



MorphoXL, version 2.2.0

User manual

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1. General provisions

1.1. The application is designed to determine the breeding suitability of the founding queens in honey bee families based on the results of the analysis of wing morphometric indicators in selected samples of worker bees and drones (Cubital index, Dumbbell index, Angular discoid displacement and a number of additional indices), as well as determining the breed affiliation of the studied colony by the method geometric morphometry.

1.2. The **MorphoXL.exe** executable file uses system modules that are installed with all versions of the Windows operating system, so it does not require separate installation. However, if these modules are missing, you will need to download and install the required modules from the [vbrun60sp6.exe](#) installation package on the Microsoft website.

1.3. Functionality: the application calculates the morphometric indices for each of the studied beewings (up to 100 pieces in a sample), after which it performs statistical processing with the determination of mathematical expectation, standard deviations, coefficients of variation, representativeness errors, and confidence intervals. By comparing the confidence intervals with the reference breed ranges, the percentage of compliance with the breed (preset or one of the list of breeds in the classifier, depending on the selected research mode) is calculated with recommendations on the possible use of the studied beecolony in terms of breeding suitability.

The application also includes a geometric morphometry module, which allows you to reliably determine whether the studied colony belongs to one of the subspecies of honey bees, which are presented in the corresponding classifiers. The classifiers themselves are fully compatible with the classifiers of the [IdentiFly](#) application, which is recommended in [COLOSS](#) for the morphometric study of bees. This allows you to use specialized classifiers from another developer in the MorphoXL application.

1.4. The output data are the results of digitizing the wings of bees or drones - files with the coordinates of 8, 12 or 19 landmarks, on each right (or each left) front wing of the bee, generated in the universal general-purpose morphometric application **TpsDig2**, or any other application that generates files of a similar format ("*.tps").

We have also developed a similar application called **WingsDig**, which specializes exclusively in bee morphometry and is focused on capturing images of bee wings using a USB microscope. The WingsDig application has its own, quite detailed instructions for use, so the following will be mostly about work in the **TpsDig2** application.

1.5. The least informative are the "TPS" files with 8-point measurements and allow the calculation of only three main morphometric indices: Cubital index, Dumbbell index and Angular discoid displacement. More informative are the "TPS" files from 12 landmarks per wing and allow to calculate a number of additional morphometric indices. This is the well-known Precubital index and the little-known, but quite useful Mayer index, and Izmailov index, which allow to additionally assess the degree of metization of the test sample, which cannot always be seen from the result of the analysis of the main indices. As for additional and little-known morphometric indices, it should be noted that they were developed both by representatives of an unofficial domestic morphometric school and a foreign one (Mayer's index, Kazakhstan). And finally, the "TPS" files with 19-point measurements allow you to calculate both the main and additional indices, and also allow you to fairly reliably assess whether the test sample belongs to one of the subspecies of honey bees, with the help of geometric morphometry. At the same time, the points on the wing can be arranged both in the style of the IdentiFly application and in the DAWINO style (BeeMorph application, Czech Republic).

The Mayer index and the Izmailov index (see illustrations below) are essentially analogs of the angular discoid displacement, but are calculated for different regions of the wing. All other morphometric indices of classical morphometry used by this application are well described in the relevant literature. They can also be found on the application's website, in the [DAWINO](#) protocol description.

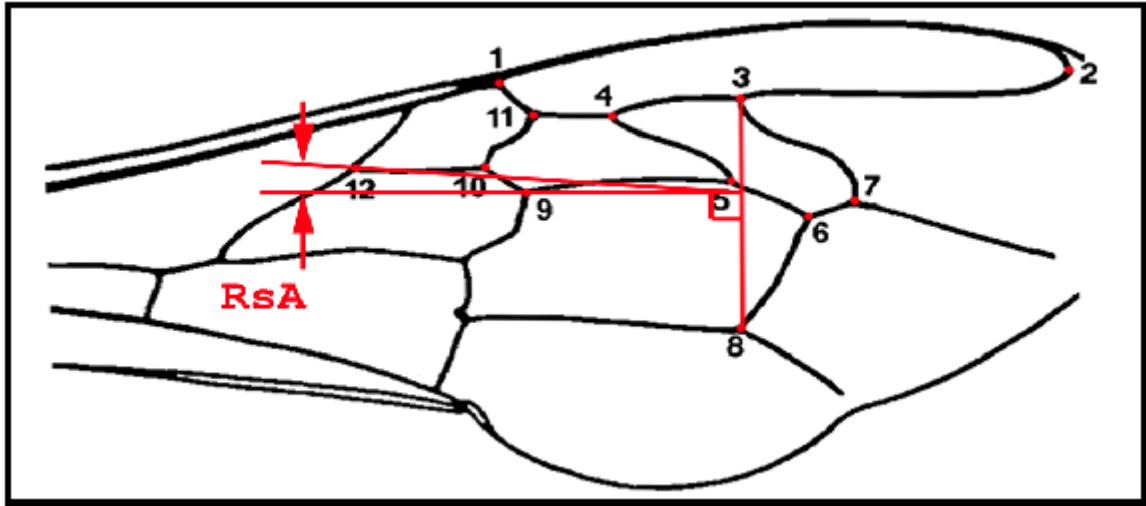


Figure 1 – Calculation scheme of the Mayer Index (RSA). Landmarks 3, 8, 9, 12

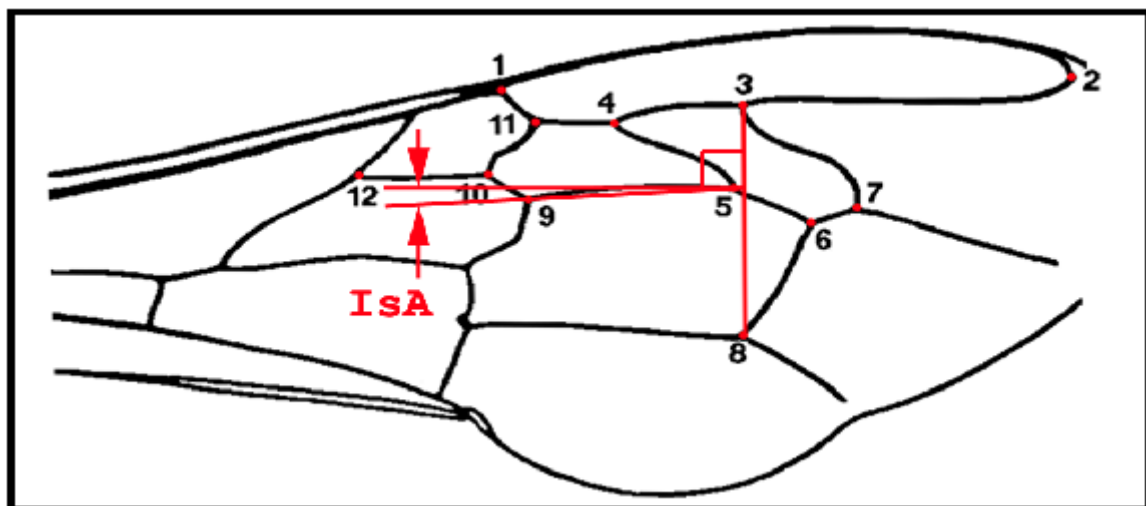


Figure 2 – Calculation scheme of the Izmailov index (IsA). Landmarks 3, 8, 5, 9

1.6. When working in the **tpsDig2** application, use the "comma" symbol (set in the "Options" > "Decimal character" menu) as a separator for the integer and fractional parts of the number. This recommendation corresponds to the settings of computer regional standards for the region of Ukraine. On the computer, they can be changed through "Control Panel\Language and regional standards\Regional parameters\Settings\ Integer and fraction separator"). When positioning the points on the wing, use the optimal scale of the image, which provides sufficiently high reliability of the measurements.

1.7. When obtaining images of wings using a scanner, it is recommended to set the maximum possible image resolution, but not less than 2400 dpi.

1.8. When working with a USB microscope, as a rule, obtaining images is performed in specialized applications that are provided with the device. In the settings of these applications, it is recommended to set the frame size to 1600x1200, which corresponds to an image resolution of approximately 6000 dpi. If this is not done, then the resulting images will be of too low resolution and not suitable for further work. Unlike such applications, the **WingsDig** application has its own module for working with USB - a microscope where the frame size is 1600x1200 as the default setting.

1.9. You can get the latest version of the free English-language **TpsDig2** application at the following link: <https://www.sbmorphometrics.org/soft-utility.html>.

1.10. MorphoXL has a dedicated menu command for saving a report in PDF format. The application automatically generates the report's table of contents and current information.

2. Preparation of wing samples

2.1. Bees for research can be taken from the seabed (not recommended) or from the nest in spring, autumn and summer. In the latter case, the accuracy of the study is significantly increased, because the factor of possible wandering of bees is excluded. Regarding taking a sample from the nest, there are certain recommendations:

- to select young bees directly from the breeding hive. The optimal age for research is considered to be about 5 days of bees;
- according to other recommendations, a large queen insulator cap is put on the brood area at the exit and after five to six days all the bees are swept out of the frame. A frame with bees in an isolator is placed in a freezer for 20 minutes to kill them. In this way, the moment of foreign material getting into the sample, as well as jamming of the wings, is excluded.

2.2. For an approximate assessment of the colony in the first year of the queen's life (preliminary assessment), as a rule, 30 worker bees are required in a sample. An accurate assessment of the family by signs (a complete study) requires the collection of at least 50 bees, and in special cases, 100 bees. A study of 50 drones is enough to assess the parental family. Preparation is carried out in the following order:

- if the bees are taken from the sea, they should be washed in warm water so that they are cleaned of wax crumbs and not sticky, and then dried;
- the wing is torn off (cut off with scissors) and carefully laid out on the tape with the upper side of the wing against the tape, the front edge of the wing towards itself (see Figure 1), after which it is pressed against it with several smoothing movements of the nail (toothpick) from the base of the wing;
- after gluing all the wings on the tape (glued in several lines, see **Figure 1**), the family number is written on a sheet of blank paper, the tape with the wings is turned over and glued to a sheet of paper (a scanner pot). In the case of working with a USB microscope, cover the tape with the wings with another strip of transparent tape. Inscriptions are made with a felt-tip pen;
- if air bubbles have accidentally formed, then carefully expel them into the empty zone and, after piercing with a needle, smooth them with a finger;
- the sample prepared for further work is shown in **Figure 2**.



Figure 2 – Wing sample prepared for scanning

2.3. Instead of gluing the wings on scotch tape, you can lay them on transparent plastic and then fix them on top with scotch tape (Belgian school). At the same time, one end of the tape is pre-fixed to the plastic, and after unfolding, the wing is stretched and gradually, displacing the air, is glued to the plastic. Another option is that the wings are pasted on scotch tape, after which the latter is pressed against a sheet of plastic (German school). The advantage of stickers on plastic (organic glass) is its perfectly even and smooth surface.

2.4. It is possible to use two object glasses: wings are laid out on one of them, they are covered with the other. To avoid loss of wings (air flow!), glycerin is used, which, however, can affect the clarity of the microscopic picture. Therefore, not the entire surface of the slide is wetted, but glycerin is applied in strips with the help of a brush. Then wings are laid out on these strips of glycerin. In this

way, a sufficiently clear microscopic picture with very precise measured values is obtained. Ruttner advises placing the cut wings in a container with alcohol to which a little sugar has been added. Then the removed wings are placed in an even row on a glass slide. Thanks to the addition of sugar, after the alcohol evaporates, they stick well to the glass plate.

2.5. The process of preparing the material is the most responsible, so use the method that gives you the highest quality.

3. Obtaining images of wings

3.1. When using a scanner, it should be set to receive images with a resolution between 3200 and 4800 dpi. At smaller values, the reliability of the results decreases significantly. The wing sample must be placed on the scanner along the longer side of the scanner field - so that when scanning the rays cross the veins of the wing - this way the image is clearer. After viewing, the image is returned to a horizontal position by means of a graphic editor and saved in the appropriate folder.

3.2. It is recommended to view the image of the wings in a graphic editor, to check for raised wings or other defects. Since their processing may give incorrect results, which will affect the overall results of the family assessment, it is better to remove them from further processing by noting in any way. For example, cross out in red in the editor.

3.3. Currently, a very progressive method of obtaining images of wings is the use of a USB microscope. They are offered in various designs and in a wide price range, starting from \$13 (see Figure 3). Each frame (wing) is further digitized in the **TpsDig2** application on separately, and the results are saved in a common file with the extension "*.tps".



Figure 3 – Digital USB microscope

Note . **WingsDig** application has its own module for working with such equipment, so it does not need third-party software that is provided with the microscope.

4. Digitization of wing images

4.1. To digitize wing images, i.e. to place landmarks on the wing and obtain their coordinates, it is recommended to use the **TpsDig2** application (or **WingsDig**). The appearance of the **TpsDig2** application icon on the computer desktop is shown in Figure 4.



Figure 4 – Icon for launching the TpsDig2 application

The window of the **TpsDig2** application is shown in Figure 5 .

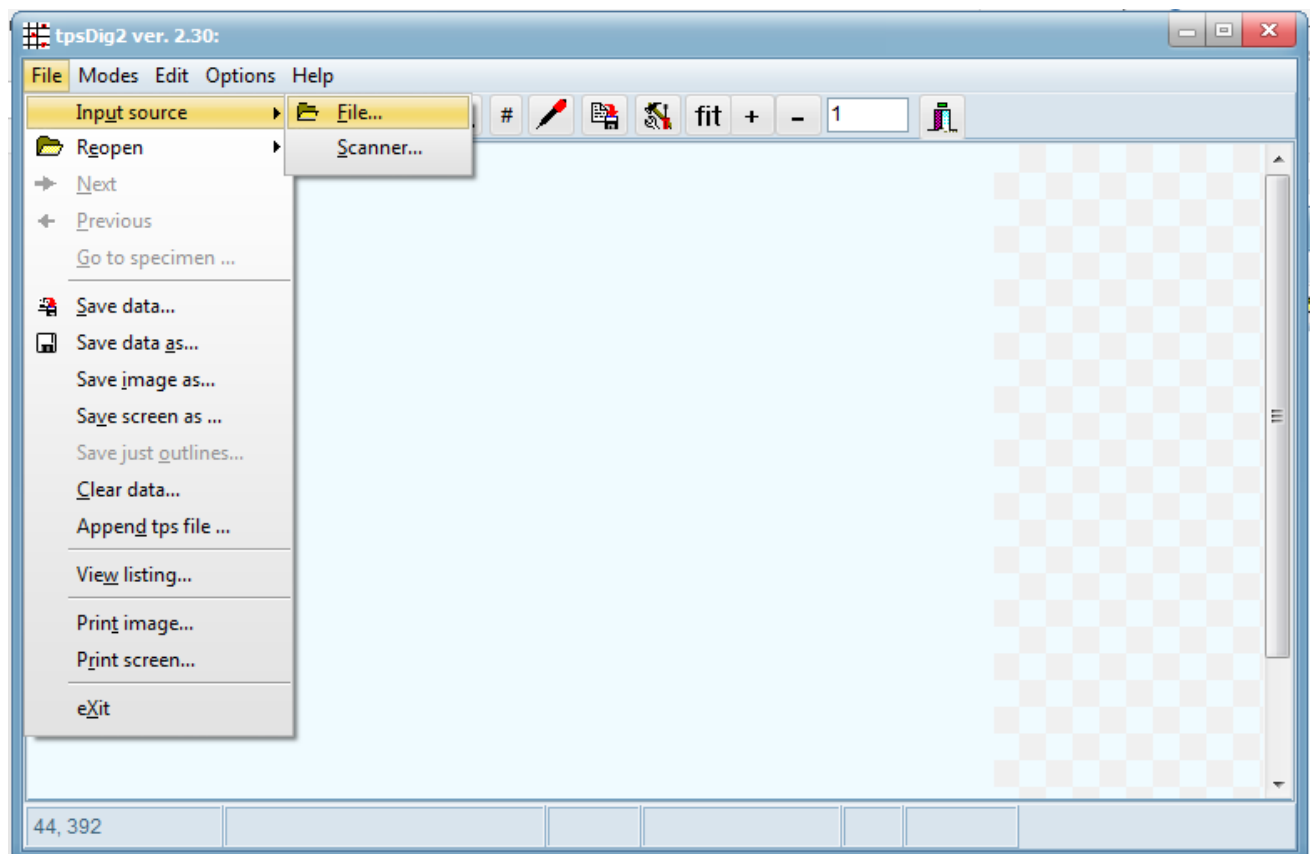



Figure 5 – General view of the TpsDig2 application

To start work, launch **tpsDig2** and download a file with wings images, as shown in **Figure 7**. In the next dialog box for opening the file, you need to specify the type of image (extension), or select the "**All graphics**" option, and find the desired image file in the computer's file system. After successful download, it is necessary select the digitizing mode (a button with a crosshair ) , as shown in **Figure 6**.

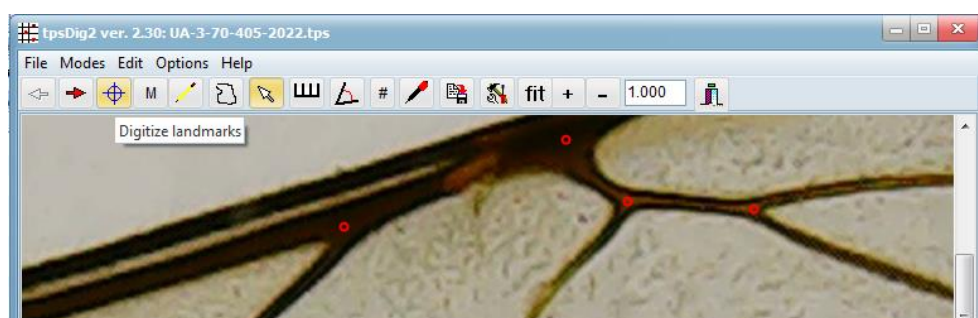


Figure 6 – Selection of the point placement mode

4. 2. The order and location of landmarks for 8-point morphometry

We put the points in a strictly defined sequence, at the intersections of the axes veins, 8 landmarks on each wing. Landmarks 1 and 2 are placed slightly differently - on the inner surfaces of the oval veins, at the maximum distance from each other. The sequence of points is as follows:

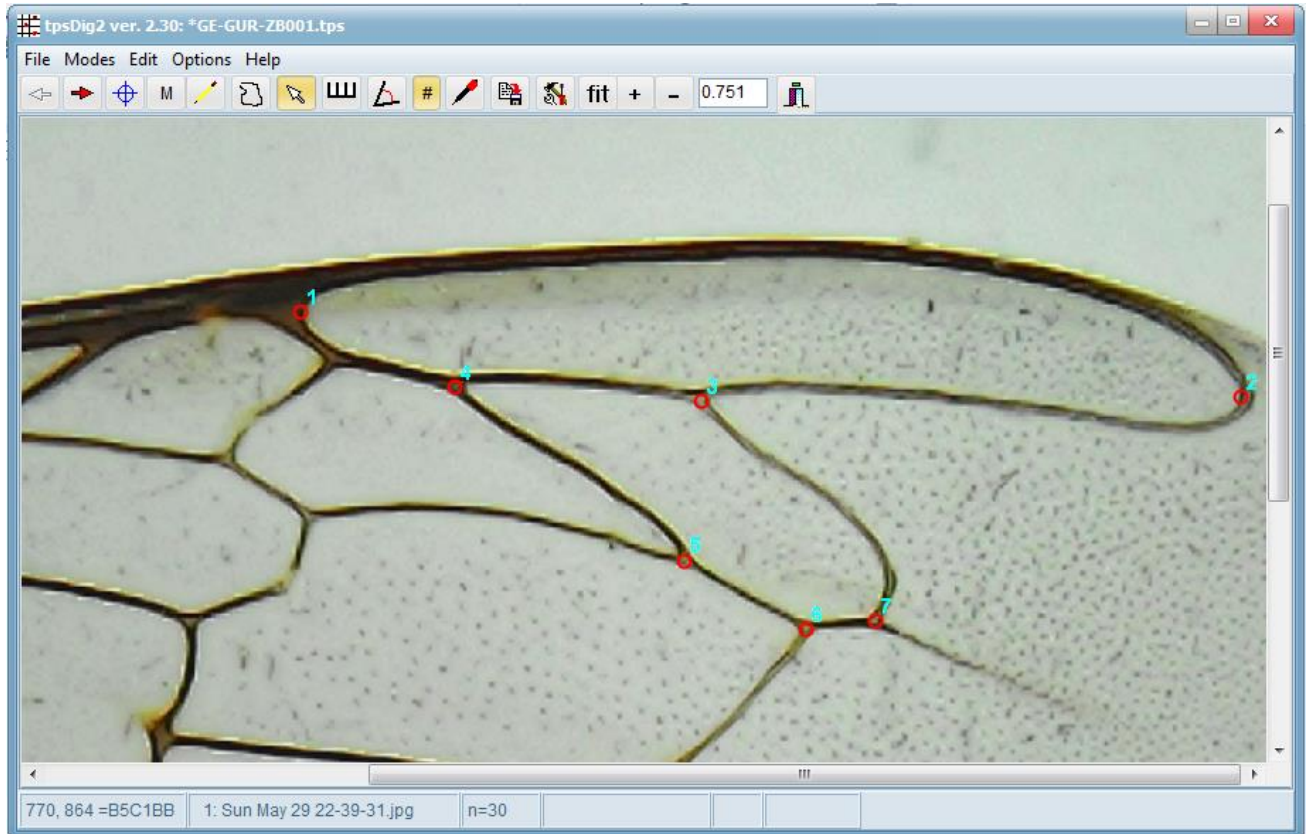



Figure 7 – The sequence of placing points in 8-point morphometry

You must first adjust the point size. According to the recommendation of Friedrich Ruttner, size of the landmark must be set in such a way that it fits completely into the nodule at the intersection of the veins, and touches the boundaries of the nodule in at least three places (see Figure 8). In this case, the centers of the point and node coincide.



Figure 8 – Examples of positioning landmarks 3 - 8

Landmark size setting dialog box (see Figure 9). is called by the menu command **"Options\Image tools"** or by a button  on the toolbar.

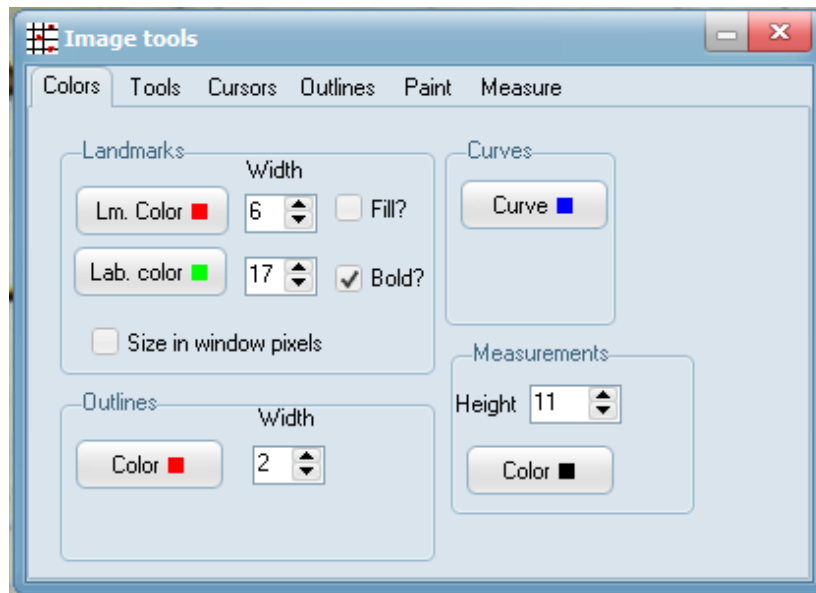



Figure 9 – Settings window

If necessary, the location of the point on the wing can be edited by changing the mode of operation of the application from the mode of placing points to the editing mode. To do this, you need to execute the menu command **"Mode\Edit"**, or press the button  on the toolbar.

Sometimes a situation arises when an extra landmark is accidentally placed on the wing. Then we switch to editing mode and use the right mouse button to call up the context menu on this extra landmark (see Fig. 10). If by chance you did not put a dot, i.e. you missed it, then the context menu is called on the next landmark (by number) and the "Insert landmark" command is executed.

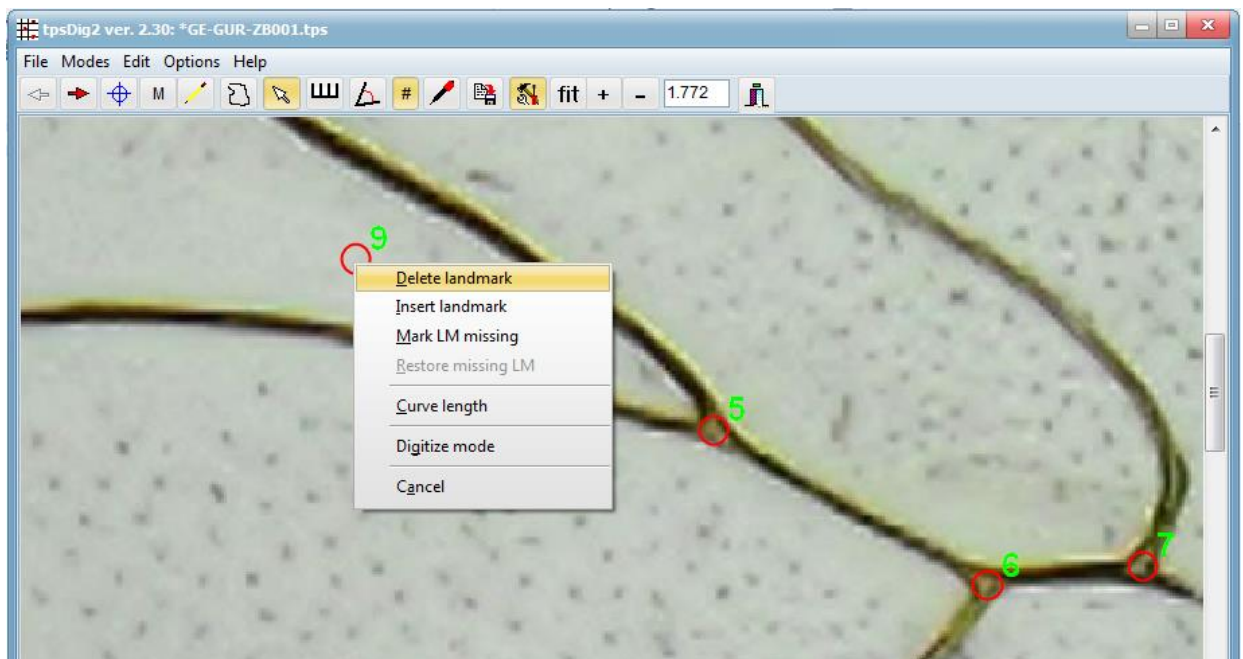



Figure 10 - Editing a Landmark

If the lack of a landmark is detected when the "tps" file is reopened, then the tpsDig2 application will no longer be able to add a new landmark by the required number, but only at the end of the list.

In this case, it is recommended to find the wing with the missing landmark, remove all previously placed landmarks on it and place them again.

After all the landmarks on the wings are arranged, save the results of our work in a file with the extension "tps". To do this, you need to call the file save dialog box by clicking the button  on the toolbar.

4.3. Order and location of Landmarks for 12-point morphometry

This format was developed specifically for the possibility of calculating all additional morphometric indices, with a minimum number of points on the wing. Basically, these are our 8 landmarks from the previous section (in red), plus 4 additional points, which are marked in blue in Figure 11.

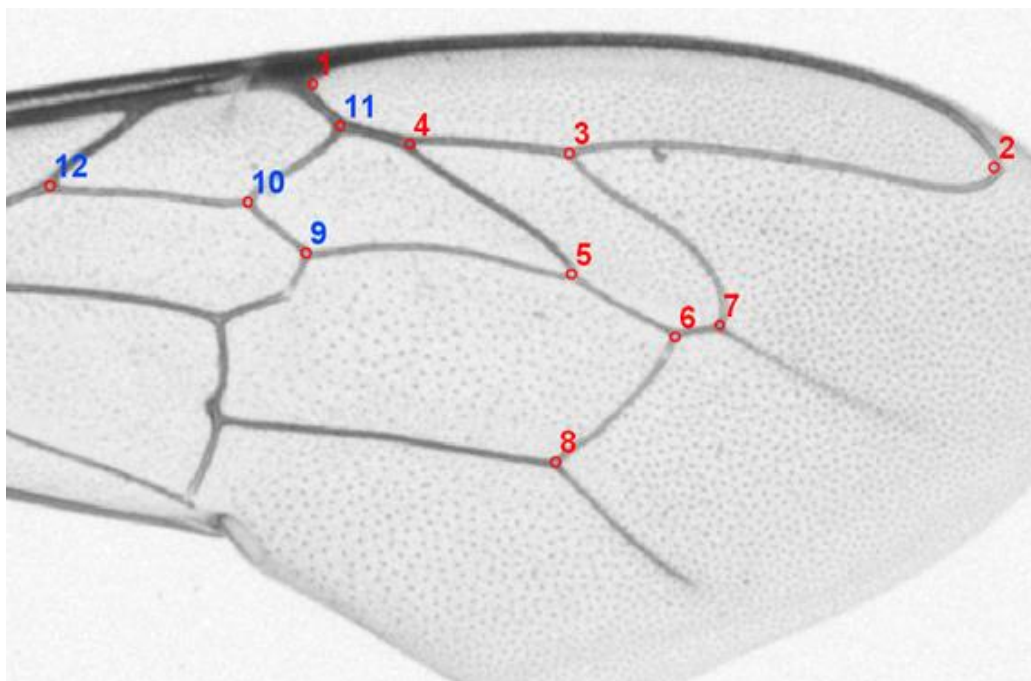


Figure 11 – The sequence of placing landmarks in 12-points morphometry

As already mentioned, additional landmarks allow you to calculate the Precubital index, the Mayer index, and the Izmailov index.

4.4. Dock times and location of landmark for 19-points morphometry

It should be noted here that for 19-point morphometry there is no single "standard" rule for the location of points on the wing, as each software developer came up with their own specific rules. For this reason, MorphoXL implements the capability with only the two most common variants. The first of them is the oldest and well-known DAWINO protocol (Figure 12) – developed by the Czech Institute of Beekeeping, which performs the analysis of breed affiliation of the test sample by the method of discriminant analysis, according to the data of classical morphometry. It processes the values of morphometric indices of the wing and a number of geometric parameters (length, width, areas of individual fields, angles). The next "standard" is offered by the IdentiFly application , which performs the analysis of breed affiliation using a more modern method - discriminant analysis based on the data of geometric morphometry, which is based on the theory of similarity of forms.

The MorphoXL application also performs the most modern method - discriminant analysis based on geometric morphometry data, but the input data can be received in any of these two formats. Along with this, the application also calculates several parameters from classical morphometry,

which are used in algorithms for determining the selection suitability of the founder queen in the studied colony.

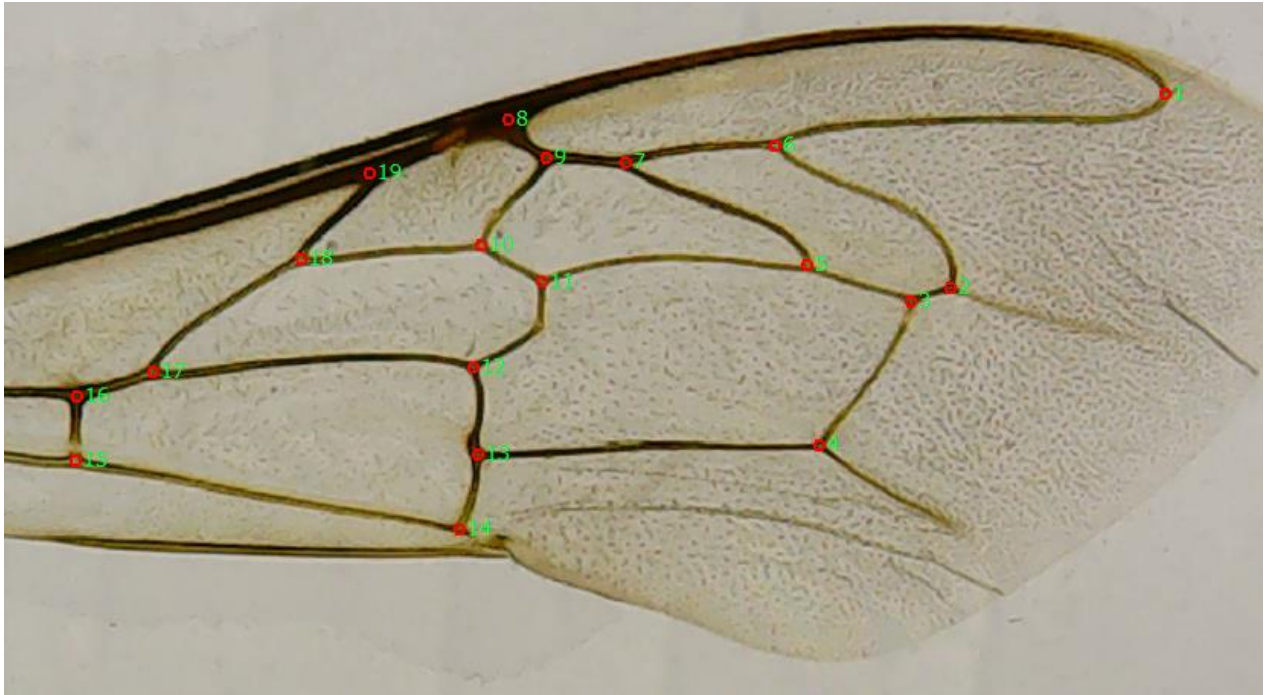


Figure 12 – Order and location of points for 19-landmark morphometry according to the DAWINO protocol

Although the numbering of the points here is quite different from those discussed above, the general rules for positioning the points remain the same, with a certain exception for landmarks 8 and 19. They are also located "in the center of the nodule at the intersection of the veins", but the size of their nodules is much larger than the rest

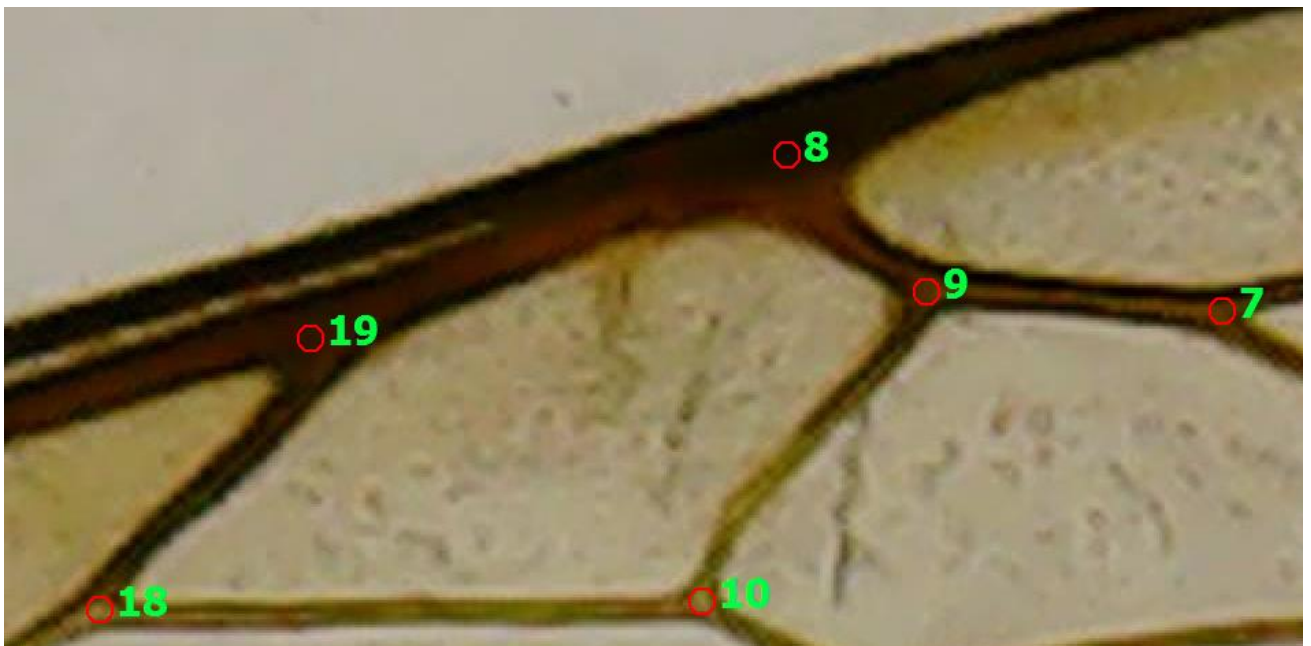


Figure 13 - The feature of the location of landmarks 8 and 19 according to the DAWINO protocol

IdentiFily application there is not only a different numbering of the same 19 landmarks, but also the image of the wing itself is turned horizontally by 180 degrees (Fig. 14).

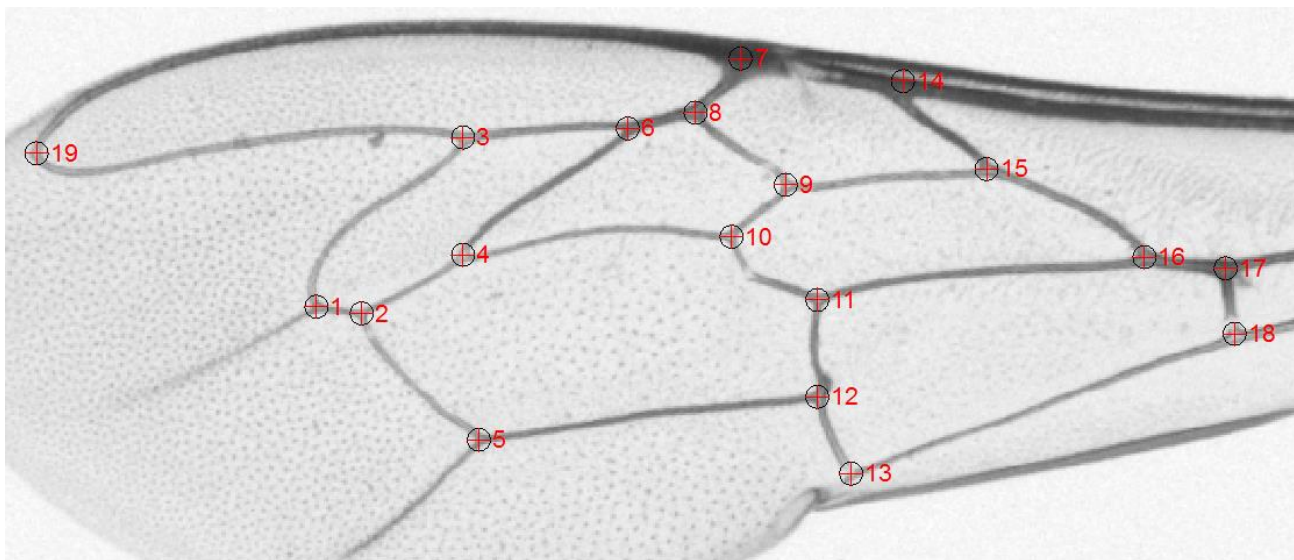


Figure 14 – Order and location of points for IdentiFily-style 19-landmark morphometry

The rule for positioning point 7 here fully corresponds to its analogue in the **DAWINO style** (where it is point 8), but for point 14 there is a certain peculiarity - the two upper parallel lines are considered one vein, so the "node at the intersection" becomes much larger, and the center of this node moves up.

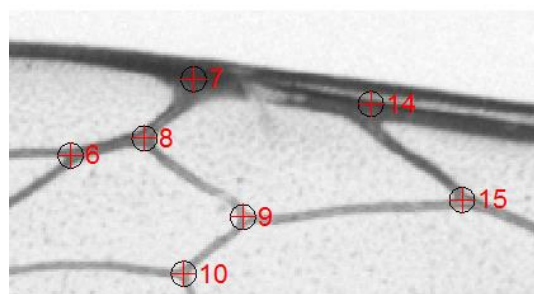




Figure 15 – IdentiFily-style layout of points 7 and 14

4.5. It is also worth noting that a **prerequisite** for further correct work with the saved "tps" file is its placement in the same folder where the wing image files are located. This is due to the fact that the "tps" file is an ordinary text file and, in addition to the coordinates of the points, it only indicates the name of the file with the image of the wing on which the points were placed. That is, it is considered that the images of the wings are located next to each other, in the same directory. Therefore, when there is a need to re-edit the file "tps", the editor searches for the desired image next to where the "tps" file was downloaded. If, for any reason, the user wants to save the image of the wings separately from the location of the "tps" file, then before saving it, it is necessary to set the option "Specify the full path to the images" in the editor.

4.6. When saving the research results on a computer disk, it is recommended to implement some kind of unified and convenient directory structure, which would include the year of the research, family or queen number, hive number in the names of folders/subfolders, etc. This systematization of folders will make it much easier to find the necessary information in the future.

4.7. Also, the **tpsDig2** application has the ability to save the results of wing digitization from several images in one file. To do this, when saving the digitization results of each subsequent image, select the previously created "tps" file in the dialog box, and answer **"Append"** to the application's message that such a file already exists. The next time such a complex file is opened by the **tpsDig2**

application, **buttons with arrows**   on the toolbar become available, which are used to move through the images.

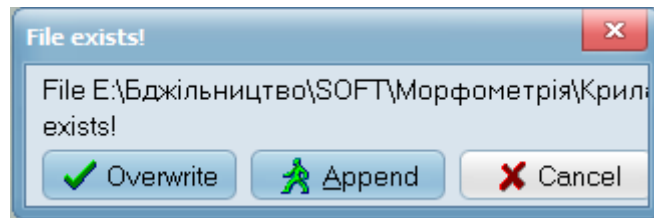


Figure 16 – Saving the digitization results to a "tps" batch file

It should be noted here that, unlike **tpsDig2**, the **WingsDig** application is maximally adapted to work with a large number of images (a separate image for each wing) in the "tps" file, so it is more convenient in this situation.

5. Processing of digitization results in the "MorphoXL" application

5.1. Launch the application by double-clicking the "MorphoXL.exe" file in any file manager window. To get started, open the menu with the appropriate commands. To do this, left-click on the bee icon on the "Report" page, or on the application window title bar if you're on another page.



As a result, a floating menu will appear, in which we select the command "Get measurement data from the TPS file".

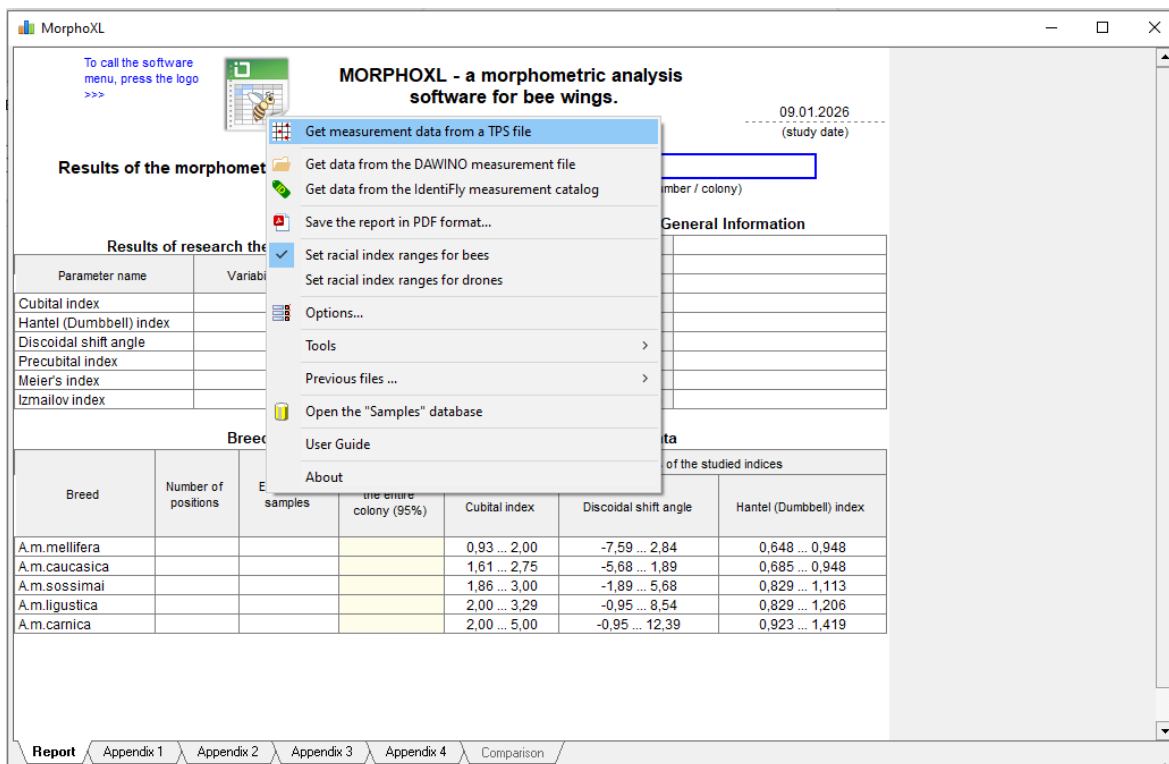


Figure 18 - Execution of the command to retrieve data from TPS files

Note: An alternative way to access the MorphoXL menu is to right-click the context menu in the title bar of the program window. This is especially useful if you are not currently on the "Report" sheet.

5.3. The application will offer to open a file with point coordinates that was created earlier in the tps file editor.

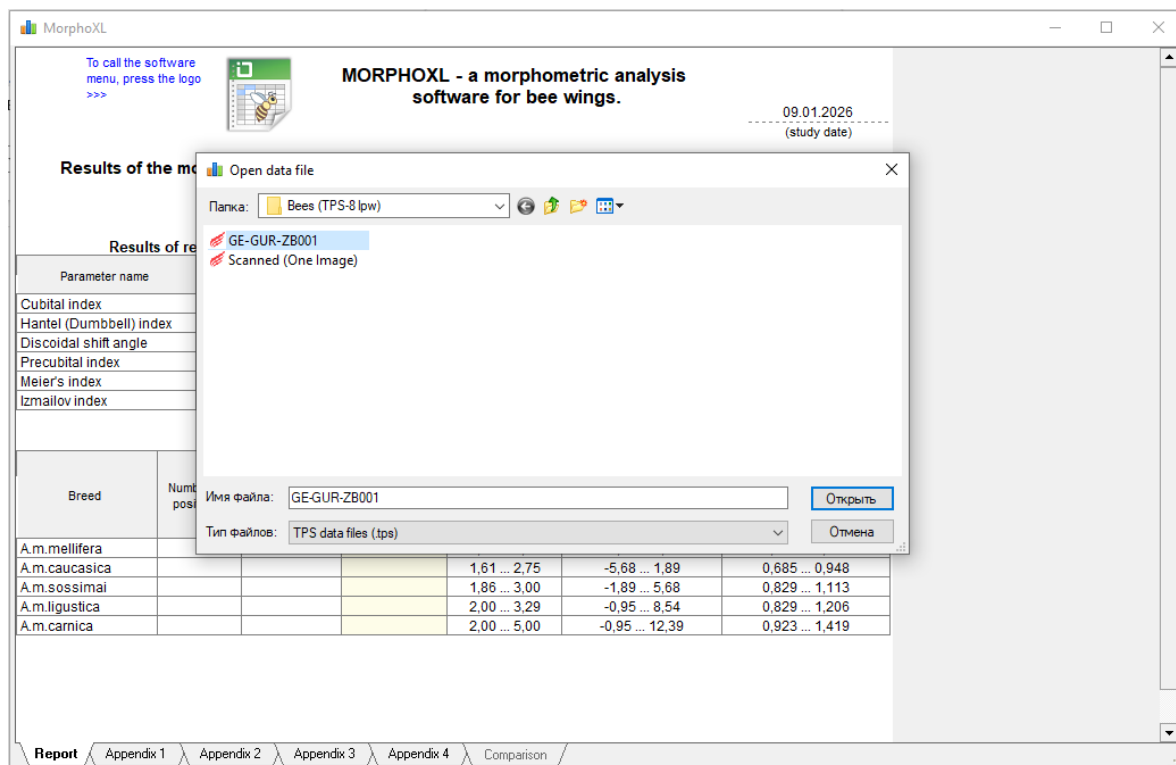


Figure 19 – Document opening dialog box

If the data is successfully downloaded and processed, a corresponding message will be issued (see **Figure 20**).

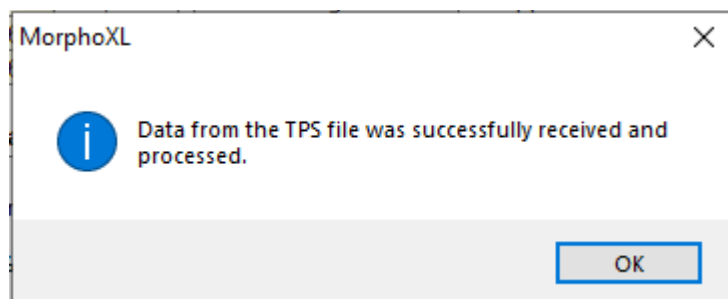


Figure 20 – The application message about the successful execution of the analysis

In a similar way, the application allows you to download and process the results of measurements in DAWINO style, which are in files with the extension ".txt" and ".csv". Also, the application has a separate command for processing the results of digitization performed in the **IdentiFly** application, although there is a peculiarity here - these data are not in a separate file, but in the files of the wing images themselves, in ".png" format. Therefore, in this case, we indicate to the **MorphoXL** application not a separate file, but the path to the directory where the wing image files processed in the **IdentiFly** application are located.

5.5. If necessary, the breed ranges can be changed by the user on the "Options" sheet, where it is allowed to adjust their limits, with the help of drop-down lists of acceptable values - see **Figure 21**. It is also allowed to change the names of the breeds, which makes it possible to adapt the application in accordance with the needs of the user, with a list of relevant breeds of bees in his area. The section "**Population studies**" deals with how to determine or clarify the limits of the breed range of any index for each individual breed (subspecies) of honey bees.

Options

Module of geometric morphometry (breed assessment)

Pedigree analysis is only performed for DAWINO or Identify style bee wing studies (19 landmarks)
 Choose a classifier compatible with the Identify software ("Classifiers" folder)

>> A-m-Sossimai and 5-subspecies.dw

☐ Enter the classification result into the "Samples" database ☐ Perform analysis automatically

Classical morphometrics module (selection suitability assessment)

Choose method of analyzing the breeding stock of a colony:

☒ Automatically (for matching to one of the breeds list)
☐ For compliance with the given breed (in the list below)

Select a breed for compliance analysis:
 mellifera

Choose subject of research:

☒ bees
☐ drones

☒ Use custom settings for morphometric index boundaries.

User correction of breed ranges (boundaries of morphometric indices)

No	Race	Ci range			DsA range			Hi range		
		Min	Max	\bar{M}	Min	Max	\bar{M}	Min	Max	\bar{M}
1	mellifera	0,93	2,00	1,47	-7,59	2,84	-2,38	0,648	0,948	0,798
2	caucasica	1,61	2,75	2,18	-5,68	1,89	-1,90	0,685	0,948	0,817
3	sossimai	1,86	2,75	2,43	-1,89	5,68	1,90	0,829	1,113	0,971
4	ligustica	2,00	3,00	2,65	-0,95	8,54	3,80	0,829	1,206	1,018
5	carnica	2,00	3,29	3,50	-0,95	12,39	5,72	0,923	1,419	1,171

*WARNING! When adjusting breed ranges, it is allowed to change only the values specified in the corresponding drop-down lists. These values are standardized and should correspond to the class scale of the derived morphometric index.

Interface language: English

Apply Cancel

Figure 21 – Changing the boundary of the breed range

When changing breed ranges, the application monitors the correctness of this operation and can issue appropriate critical messages under certain circumstances.

You can also choose one of the methods of analyzing the colony's breed affiliation: automatically (standard mode) - when the application itself determines the predominant breed for the colony being studied, or "In accordance with the specified breed", which is selected from the drop-down list. This option is useful when we are investigating a colony with a known breed and we need to confirm it.

After changing breed ranges, the analysis results are updated automatically. Custom breed range settings will be saved in the application settings file (the "**MorphoXL.ini**" file) and will be used in future sessions. To restore the original breed range settings, you must disable the option "**Use custom settings for morphometric index boundaries**" button in the same window. The application typically sets these values to default, but if the "**MorphoXL.ini**" file contains custom settings for both bees and drones, they take precedence. You can also change the study focus (**bees/drones**) using the application menu..

Note: The WingsDig application allows you to save additional information about the test sample in "tps" files, including "**Research subject**". When processing such files, the MorphoXL application automatically changes the corresponding settings, which informs the user.

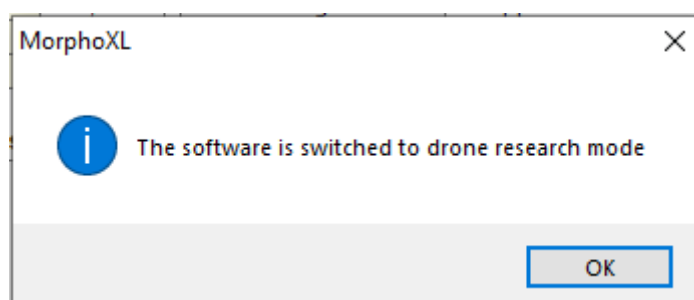


Figure 22 - Notification of a change in the subject of research

5.6. In the "Settings" window, you can also change the interface language of the MorphoXL application by selecting it from the list of available languages (**Figure 21**). All available language files with the "*.lng" extension are located in the application folder.

Figure 23 – Changing the interface language

You can also specify/change the path to the location of the "tps" file editor so that it can be called using the MorphoXL drop-down menu (Fig. 24). Configure the mode of operation of the geometric morphometry module: enable/disable the module, choose another classifier, choose the mode of saving the analysis results to the database. The accumulation of such results makes it possible in the future to develop new classifiers for the identification of previously unresearched individual subspecies or geographical populations of bees.

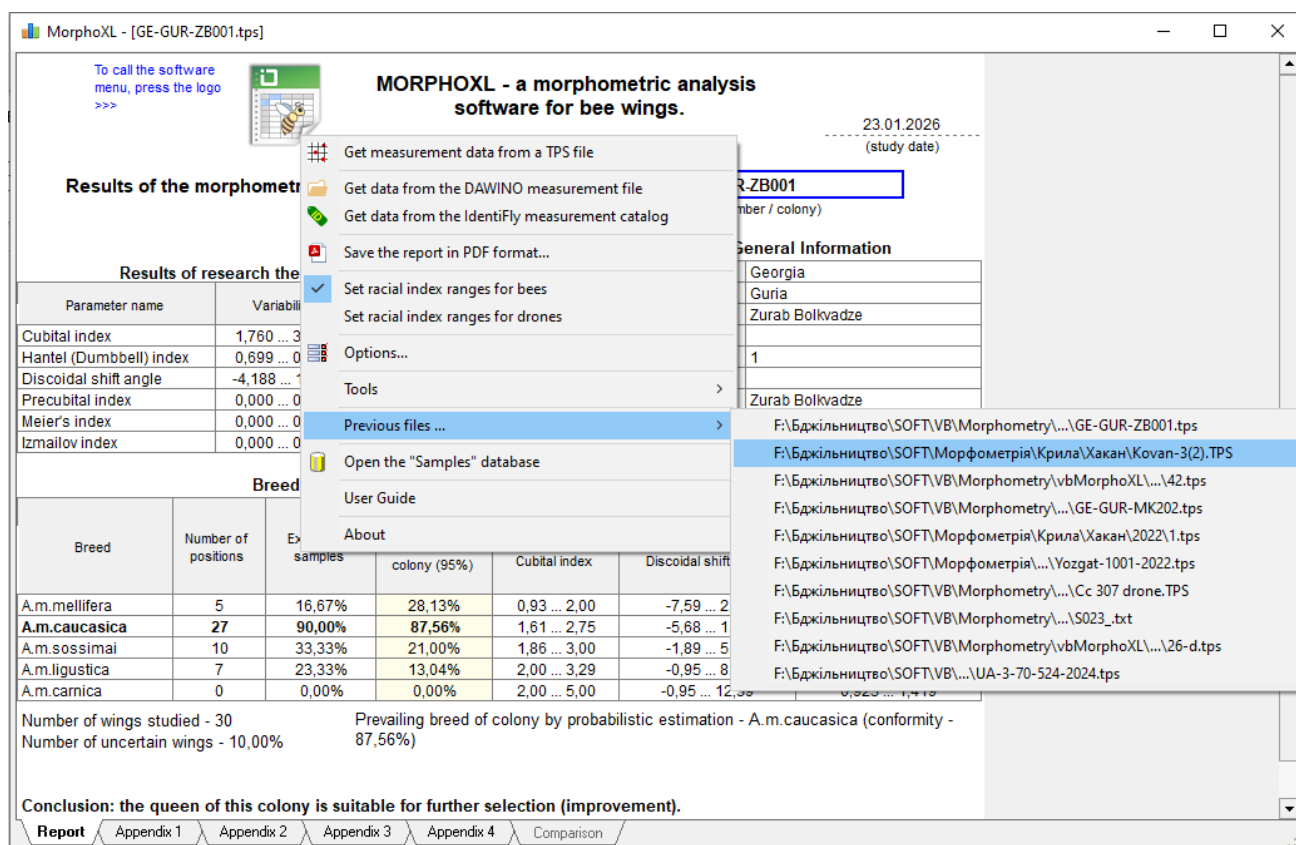


Figure 24 – Additional tools in the MorphoXL menu

The application saves the history of work with "tps", "txt", "csv" documents, which allows you to avoid complicated navigation through the file structure of the computer, if it is necessary to review it again. To do this, use the "Previous files..." menu command.

6. Analysis of results

6.1. We receive the analysis of the results of the study of breeding suitability and breed affiliation on the "Report" sheet, which indicates the percentage of compliance with the prevailing breed according to the probability assessment, as well as recommendations for the further breeding use of the studied beecolony.

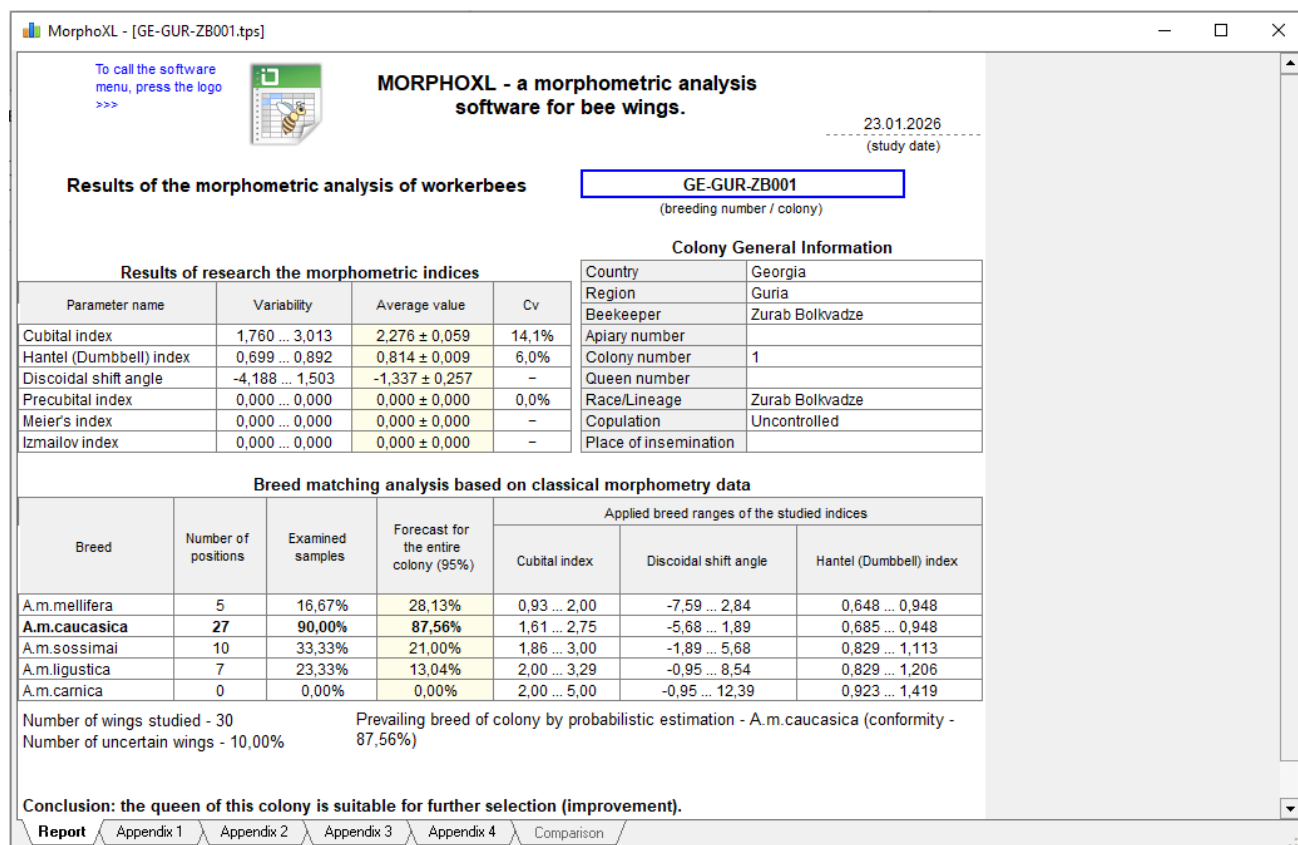


Figure 25 – MorphoXL main page

Possible recommendations of the application for the further use of the studied colony are presented below in order of increasing breeding value:

1. " the uterus of this family is not suitable for reproduction "
2. " the mother of this family is not promising for selection "
3. « the uterus of this family is suitable for further selection (improvement)
4. " the uterus of this family is suitable for reproduction "
5. " the mother of this family may be the founder of the selection line "

6.2. In the event that the application recommends the use of " ... by the founder of the selection line" or " ... for reproduction", you can be congratulated. You have found a colony that is unique in terms of the consolidation of breed-defining traits, and all you have to do is check this excellent result with the help of the geometric morphometry module, and in some cases, genetic studies. Well, if the verdict of the application is not satisfactory, then first of all, you need to make sure that you did not make any mistakes when studying the wings of this colony.

6.3. When assessing breeding suitability, the application analyzes the degree of hybridization for each of the studied indices. **Possible values of hybridization:** " hybrid ", " permissible ", " insignificant ", " absent ". Another evaluated feature is **the integrity of the colony** , which characterizes the degree of homogeneity of the colony and **can take the following values:** " disturbed ", " normal ", " ideal ". The last indicator is calculated for the Cubital and Dumbbell indices and the given rating scale fully corresponds to the value of the coefficient of variation of the studied index: for **Ci** - ">20%", "12.5%...20%", "<12.5% " and for **Hi** - ">7.5%", "6.5%...7.5%", "<6.5%".

A low colony score is often the result of several unidentified wings. In this case, the records for these wings will be shown in red font on the **"Appendix 3"** sheet. In this case, it's necessary to check the correct positioning of the dots on the images of these problematic wings. So, open the "tps" file editor and load the required file. In the editor, find the problematic wing and check the quality of the positioning—it's quite possible that the wing itself is not to blame, but rather a dexterous hand, fatigue, or inattention. If you see an error, correct it and reanalyze the sample.

6.4. If the application reports excessive hybridization or low colony integrity, we look for the cause in one of the three graphs (see Figure 26) located at the bottom of the **"Report"** sheet. In this case, we look at the graph with the desired index, where we find points that fall outside the red (confidence intervals) or even blue (breed ranges) rectangle. Next, we analyze the accuracy of its digitization in the tpsDig2 application, as described above, in section 6.3.

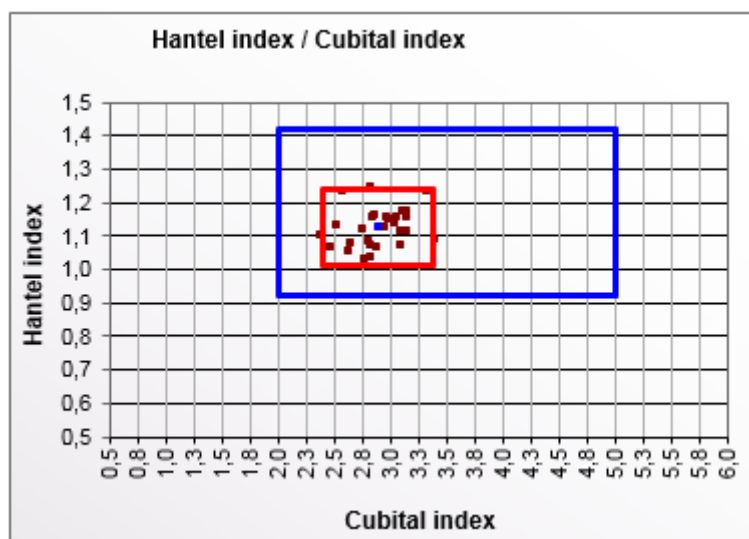


Figure 26 – Distribution of Hi/Ci (breed ranges and confidence intervals for two indices)

6.5. If all possible reasons have been exhausted and this did not lead to an improvement in the evaluation by the application, then such a family should not be used in further selection work.

6.6. Graphs of the frequency distribution of the main morphometric indices by classes (variation curves) are on the "Appendix 1" sheet. They are useful for visual detection of impurities of other breeds, by peaks in classes not typical for the predominant breed. Figure 27 shows the variation curve of the distribution of the **Cubital index** by class. Between the two green vertical lines on this graph is the so-called "clean line range". According to Ruttner's method, at least 66% of the bees of the studied sample should fall into this area in a pure-pore colony. An increase in the number of bees in this range is one of the factors that affects the higher breeding value of the colony. To the left and right of the "clean line range" are the "critical areas" (between the green and blue lines). In each of them, the number of bees should not exceed 15%. Outside the critical areas, i.e. beyond the blue lines, no more than 2% of the bees should fall on both sides.

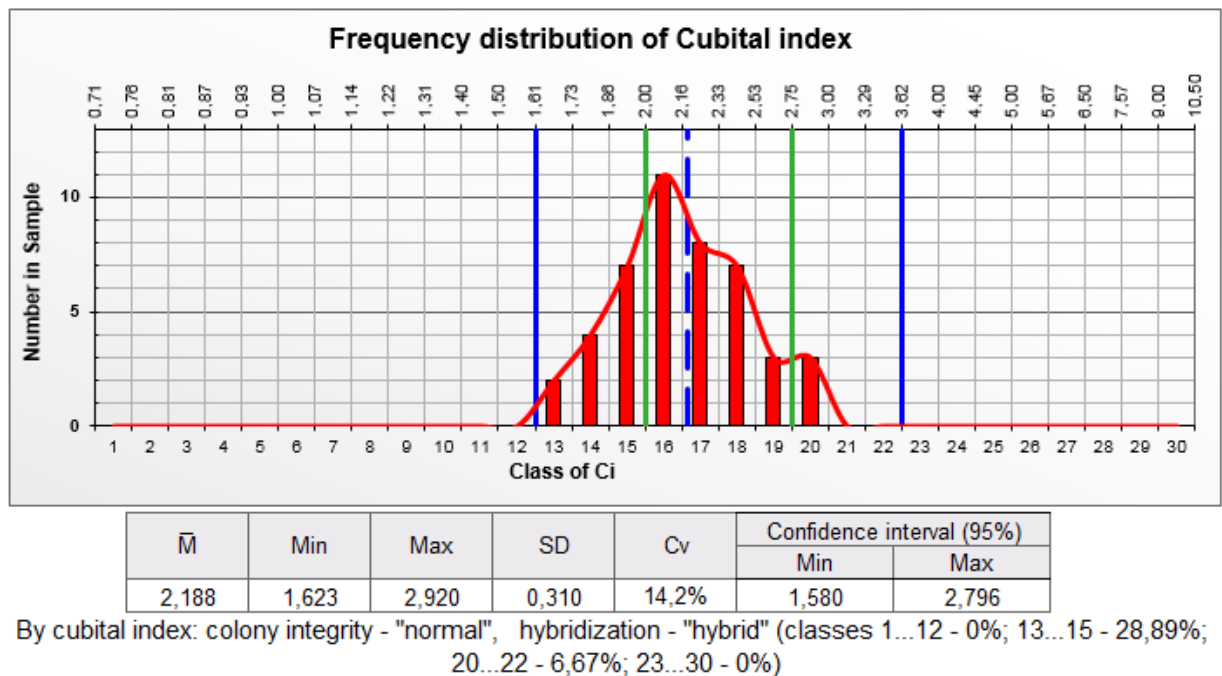


Figure 27 – Variation curve of Cubital index

In a similar way, the variation curves for the **Dumbbell index** and **Angular discoidal displacement** are analyzed.

The sheets "Appendix 3" and "Appendix 4" show variation curves for additional morphometric indices (Fig. 28), which we are able to examine only for 12-landmark and 19- landmark studies. These are the **Precubital index**, the **Mayer index**, and the **Izmailov index**. As already mentioned, additional indexes allow to additionally and more thoroughly investigate the possible hybridization of the studied sample with another subspecies of bees.

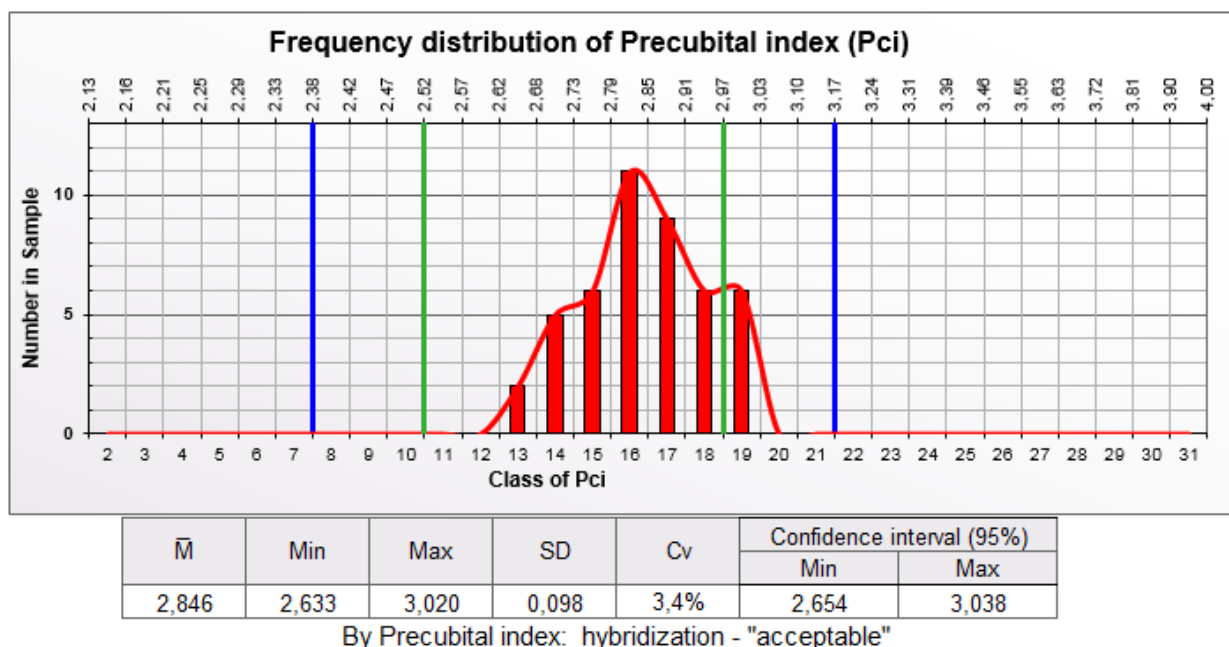


Figure 28 – Variation curve of additional Precubital index

It should be noted here that the above precubital index (Fig. 28) have a pronounced property of serving as "presence markers" in the test sample for subspecies *A.m.mellifera* and *A.m.caucasica* respectively. At the same time, this property is preserved even when it is not possible to track this methization according to the rest of the indices. Tests belonging (conditionally) to classes 19-23 will be evidence of such methization according to the precubital index, which will indicate for other subspecies of honey bees about the methization of *A.m.mellifera*.

7. Module of geometric morphometry

If the previous sections were based on the analysis of individual elements of classical morphometry and mainly served to determine the selection suitability of the queen-founder of the studied colony, then this section aims to most reliably determine whether the studied colony belongs to one of the subspecies (or evolutionary lines) of bees presented in used classifier. This method of analysis is used only for 19-point studies of worker bees, both in the **IdentiFly style** and in the **DAWINO style**. The results of the research, according to this method, are provided by the application on the "Appendix 4" sheet.

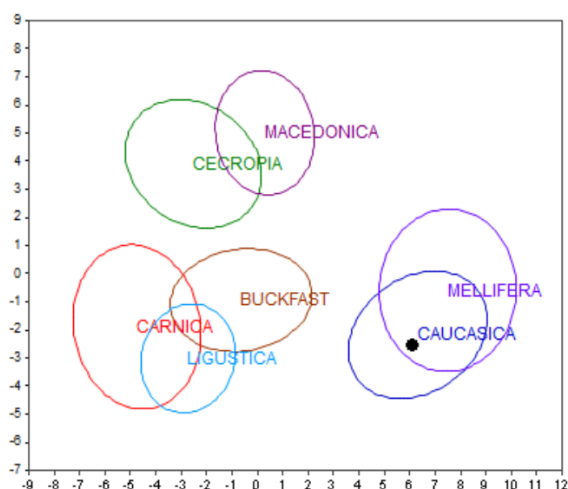
(Appendix 4)

Linear discriminant analysis based on geometric morphometry data

Sample: GE-GUR-MK202
Classifier: Apis mellifera 7 subspecies (Central Europe).dw
Date: 23.01.2026

CVA scores of studied sample

CV1	CV2	CV3	CV4	CV5	CV6
6,032785	-2,538063	-4,339868	-0,124134	-0,621689	-0,145379



Classification results

	By classification functions	Mahalanobis distances	Posterior probabilities, %
CAUCASICA	150382,19	0,7130	100,00
MELLIFERA	150337,46	10,2633	0,00
CECROPIA	150316,69	11,2546	0,00
CARNICA	150311,46	12,0221	0,00
MACEDONICA	150327,18	12,2340	0,00
LIGUSTICA	150321,88	14,1879	0,00
BUCKFAST	150336,13	16,7189	0,00

Figure 29 – Geometric morphometry module report

Note : for the correct interpretation of the above report, it should be noted that the graphic part is only partially informative, as it illustrates to us the projection of a multidimensional space onto a two-dimensional plane. Therefore, the final and comprehensive results of the analysis are in the "Classification results" table, and the graphic part only partially illustrates them.

As mentioned, the geometric morphometric analysis results are stored in the "Samples.csv" database for further processing. It is located in the application's "Database" subfolder. To view the information stored in this database, use the "Open Samples Database" menu command.

After executing this command, a database window will open, displaying all accumulated geometric morphometric research results in tabular form. Right-clicking will bring up a context menu with a set of corresponding commands for table cells, row headers, and column headers.

Geometric Morphometry Results Database - [Samples.csv]

SAMPLE	RACE	PURITY_R	POPUL	WINGS	X1	Y1	X2	Y2	X3	Y3	X4	Y4	X5	Y5	X6	Y6	X7
C0010	CAUCASICA	53.54	DAWNO	12	-0.281554	-0.057055	-0.248446	-0.059104	-0.190853	0.066314	-0.178567	-0.020108	-0.155228	-0.145718	-0.064382	0.077421	0.0140
C0011	CAUCASICA	94.07	DAWNO	12	-0.274799	-0.054103	-0.239696	-0.056397	-0.190002	0.065705	-0.180375	-0.021102	-0.153167	-0.142536	-0.069266	0.076740	0.0116
C0105	CAUCASICA	98.86	DAWNO	12	-0.276041	-0.054529	-0.244011	-0.056521	-0.190473	0.063662	-0.176882	-0.019886	-0.161287	-0.144199	-0.069142	0.076918	0.0157
C0109	CAUCASICA	95.19	DAWNO	10	-0.279282	-0.054984	-0.245580	-0.055502	-0.196309	0.063869	-0.178561	-0.018624	-0.161961	-0.142113	-0.067790	0.076863	0.0122
C0110	CAUCASICA	89.02	DAWNO	10	-0.276497	-0.053571	-0.241376	-0.055419	-0.192653	0.063631	-0.178904	-0.019543	-0.161429	-0.141127	-0.069433	0.074389	0.0100
C0111	CAUCASICA	93.96	DAWNO	9	-0.279759	-0.055896	-0.245826	-0.057985	-0.197134	0.063971	-0.178611	-0.019897	-0.164183	-0.143105	-0.070242	0.076193	0.0156
C0114	CAUCASICA	95.20	DAWNO	11	-0.279756	-0.055105	-0.244637	-0.057264	-0.195192	0.064185	-0.178589	-0.019080	-0.160177	-0.141541	-0.068871	0.075994	0.0129
C0115	CAUCASICA	97.20	DAWNO	12	-0.278916	-0.054182	-0.240700	-0.056735	-0.191004	0.065297	-0.179189	-0.019512	-0.159474	-0.141373	-0.066260	0.076854	0.0088
C0276	CAUCASICA	92.75	DAWNO	15	-0.278379	-0.054162	-0.242769	-0.055527	-0.197585	0.064065	-0.179594	-0.020121	-0.157903	-0.144611	-0.069871	0.076285	0.0146
C0277	CAUCASICA	87.72	DAWNO	15	-0.280660	-0.056143	-0.244867	-0.057410	-0.192269	0.066363	-0.183595	-0.022574	-0.154963	-0.146581	-0.072299	0.076827	0.0162
C0278	CAUCASICA	97.89	DAWNO	15	-0.279265	-0.056794	-0.244352	-0.056753	-0.186574	0.065871	-0.184738	-0.022654	-0.160966	-0.143843	-0.063477	0.077526	0.0143
C0280	CAUCASICA	97.79	DAWNO	15	-0.280476	-0.055932	-0.243249	-0.056737	-0.195438	0.061820	-0.178638	-0.019628	-0.161474	-0.144717	-0.064530	0.077068	0.0125
C0281	CAUCASICA	85.85	DAWNO	15	-0.276425	-0.053458	-0.245299	-0.058049	-0.193230	0.063917	-0.184058	-0.022554	-0.161906	-0.142826	-0.070174	0.076179	0.0104
C0282	CAUCASICA	97.69	DAWNO	15	-0.278595	-0.056137	-0.244443	-0.055958	-0.194273	0.064157	-0.182877	-0.020965	-0.158205	-0.142865	-0.071258	0.076993	0.0109
C0283	CAUCASICA	92.86	DAWNO	15	-0.279576	-0.056183	-0.242267	-0.056670	-0.195007	0.062944	-0.177938	-0.019024	-0.156581	-0.144975	-0.069796	0.075725	0.0099
C0285	CAUCASICA	77.91	DAWNO	15	-0.278412	-0.054397	-0.242799	-0.056371	-0.192175	0.064525	-0.183966	-0.021797	-0.157886	-0.145440	-0.072969	0.074982	0.0113
C0284	CAUCASICA	94.49	DAWNO	15	-0.278122	-0.056186	-0.243861	-0.057292	-0.199292	0.063998	-0.185657	-0.023446	-0.154138	-0.145167	-0.069153	0.076070	0.0152
C0286	CAUCASICA	73.90	DAWNO	15	-0.278761	-0.053675	-0.241058	-0.055226	-0.191206	0.062590	-0.182501	-0.021244	-0.160886	-0.143818	-0.074290	0.075406	0.0112
K7296	CAUCASICA	89.12	DAWNO	15	-0.278442	-0.056766	-0.240766	-0.056796	-0.192530	0.065508	-0.180404	-0.018489	-0.157092	-0.145542	-0.072450	0.075093	0.0084
K7297	CAUCASICA	92.74	DAWNO	15	-0.281116	-0.057718	-0.243496	-0.058500	-0.186918	0.065472	-0.177344	-0.018431	-0.157615	-0.143052	-0.072105	0.075178	0.0088
K7299	CAUCASICA	96.73	DAWNO	15	-0.282563	-0.056790	-0.243678	-0.057327	-0.198204	0.066258	-0.182796	-0.020627	-0.157336	-0.144419	-0.063789	0.076403	0.0134
K7300	CAUCASICA	99.74	DAWNO	14	-0.276874	-0.054873	-0.239993	-0.055972	-0.201182	0.063680	-0.176305	-0.017968	-0.156560	-0.140172	-0.068037	0.075579	0.0080
K7301	CAUCASICA	97.09	DAWNO	15	-0.280622	-0.057491	-0.243973	-0.058123	-0.194998	0.064264	-0.181700	-0.018712	-0.158396	-0.142034	-0.068159	0.074790	0.0118
K7302	CAUCASICA	93.13	DAWNO	15	-0.282649	-0.056343	-0.245170	-0.057782	-0.191805	0.064294	-0.181149	-0.018053	-0.159234	-0.139278	-0.069117	0.074657	0.0117
K7303	CAUCASICA	63.04	DAWNO	15	-0.280266	-0.055567	-0.241321	-0.055388	-0.202110	0.061902	-0.183331	-0.019094	-0.153939	-0.140778	-0.071324	0.074786	0.0116
K7304	CAUCASICA	62.94	DAWNO	15	-0.281445	-0.057594	-0.244849	-0.058967	-0.189616	0.065282	-0.179864	-0.019530	-0.160173	-0.143760	-0.065182	0.075286	0.0106
K7305	CAUCASICA	93.68	DAWNO	15	-0.279400	-0.056454	-0.242250	-0.058100	-0.188927	0.065243	-0.179788	-0.019050	-0.158136	-0.143629	-0.065363	0.076811	0.0072
K7306	CAUCASICA	81.20	DAWNO	15	-0.279092	-0.056487	-0.240935	-0.056575	-0.193368	0.063271	-0.174725	-0.015682	-0.166532	-0.142206	-0.070703	0.074326	0.0105
K7307	CAUCASICA	89.00	DAWNO	15	-0.279692	-0.056622	-0.244607	-0.056338	-0.193030	0.064812	-0.184277	-0.020109	-0.158889	-0.140908	-0.068790	0.076286	0.0101

Figure 30 – Database window (editing)

Similarly, the database window title bar displays a context menu with a set of commands for manipulating the database itself (create a new one, open another, save under a different name, export). It's also worth noting that if you give the "Samples.csv" file a different name, MorphoXL will create a new, empty database with the previous name "Samples.csv" during operation. This allows you to more easily accumulate and organize information according to your research topics, if needed.

Geometric Morphometry Results Database - [Samples.csv]

Create Database

Open Database

Save

Save As...

Export to Excel

Close Database

Close All

SAMPLE	RACE	PURITY_R	POPUL	WINGS	X1	Y1	X2	Y2	X3	Y3	X4	Y4	X5	Y5	X6	Y6	X7
C0010	CAUCASICA	53.54	DAWNO	12	-0.281554	-0.057055	-0.248446	-0.059104	-0.190853	0.066314	-0.178567	-0.020108	-0.155228	-0.145718	-0.064382	0.077421	0.0140
C0011	CAUCASICA	94.07	DAWNO	12	-0.274799	-0.054103	-0.239696	-0.056397	-0.190002	0.065705	-0.180375	-0.021102	-0.153167	-0.142536	-0.069266	0.076740	0.0116
C0105	CAUCASICA	98.86	DAWNO	12	-0.276041	-0.054529	-0.244011	-0.056521	-0.190473	0.063662	-0.176882	-0.019886	-0.161287	-0.144199	-0.069142	0.076918	0.0157
C0109	CAUCASICA	95.19	DAWNO	10	-0.279282	-0.054984	-0.245580	-0.055502	-0.196309	0.063869	-0.178561	-0.018624	-0.161961	-0.142113	-0.067790	0.076863	0.0122
C0110	CAUCASICA	89.02	DAWNO	10	-0.276497	-0.053571	-0.241376	-0.055419	-0.192653	0.063631	-0.178904	-0.019543	-0.161429	-0.141127	-0.069433	0.074389	0.0100
C0111	CAUCASICA	93.96	DAWNO	9	-0.279759	-0.055896	-0.245826	-0.057985	-0.197134	0.063971	-0.178611	-0.019897	-0.164183	-0.143105	-0.070242	0.076193	0.0156
C0114	CAUCASICA	95.20	DAWNO	11	-0.279756	-0.055105	-0.244637	-0.057264	-0.195192	0.064185	-0.178589	-0.019080	-0.160177	-0.141541	-0.068871	0.075994	0.0129
C0115	CAUCASICA	97.20	DAWNO	12	-0.278916	-0.054182	-0.240700	-0.056735	-0.191004	0.065297	-0.179189	-0.019512	-0.159474	-0.141373	-0.066260	0.076854	0.0088
C0276	CAUCASICA	92.75	DAWNO	15	-0.278379	-0.054162	-0.242769	-0.055527	-0.197585	0.064065	-0.179594	-0.020121	-0.157903	-0.144611	-0.069871	0.076285	0.0146
C0277	CAUCASICA	87.72	DAWNO	15	-0.280660	-0.056143	-0.244867	-0.057410	-0.192269	0.066363	-0.183595	-0.022574	-0.154963	-0.146581	-0.072299	0.076827	0.0162
C0278	CAUCASICA	97.89	DAWNO	15	-0.279265	-0.056794	-0.244352	-0.056753	-0.186574	0.065871	-0.184738	-0.022654	-0.160966	-0.143843	-0.063477	0.077526	0.0143
C0280	CAUCASICA	97.79	DAWNO	15	-0.280476	-0.055932	-0.243249	-0.056737	-0.195438	0.061820	-0.178638	-0.019628	-0.161474	-0.144717	-0.064530	0.077068	0.0125
C0281	CAUCASICA	85.85	DAWNO	15	-0.276425	-0.053458	-0.245299	-0.058049	-0.193230	0.063917	-0.184058	-0.022554	-0.161906	-0.142826	-0.070174	0.076179	0.0104
C0282	CAUCASICA	97.69	DAWNO	15	-0.278595	-0.056137	-0.244443	-0.055958	-0.194273	0.064157	-0.182877	-0.020965	-0.158205	-0.142865	-0.071258	0.076993	0.0109
C0283	CAUCASICA	92.86	DAWNO	15	-0.279576	-0.056183	-0.242267	-0.056670	-0.195007	0.062944	-0.177938	-0.019024	-0.156581	-0.144975	-0.069796	0.075725	0.0099
C0285	CAUCASICA	77.91	DAWNO	15	-0.278412	-0.054397	-0.242799	-0.056371	-0.192175	0.064525	-0.183966	-0.021797	-0.157886	-0.145440	-0.072969	0.074982	0.0113
C0284	CAUCASICA	94.49	DAWNO	15	-0.278122	-0.056186	-0.243861	-0.057292	-0.199292	0.063998	-0.185657	-0.023446	-0.154138	-0.145167	-0.069153	0.076070	0.0152
C0286	CAUCASICA	73.90	DAWNO	15	-0.278761	-0.053675	-0.241058	-0.055226	-0.191206	0.062590	-0.182501	-0.021244	-0.160886	-0.143818	-0.074290	0.075406	0.0112
K7297	CAUCASICA	89.12	DAWNO	15	-0.278442	-0.056766	-0.240766	-0.056796	-0.192530	0.065508	-0.180404	-0.018489	-0.157092	-0.145542	-0.072450	0.075093	0.0084
K7298	CAUCASICA	92.74	DAWNO	15	-0.281116	-0.057718	-0.243496	-0.058500	-0.186918	0.065472	-0.177344	-0.018431	-0.157615	-0.143052	-0.072105	0.075178	0.0088
K7299	CAUCASICA	96.73	DAWNO	15	-0.282563	-0.056790	-0.243678	-0.057327	-0.198204	0.066258	-0.182796	-0.020627	-0.157336	-0.144419	-0.063789	0.076403	0.0134
K7300	CAUCASICA	99.74	DAWNO	14	-0.276874	-0.054873	-0.239993	-0.055972	-0.201182	0.063680	-0.176305	-0.017968	-0.156560	-0.140172	-0.068037	0.075579	0.0080
K7301	CAUCASICA	97.09	DAWNO	15	-0.280622	-0.057491	-0.243973	-0.058123	-0.194998	0.064264	-0.181700	-0.018712	-0.158396	-0.142034	-0.068159	0.074790	0.0118
K7302	CAUCASICA	93.13	DAWNO	15	-0.282649	-0.056343	-0.245170	-0.057782	-0.191805	0.064294	-0.181149	-0.018053	-0.159234	-0.139278	-0.069117	0.074657	0.0117
K7303	CAUCASICA	63.04	DAWNO	15	-0.280266	-0.055567	-0.241321	-0.055388	-0.202110	0.061902	-0.183331	-0.019094	-0.153939	-0.140778	-0.071324	0.074786	0.0116
K7304	CAUCASICA	62.94	DAWNO	15	-0.281445	-0.057594	-0.244849	-0.058967	-0.189616	0.065282	-0.179864	-0.019530	-0.160173	-0.143760	-0.065182	0.075286	0.0106
K7305	CAUCASICA	93.68	DAWNO	15	-0.279400	-0.056454	-0.242250	-0.058100	-0.197878	0.065243	-0.179788	-0.019050	-0.158136	-0.143629	-0.065363	0.076811	0.0072
K7306	CAUCASICA	81.20	DAWNO	15	-0.279092	-0.056487	-0.240935	-0.056575	-0.193368	0.063271	-0.174725	-0.015682	-0.166532	-0.142206	-0.070703	0.074326	0.0105
K7307	CAUCASICA	89.00	DAWNO	15	-0.279692	-0.056622	-0.244607	-0.056338	-0.193030	0.064812	-0.184277	-0.020109	-0.158889	-0.140908	-0.068790	0.076266	0.0101

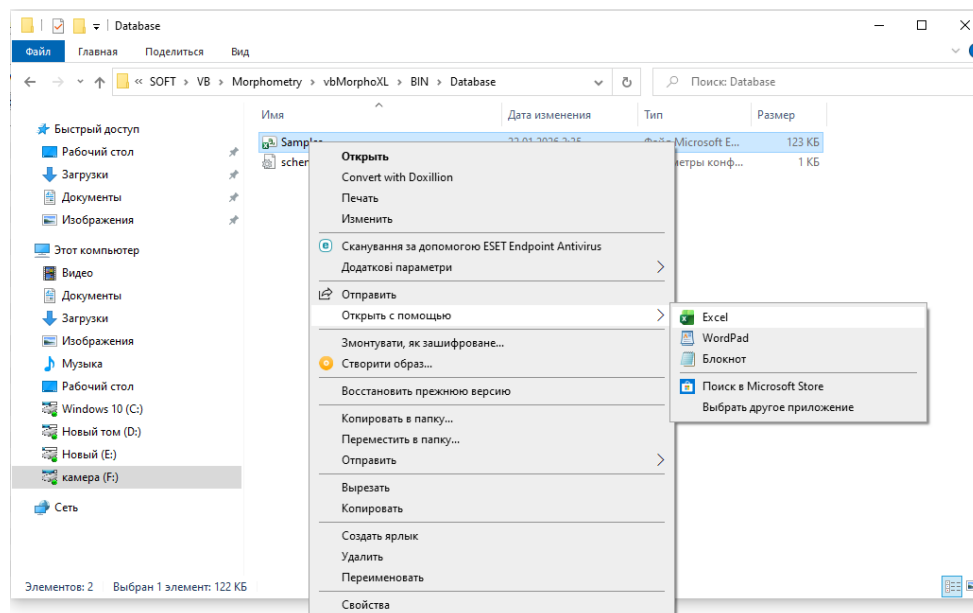


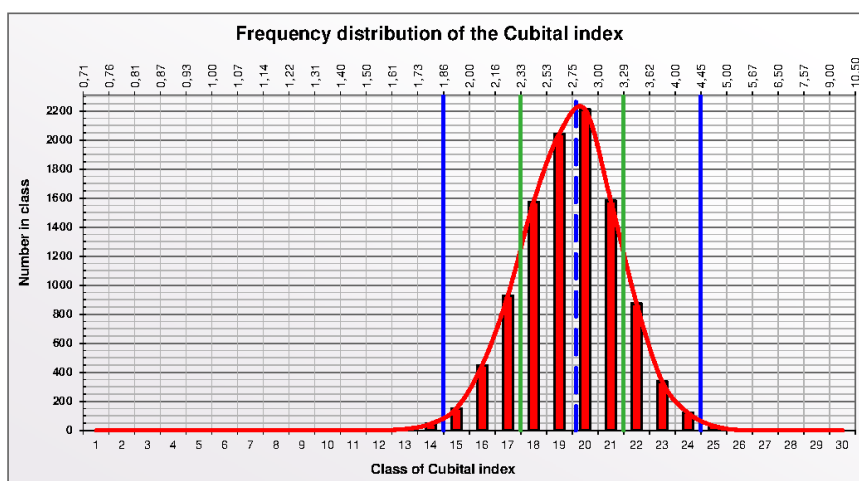
Figure 32 – Opening a *.csv database in Excel

8. Population research, determination of breed ranges

A situation may arise when, for one of the breeds, it is necessary to specify whether to determine the limits of the breed range for a certain morphometric index. For this, it is necessary to perform morphometric studies of bees of this subspecies within their natural range and to construct a variation curve for the required morphometric feature. The method of determining the breeding range based on the variation curve of the population is described in the work of F. Ruttner "Breeding technique and selective selection of bees". Below is an example of determining the boundaries of the cubital index within the natural population of Carpathian bees.

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Generalizing variation curves based on the results of morphometric studies of wing venation in Carpathian bee colonies (including Rakhivskiy, Vuchkivskiy, Synevir, Hoverla breed types) as of 2019. In general, 10363 bees in 406 colonies were studied.



\bar{M}	Min	Max	SD	Cv	Confidence interval (95%)	
					Min	Max
2,786	1,495	5,552	0,459	16,5%	1,886	3,686

Full range of values - classes 15...24, covers 99,12% bees
 Breed range - classes 17...22, covers 88,92% of bees
 Range of typical values (limit $\geq 66\%$ of bees) - classes 18...21, covers 71,52% of bees

Any other necessary morphometric characteristics of natural bee populations within their natural range are determined by a similar method.

9. License

MorphoXL is shareware software. The unregistered version does not impose any time restrictions on the user, but the functionality of the application will be limited only until the breeding suitability is determined, according to the 8-point studies of worker bees. At the beginning of use, the user is given a trial period lasting one day, after which the application will automatically be switched to limited functionality mode. You can get information about the application registration status in the dialog box using the "About" menu command.

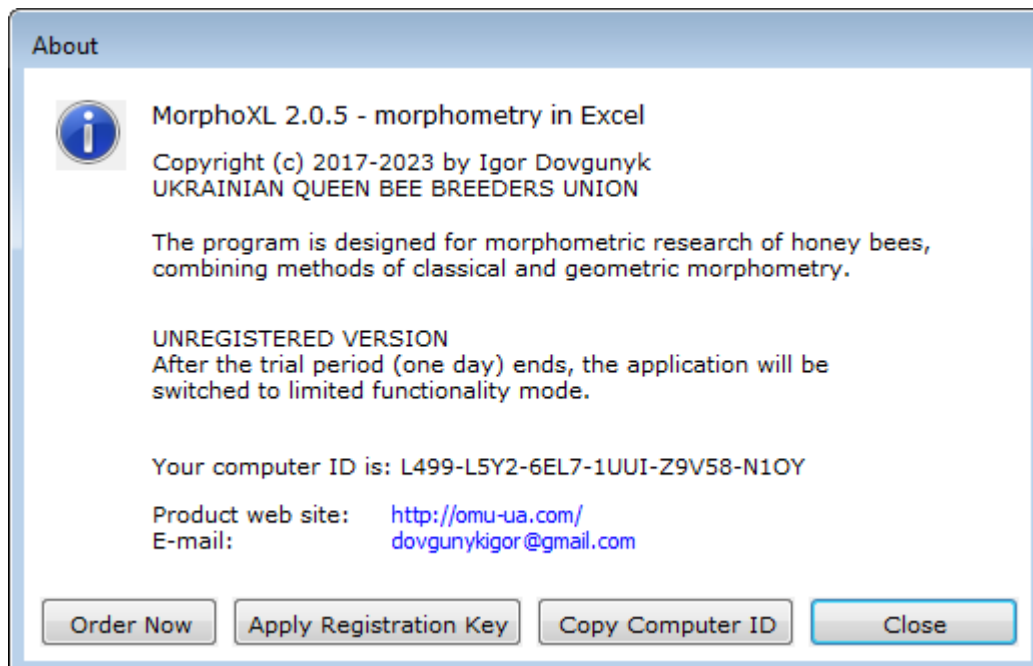


Figure 30 – About dialog box

A registration key is used to register the application. Each individual computer has its own unique identifier "Computer ID". To receive the registration key, you need to send the user's computer ID to email address specified in the dialog box above. If you double-click on this e-mail address and on the condition that there is a configured mail application on the computer (for example, Microsoft Outlook), then the application will start with an already built-in electronic letter template, along with an identifier. The identifier can also be downloaded to the clipboard by clicking the "Copy Computer ID" button in the dialog box. Your message should include the following information:

Computer ID: ...

Your name: ...

Your email: ...

After receiving the registration key, it must be loaded into the MorphoXL application. To do this, in the above dialog box, you need to click the "Apply Registration Key" button, where in the next dialog box specify the received registration key file "MorphoXLKey.dat". After successful registration and reboot, the application will go into full-featured mode.

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Igor Dovgunyk, Lviv, 2017-2026

UNION OF UKRAINIAN QUEEN BEE BREEDERS