forum

Proceeding Paper

Measurement of the Exterior of Bees: Comparison of Methods †

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† Presented at the 2nd International Electronic Conference on Diversity (IECD 2022)—New Insights into the Biodiversity of Plants, Animals and Microbes, 1–15 March 2022; Available online: https://iecd2022.sciforum.net/.

Abstract: In a comparison of two measurement methods using nonparametric criteria, we established that the measurement results for some exterior features obtained through one method were significantly different from the results obtained using another measurement method. Measurement using Altami Studio 3.4.0® (OOO"Altami", Saint Petersburg, Russia) allows results to be generated with less trait variability between repeated measurements by one operator, as well as between operators, compared to MBS-9 (LOMO, St. Petersburg, Russia). With the help of an analysis of variance, we 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 operators to be identified who are prone to less variability of results, as well as control the accuracy of measurements.

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

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Citation: Berezin, A.; Mitrofanov, D. Measurement of the Exterior of Bees: Comparison of Methods. *Biol. Life Sci. Forum* **2022**, *15*, 27. https://doi.org/10.3390/IECD2022-12373

Academic Editor: Matthieu Chauvat

Published: 14 March 2022

1. Introduction

The founder of the scientific approach to the study of the 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 by employing a micrometer lens [2]. A 6× or 10× magnifier with divisions [3], a projector, and a ruler were used elsewhere in the world to measure exterior features [3,4]. Recently, 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 the 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 [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, when using it as a source to obtain a digital image, it is necessary to determine the location of the object in advance. For example, one can select its central part [10] and/or test an existing device to select areas for the optimal placement of micro specimens [11].

After obtaining a digital image of an object, it is measured in software designed to work with images with sufficient accuracy [12] and the required functions. Before starting the work, the program is calibrated according to an 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; the related procedure depends on the measurement method used, with the choice of method of little importance [12].

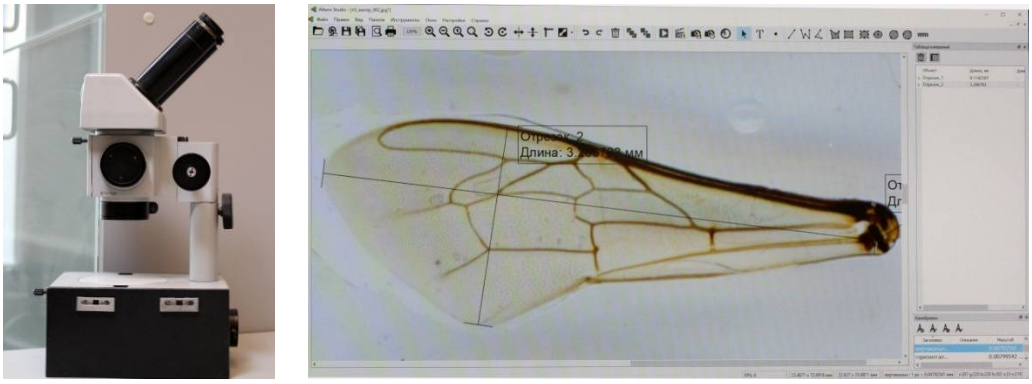
There are some scientific publications in which the authors compare methods for measuring the length of segments of the "media" vein—"A" (YZ) and "B" (XY)—on the basis of which the "cubital index" is calculated. Lomaev and colleagues compared the measurement of these signs with the MBS-9 stereo microscope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

We set out with the following aims:

- 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 in [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 in affecting the measurement results;
- to determine the advantages of one method over another.

2. Materials and Methods

The following hardware and software were used: an MBS-9 stereoscopic microscope (Figure 1a) supplied with an eyepiece with diopter adjustment and with a snap-in scale (the scale division value is 0.1 mm); an Epson Perfection V600 Photo (Seiko Epson Corporation, Jawa Barat, Indonesia) flatbed scanner (with a resolution of 3200 dpi in 24-bit color mode); Altami Studio 3.4.0® software (has a certificate of conformity), which was used to measure images of objects (Figure 1b), having been calibrated before the measurements were taken.



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

Figure 1. (**a**) MBS-9 microscope; (**b**) work screen 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 also calculated [16]. Our analysis of repeatability and reproducibility, and calculation of *U* (Mann–Whitney) and *T* (Wilcoxon) criteria, were performed using Statistica 13.0® software (StatSoft, Moscow, Russia). All measurements were performed according to a modified and supplemented method developed by Alpatov [17].

Four slides were loaded with body parts of 30 bees from one colony. Then, four operators measured the samples three times using MBS-9. The specimens were placed one at a time on the scanner glass in a certain place [11] and their digital images were obtained. Next, 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 millimeters, dividing the wing length by 10 and the rest of the measured data of the exterior features by 20.

3. Results

Averages (M) were obtained from the three measurements taken (by one operator), and those averages were compared using non-parametric criteria. The results are presented in Table 1.

Table 1. Differences between two measurement methods, assessed using non-parametric criteria with the Mann–Whitney (U) and Wilcoxon (T) tests ($n_{MBS\text{-}9} = n_{Altami\ Studio} = 12$).

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

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

Two opposite non-directional hypotheses were tested: H_0 (or zero) M_1 does not differ from M_2 but H_1 (alternative or experimental) M_1 differs from M_2. The obtained empirical values for the criteria were compared with the critical values found in the tables (according to Gravetter and Wallnau [18]), and denoted by numbers from 1 to 4 (superscript). As our hypotheses were non-directional, critical values were taken for a two-sided distribution [19]. 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 for each of the four operators, and between the operators for 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 graphical representations 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]
$S^2_{interaction\ operator\ method}$	1.3	75.0	83.8	65.3	43.3	0.0	18.3	6.5	88.6
$F_{interaction\ operator\ method}$	1.4	14.8 [3]	16.5 [3]	6.7 [2]	29.4 [3]	0.3	4.2 [1]	2.4	32.8 [3]
$S^2_{between\ methods}$	46.2	0.0	0.0	0.0	43.9	11.6	64.7	67.2	3.1
$F_{between\ methods}$	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 Table IX in [16]. [1, 2, 3]—the confidence is at the first (0.95), second (0.99) and third (0.999) significance levels, respectively.

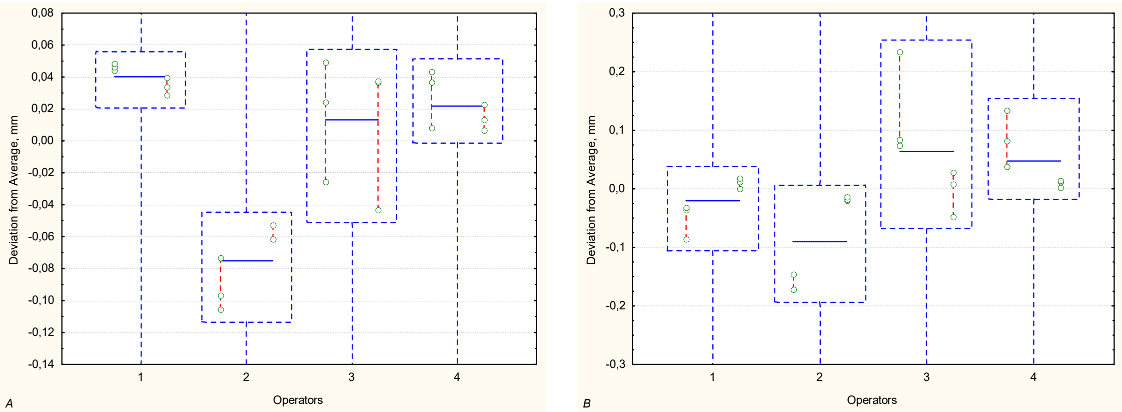


Figure 2. Final graphs of measurements of repeatability and reproducibility: (**A**)—L_x, (**B**)—L_{kr}.

In Figure 2, the three points (green) connected by a vertical dashed line (red) 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. Measurements taken by one operator are enclosed in a dotted frame (in blue). The average deviation of measurements for each operator is represented by a horizontal solid line (in blue) in each frame. The frame height is an indicator of the measurement variability.

4. Discussion

The parallel use of the Mann–Whitney (U) test for independent samples and Wilcoxon (T) test for dependent samples (Table 1) was justified by the fact that the measurement results obtained by different methods could, on the one hand, be considered independent, and on the other hand, could be considered dependent on each other because they were 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 the U criterion are given for two significance levels, in the given investigation its power turned out to be equal to the power of criterion T. There was a slight difference in the average values of the measured attributes obtained by the two measurement methods, which amounted to a few hundredths of a millimeter.

Analysis of the coefficient of variation (C_V) showed that:

(1) when comparing the measurements produced by one operator, in most cases, C_Vs were less when measured on a computer using Altami Studio 3.4.0® than when measured with MBS-9;

(2) C_Vs 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 when using Altami Studio 3.4.0® software compared to when taking 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 "method" factor turned out to be statistically significant in seven cases as well, with 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 "operator" and "method" factors turned out to be statistically significant in six cases, with four cases being at the third level of significance, which can be explained by a combination of the "human" factor and the utility of the measurement method, or the tendency of certain operators to carry out measurements more accurately using one or another method. The variability of the measurement results of the exterior sign "proboscis length" was significantly affected by the measurement method and the operator. 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 approach using software allows it to be observed with maximum accuracy. When measuring the length of an object using MBS-9, the measurement cutoff point often falls between the graduations of the scale. So, when counting the sections, they are rounded up 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.

By analyzing the obtained graphs (Figure 2A,B), we can conclude that the minimum deviation from the average value when following the measurement process, in the majority of results, was produced by operators 1 and 4, which was indicated by the smaller sizes of their frames compared to operators 2 and 3. Accordingly, operators 1 and 4 are better candidates to record important measurements.

5. Conclusions

Taking measurements using software has a number of advantages over MBS-9:

- the ability to measure the curved proboscis correctly;
- scanned body parts of bees may be conveniently stored in an electronic form, exchanged by email with other researchers, and used for training employees within the organization so they can obtain identical results;
- less labor and time costs;
- 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 with MBS-9.

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

Author Contributions: Conceptualization, A.B.; methodology, A.B.; validation, A.B. and D.M.; formal analysis, A.B. and D.M.; investigation, A.B.; writing—original draft preparation, A.B.; writing—review and editing, D.M. All authors have read and agreed to the published version of the manuscript.

Funding: The work was performed as part of state assignment No. 0642-2019-0002.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Federal State Budgetary Scientific Institution "Federal Beekeeping Research Centre" (FSBSI "FBRC"), Pochtovaya Street, 22, Ryazan Region, 391110 Rybnoe, Russia.

Acknowledgments: The authors are sincerely grateful to the reviewers for their comments on the text, as well as to E. Larkina, A. Laskin, N. Markina, and I. Yazykov for participating in the tests as operators.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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