



MorphoXL, version 2.0.0

User manual

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1. General provisions

1.1. The program 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. Field of application: selection of honeybees.

1.3. Functionality: the program 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 program 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](#) program, which is recommended in [COLOSS](#) for the morphometric study of bees. This allows you to use specialized classifiers from another developer in the MorphoXL program.

1.4. The executable file of the program is a Microsoft book Excel named "MorphoXL.xls". The **MorphoXL** program was developed in the Microsoft Excel-2003 environment, using automation with VBA 6.5 (Microsoft Visual Basic for Applications), and the **rtIMXL.dll** runtime library created in the VB 6 environment ((Microsoft Visual Basic). To work in 64-bit versions of **Office**, the program additionally uses the **COM32on64.exe** automation server . The program functions normally in the EXCEL environment of all subsequent releases of "office software packages", including **Office-2016** - both in 32-bit and 64-bit versions.

1.5. For the correct operation of the program, **the medium level of security** must be selected in the Excel environment (Figure 1a, 1b) , and when opening the program, on the warning of the security system, click the "**Enable macros**" button in the Excel 2003 dialog box , or "**Enable content**" in newer versions of Excel .

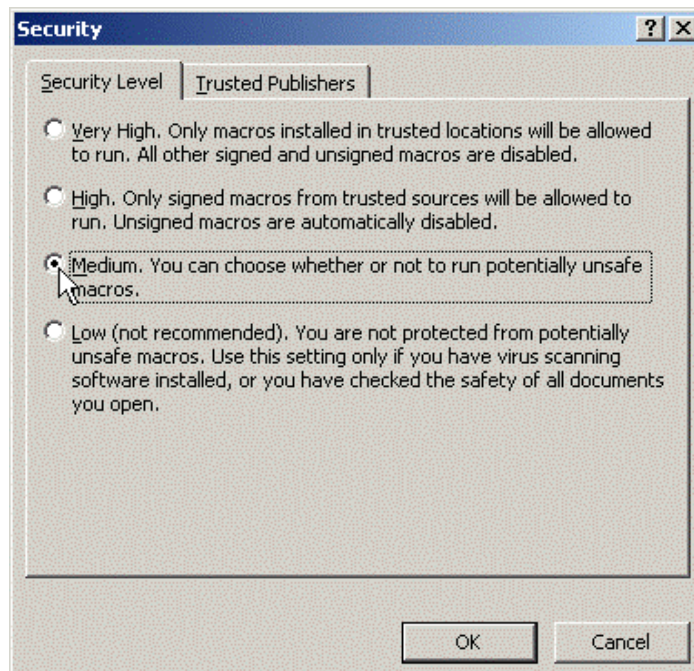


Figure 1a – Security settings in Excel 2003

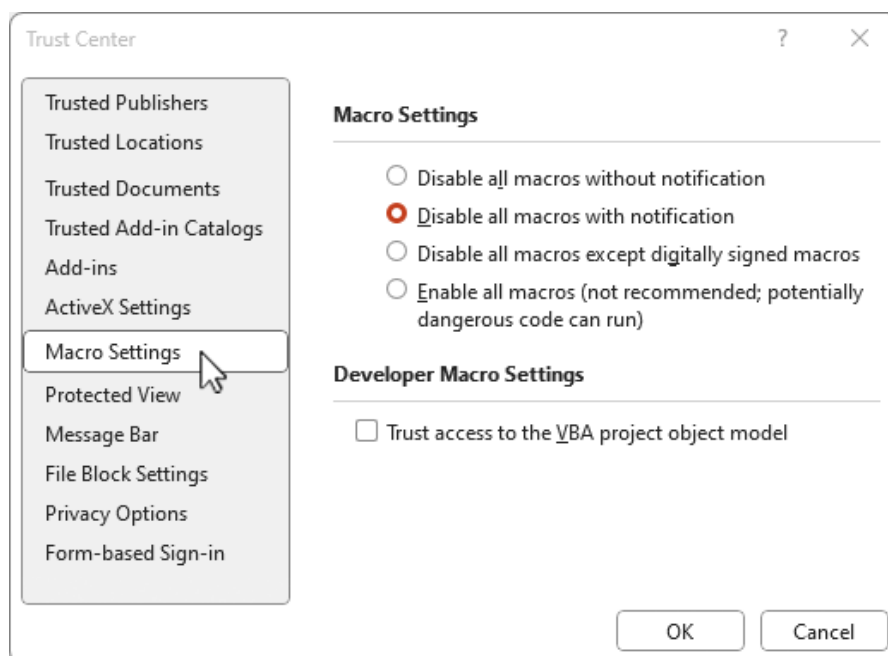


Figure 1b – Security settings in newer versions of Excel

1.6. 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 program TpsDig2, or any other program that generates files of a similar format (*.tps). We have also developed a similar program called WingsDig, which specializes exclusively in bee morphometry and is focused on capturing images of bee wings using a USB microscope. The WingsDig program has its own, quite detailed instructions for use, so the following will be mostly about work in the TpsDig program.

1.7. 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 discoidal 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, Izmailov index, and Kuzmych 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 program and in the DAWINO style (BeeMorph program, Czech Republic).

1.8. When working in the **tpsDig2** program, 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.

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

1.10. When working with a USB microscope, as a rule, obtaining images is performed in specialized programs that are provided with the device. In the settings of these programs, 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 programs, the program **WingsDig** has its own module for working with USB - a microscope where the frame size is 1600x1200 as the default setting.

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

1.12. If necessary, all pages of the MorphoXL program (sheets of the Excel book) can be printed or saved to a file by standard means (File\Print...). In the "Print Setup" dialog box, either a real printer or a program that outputs information to a file (virtual printer) is selected from the list as the name of the printer. For example, Microsoft Office Document Image Writer, Adobe Acrobat Writer, or PDFCreator. Also, the MorphoXL program has a corresponding menu command that implements the saving of the report in the "PDF" format by standard Microsoft means Office, or the virtual printer "PDFCreator", if it is installed on the system. At the same time, the program automatically forms the content and actual informative content of the report.

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** program on separately, and the results are saved in a common file with the extension "*.tps".



Figure 3 – Digital USB microscope

Note . **WingsDig** program 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** program (or **WingsDig**). The appearance of the **TpsDig2** program icon on the computer desktop is shown in Figure 4.



Figure 4 – Icon for launching the TpsDig2 program

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

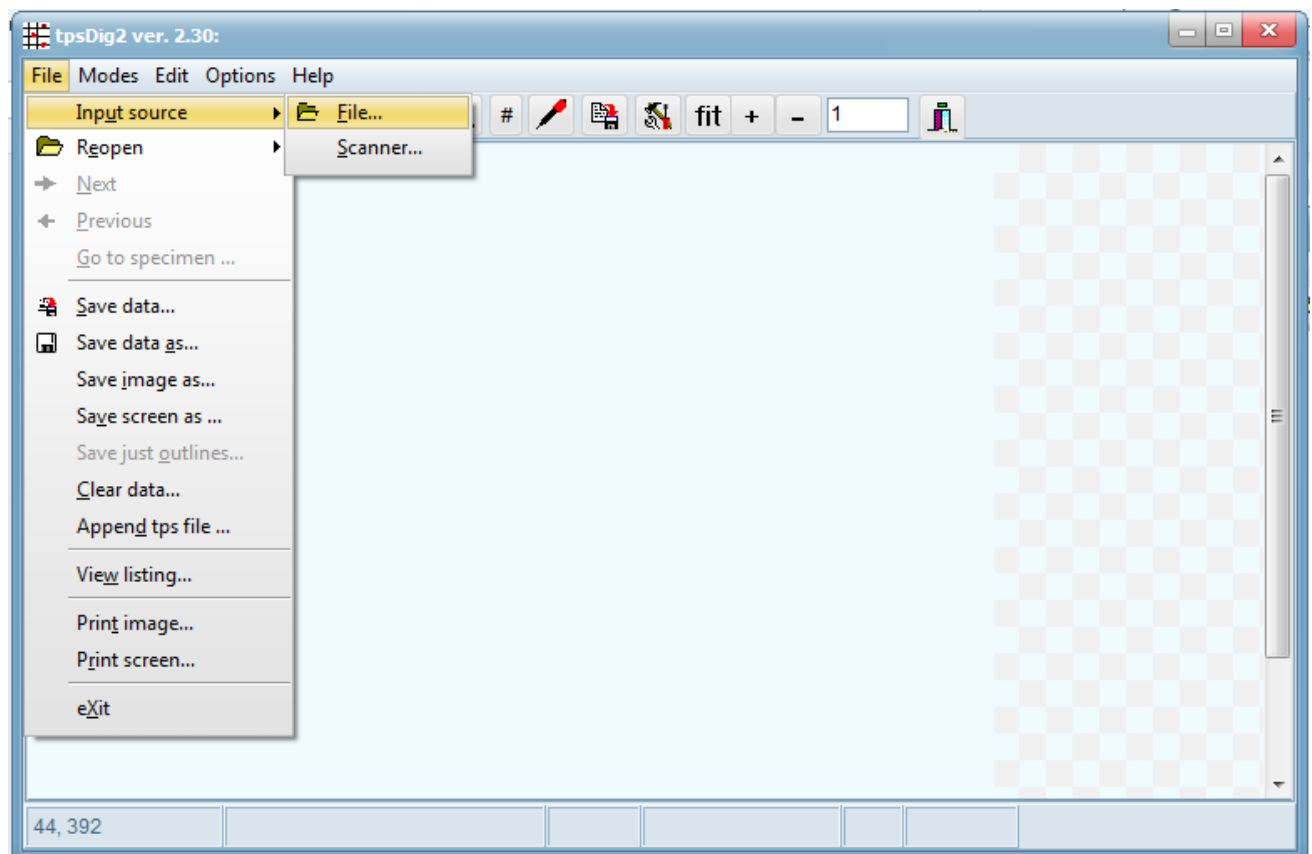



Figure 5 – General view of the TpsDig2 program

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**.

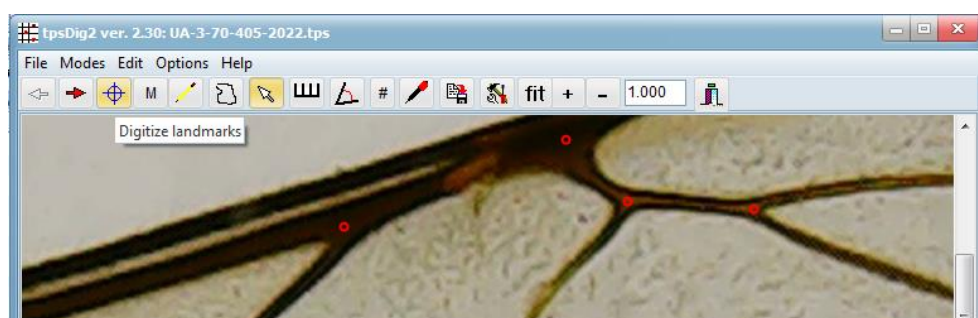


Figure 6 – Selection of the point placement mode

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:

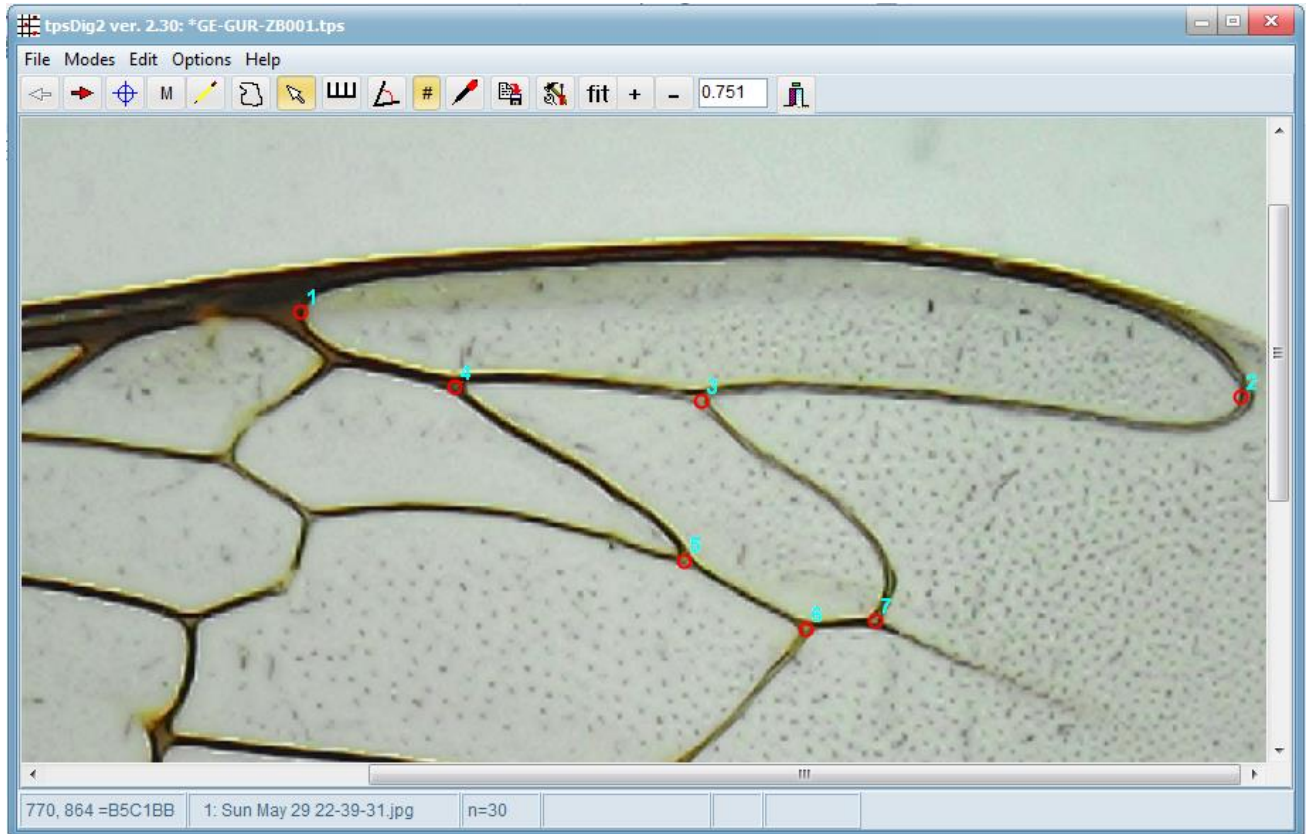


Figure 7 – The sequence of placing points in 8-point morphometry

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.

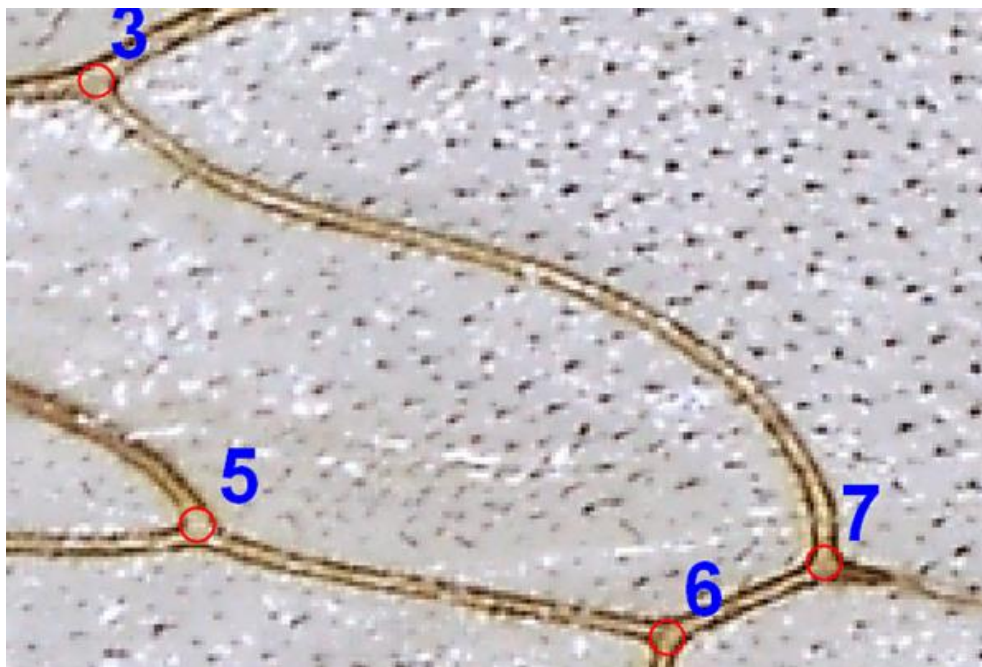



Figure 8 – Examples of positioning landmarks 3 - 8

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

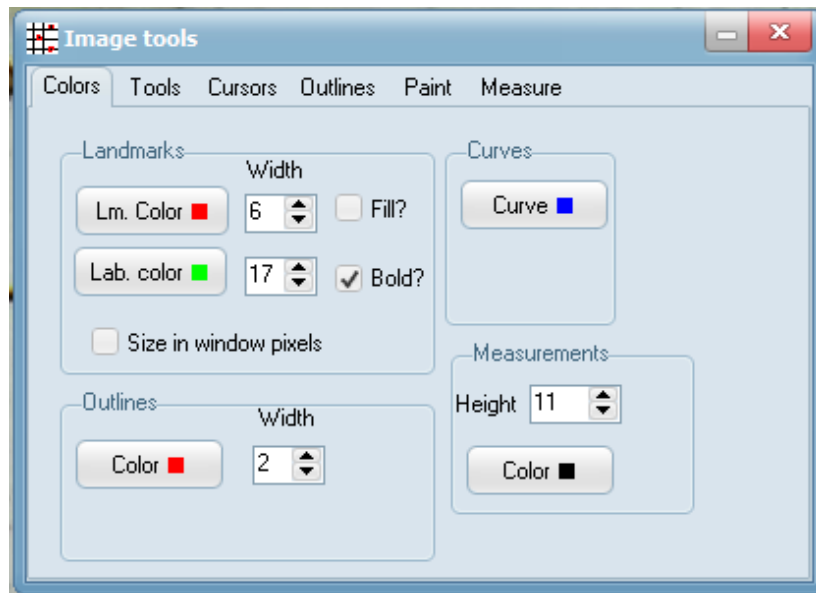



Figure 9 – Settings window

If necessary, the location of the point on the wing can be edited by changing the mode of operation of the program 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.

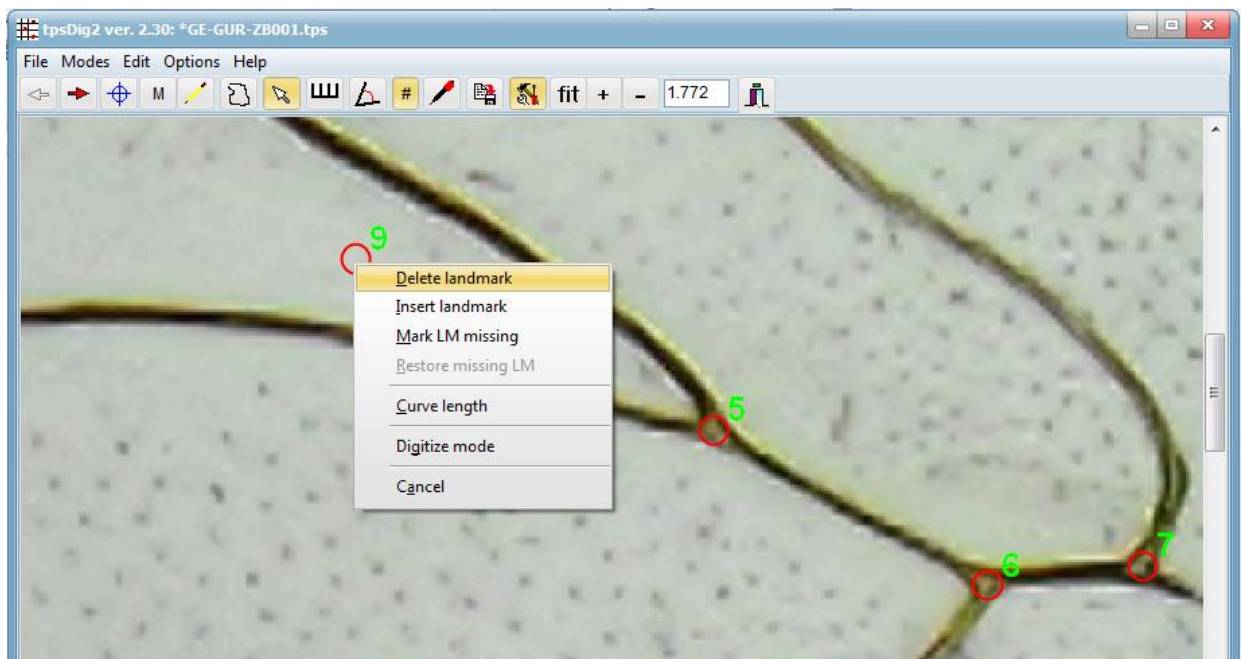



Figure 10 - Editing a Landmark

If the lack of a landmark is detected when the "tps" file is reopened, then the tpsDig2 program 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.

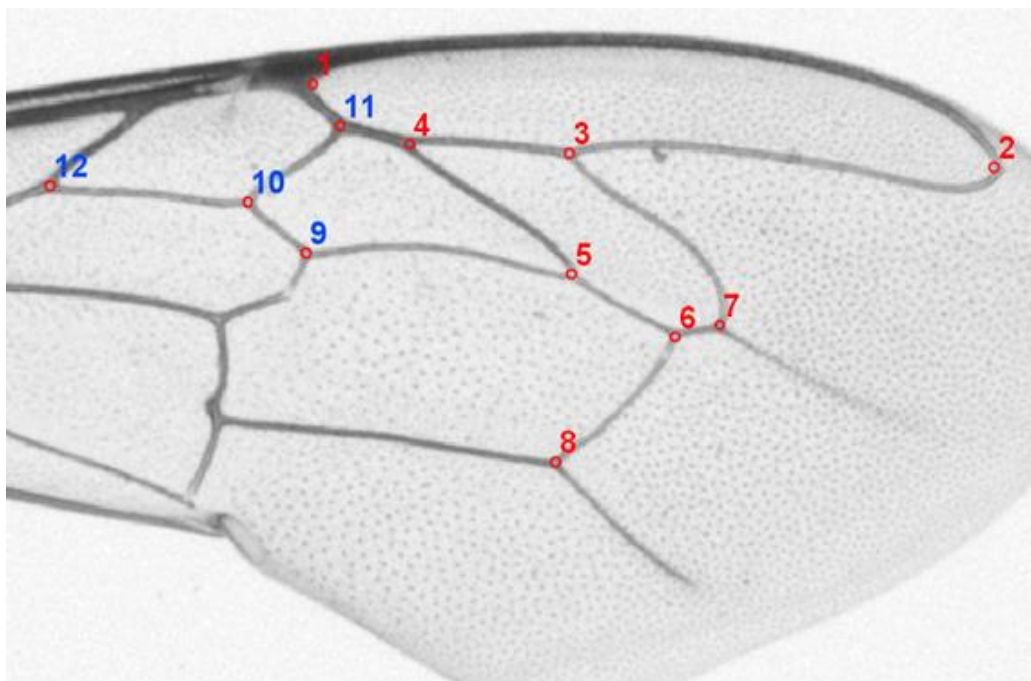


Figure 11 – The sequence of placing landmarks in 12-points morphometry

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

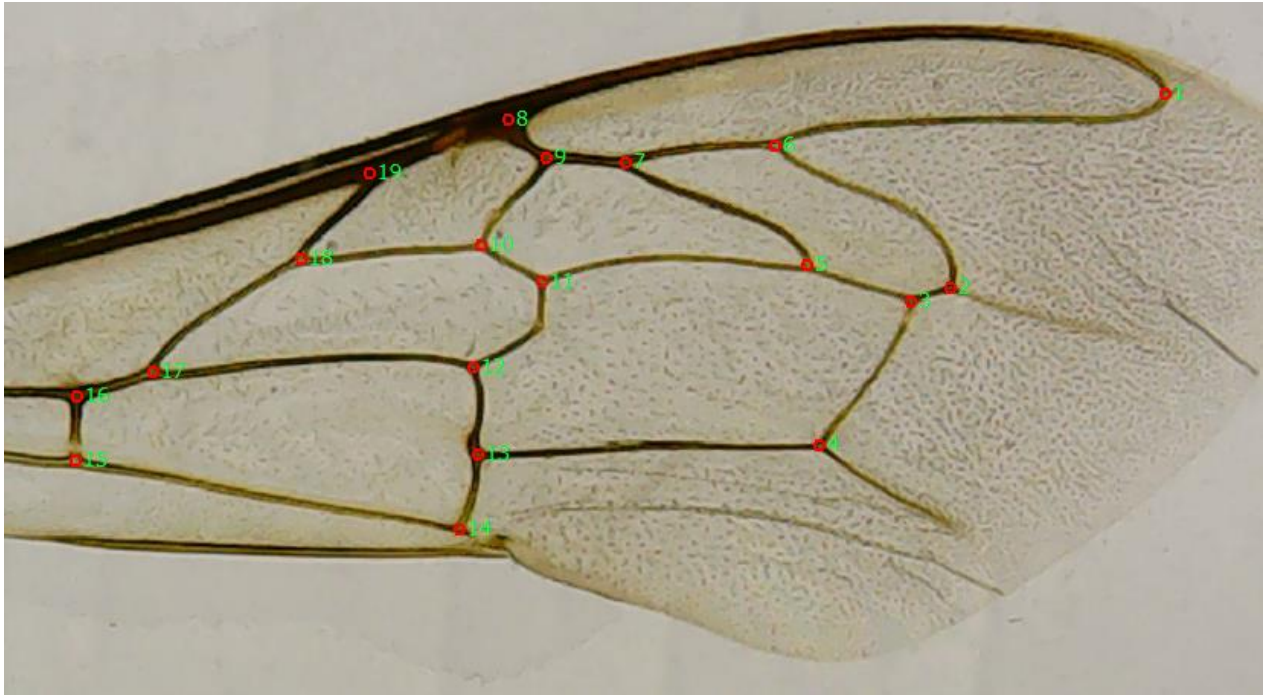


Figure 12 – Order and location of points for 19-landmark morphometry according to the DAWINO protocol

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

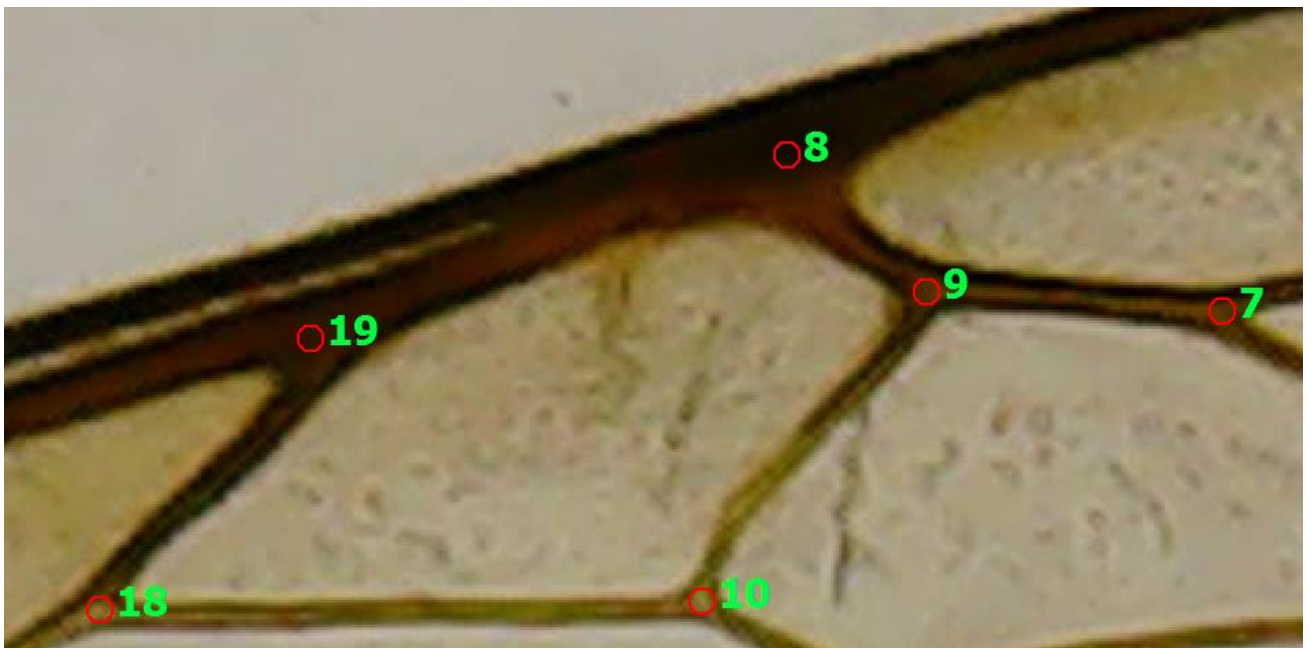


Figure 13 - The feature of the location of landmarks 8 and 19 according to the DAWINO protocol

IdentiFily program 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).

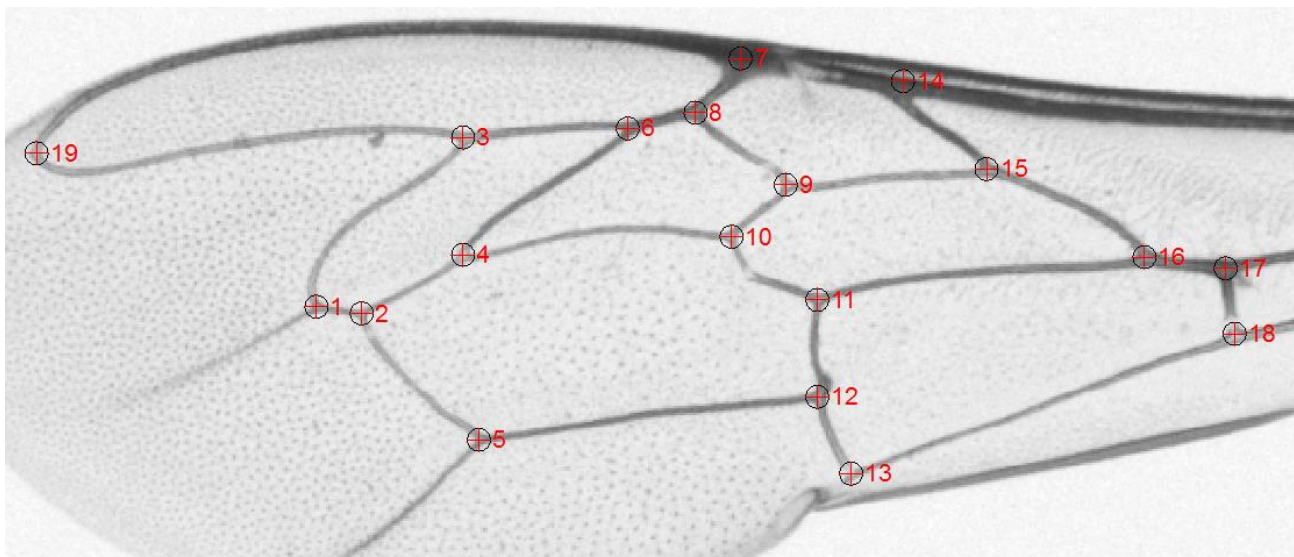


Figure 14 – Order and location of points for IdentiFily-style 19-landmark morphometry

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.

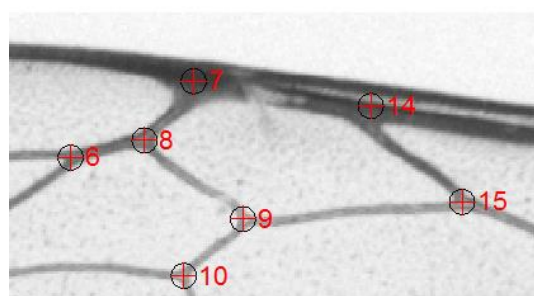




Figure 15 – IdentiFily-style layout of points 7 and 14

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** program 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 program's message that such a file already exists. The next time such a complex file is opened by the **tpsDig2** program,

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

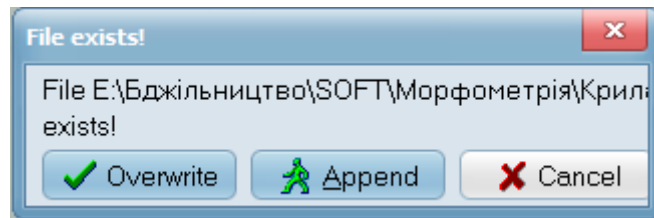


Figure 16 – Saving the digitization results to a "tps" batch file

It should be noted here that, unlike **tpsDig2**, the **WingsDig** program 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 program "MorphoXL"

5.1. Start Excel and open the "MorphoXL.xls" file in it. The same effect can be achieved by double-clicking on the "MorphoXL.xls" file in the window of any file manager. If the security level is set as recommended in section 1.5, the Excel environment will display a security message about the presence of macros in the book "MorphoXL.xls". If your version of the program is obtained from legal sources, then feel free to press the **"Enable macros"** button (see **Figure 17a**), for Excel 2003 and earlier versions. For newer versions, starting with Excel 2007, press the **"Enable content"** button (see **Figure 17b**). **Otherwise, the program will not work.**

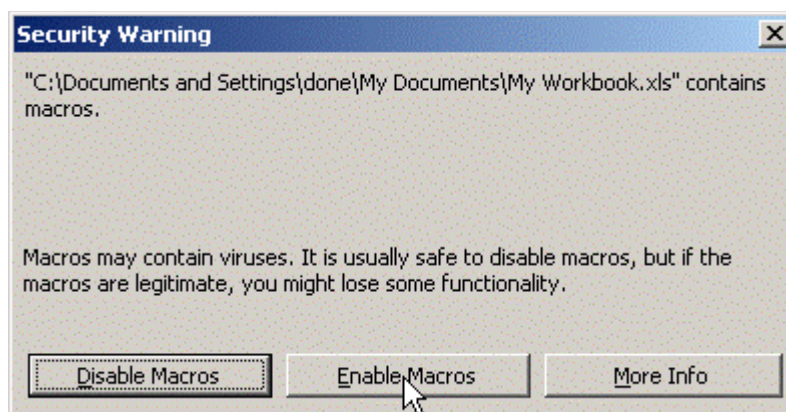


Figure 17a - Security warning in Excel 2003

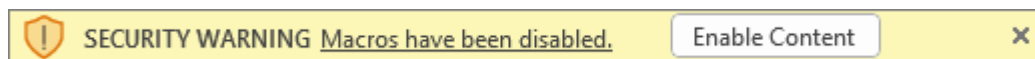


Figure 17b - Security warning in newer versions of Excel

5.2. To start work, you need to call up the program menu with a set of appropriate commands. To do this, you need to click the left mouse button on the icon



or pressing the **"Ctrl"** and **"t"** keys on the keyboard at the same time (the keyboard layout must be English).

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

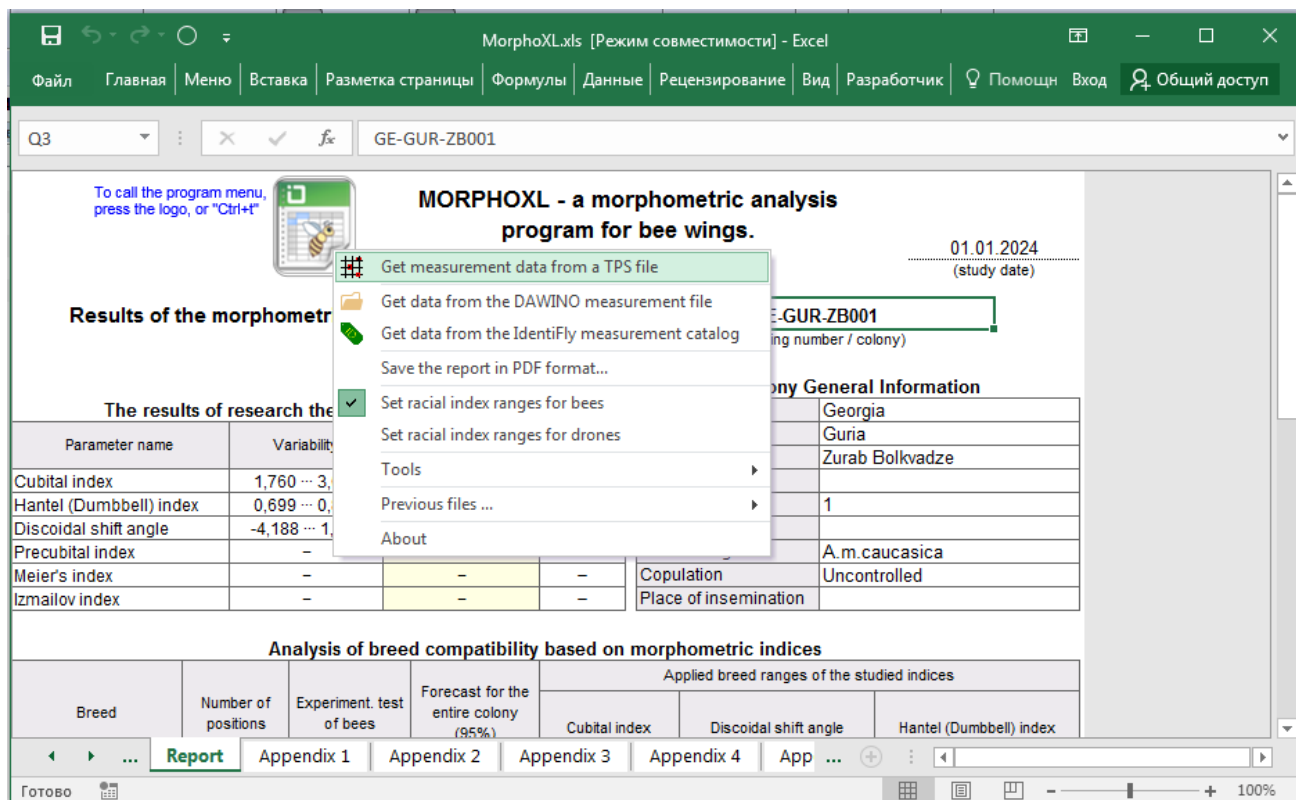


Figure 18 - Execution of the command to retrieve data from TPS files

5.3. The program will offer to open a file with point coordinates that was created earlier in the editor "tps" files - see **Figure 19**.

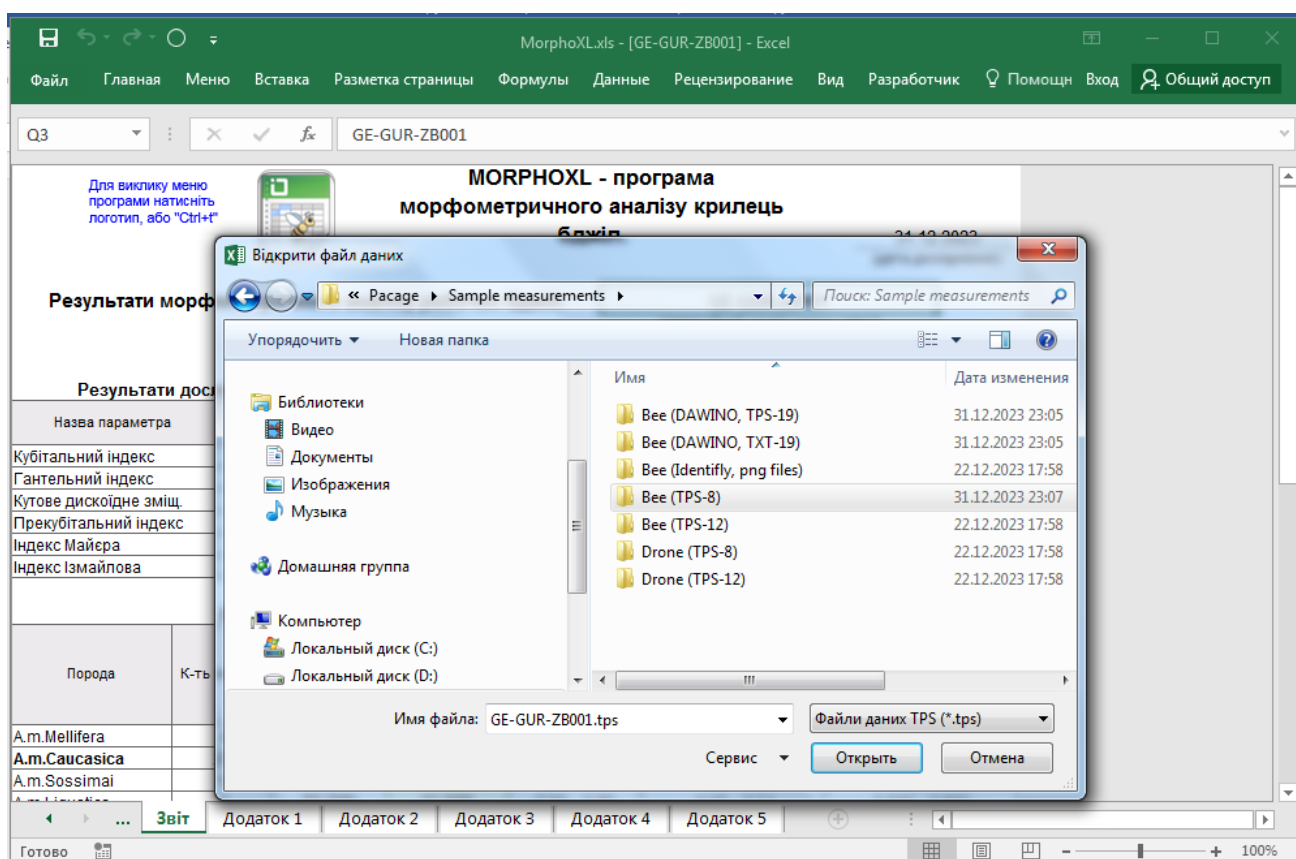


Figure 19 – Document opening dialog box

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

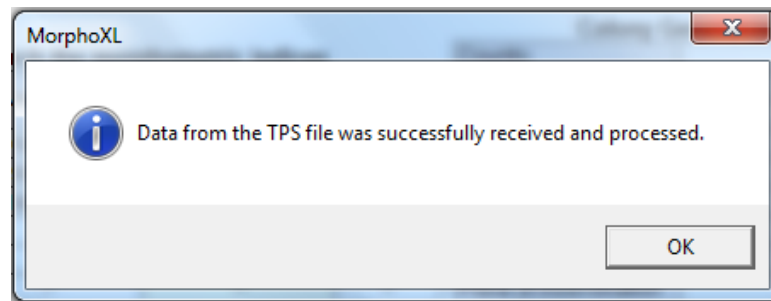


Figure 20 – The program message about the successful execution of the analysis

In a similar way, the program 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 program has a separate command for processing the results of digitization performed in the **IdentiFly** program, 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** program not a separate file, but the path to the directory where the wing image files processed in the **IdentiFly** program 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 program 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.

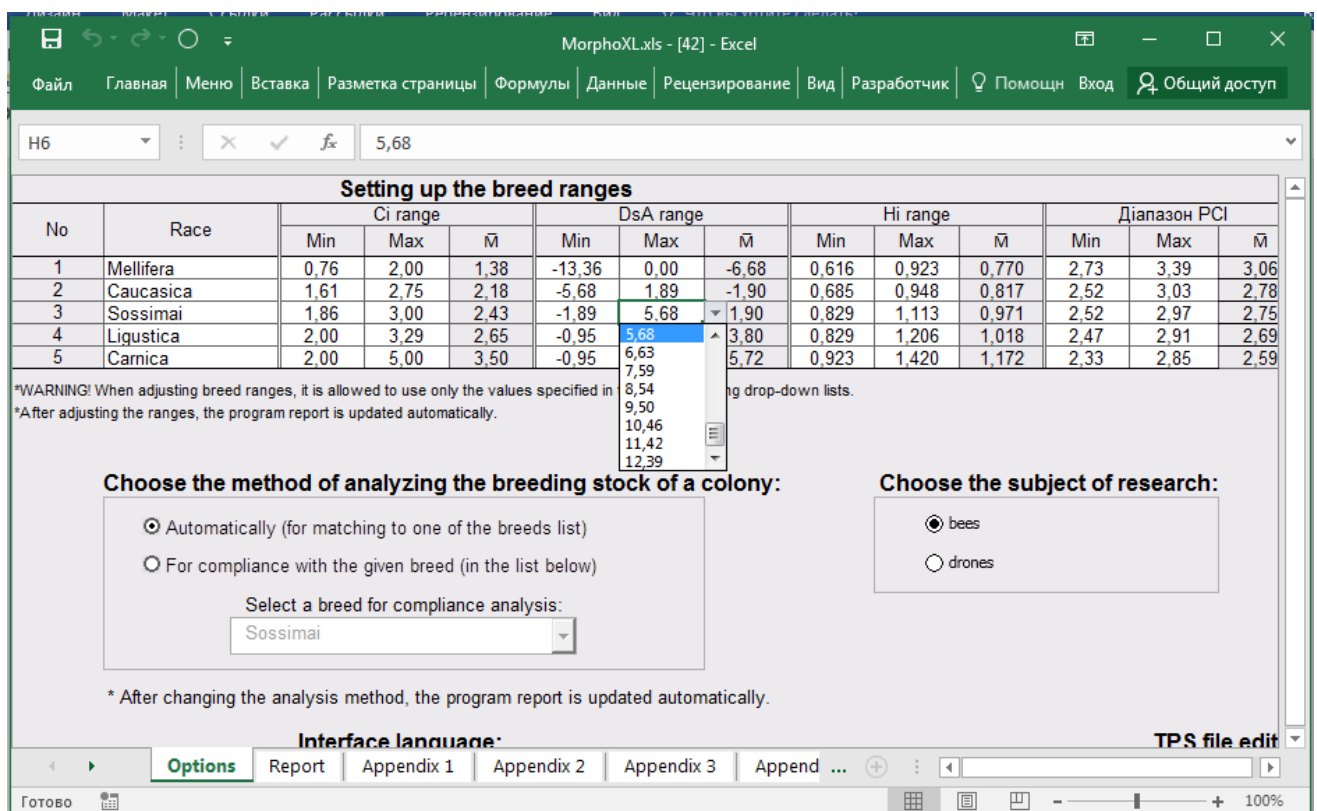


Figure 21 – Changing the boundary of the breed range

When changing breed ranges, the program 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 program 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 the breed ranges, the analysis results are updated automatically. The user can save his own settings of breed ranges in the program settings file (the "MorphoXL.ini" file) by clicking the "Save index changes" button. This will be useful when the user changes the research subject to "bees/drones" and the program will automatically adjust the limits of the breed ranges accordingly. Usually, the program sets the default values there, but if there are custom settings in the "MorphoXL.ini" file, they are given priority. You can also change the research subject "bees/drones" using the program menu.

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

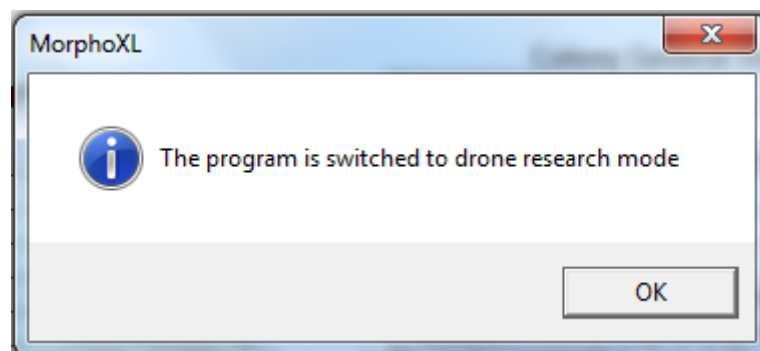


Figure 22 - Notification of a change in the subject of research

5.6. On the "Options" sheet, you can also change the interface language of the MorphoXL program by choosing it from the list of available languages (Figure 23). All available language files with the extension "*.lng" are in the program folder.

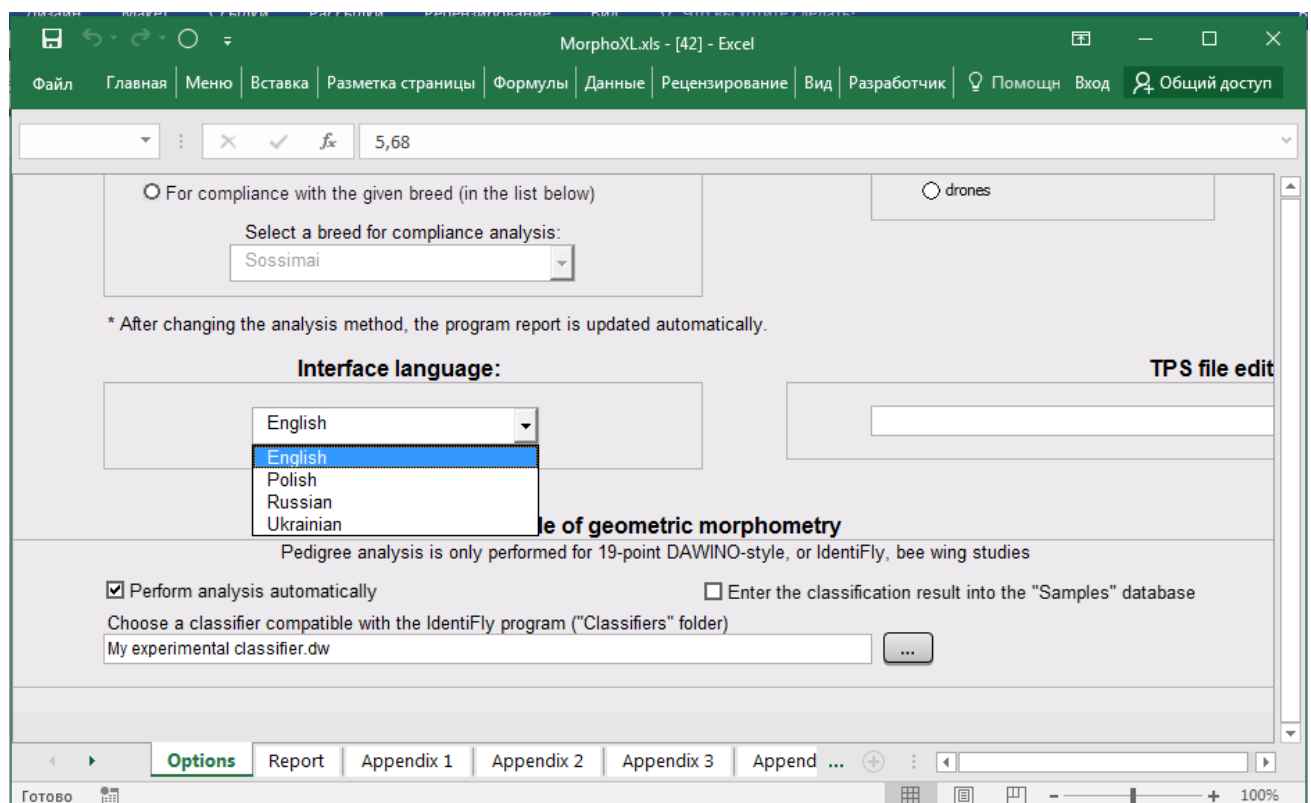


Figure 23 – Changing the interface language

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.

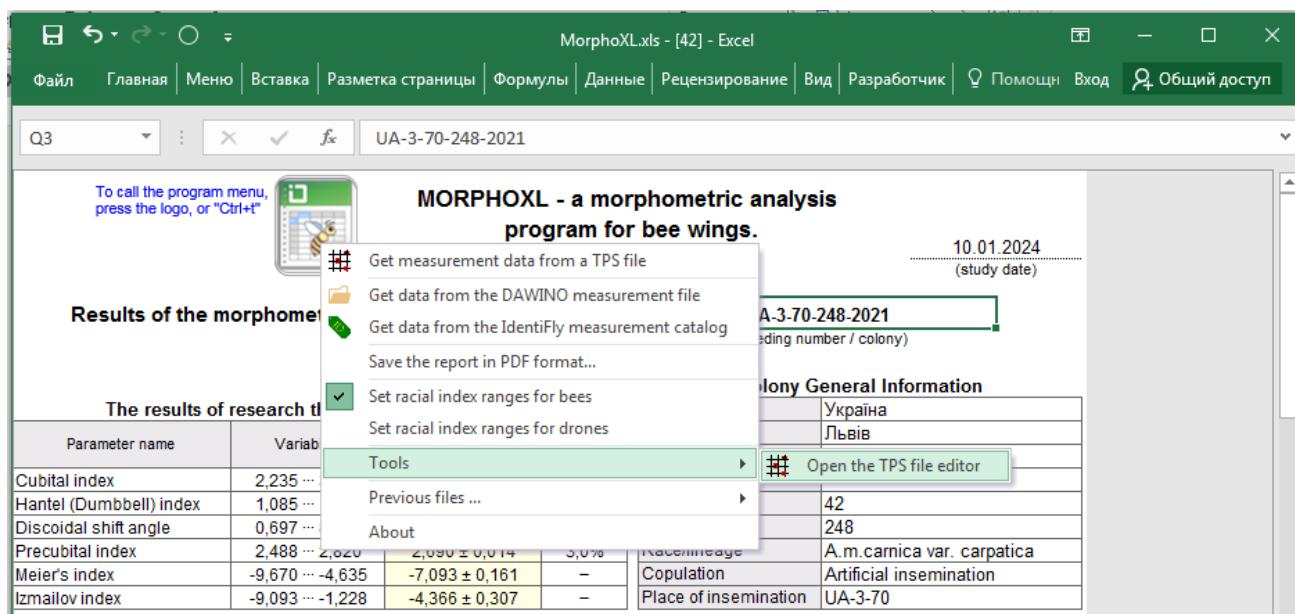


Figure 24 – Additional tools in the MorphoXL menu

The program 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.

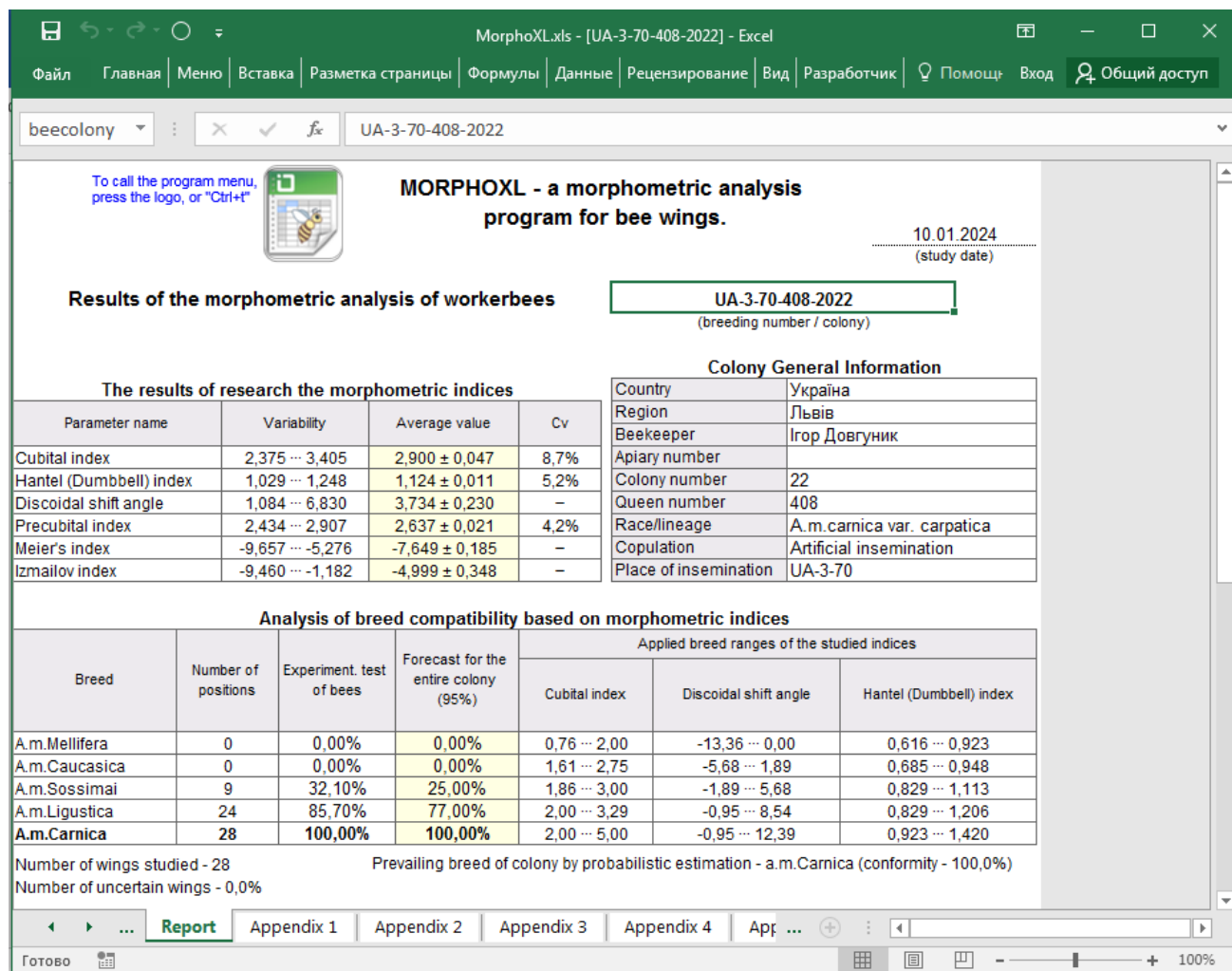


Figure 25 – MorphoXL main page

Possible recommendations of the program 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 program 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 program 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 program 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 "undetermined" wings. In this case, the entries for these wings will be shown in red font on the "Appendix 2" sheet. In this case, it is necessary to once again check the correctness of the positioning of the landmarks on the images of these problematic wings. So, open the "tps" file editor and upload the desired file to it. In the editor, we find the problematic wing and check the quality of positioning - since it is quite likely that the fault is not the wing itself, but a not very skillful hand, or fatigue, or inattention. If you see an error, correct it and run the sample again.

6.4. If the program complains about too high hybridization or too low integrity of the colony, then we look for the reasons on one of the three graphs (see Figure 26), which are located at the bottom of the "Report" sheet. In this case, we look at the graph with the required index, where we find points that go beyond the boundaries of the red (confidence intervals) or even the blue (breed ranges) rectangle. We bring the cursor to each of them one by one, and it shows the index values for this point in brackets: first along the horizontal axis, then along the vertical axis. Remember these indexes, go to the "Appendix 2" sheet. Call up the search dialog box (press the "Ctrl" + "F" keys at the same time), enter the value of the desired index there and find the desired wing. Next, we analyze the correctness of its digitization in the **tpsDig2** program - as already described above, in clause 6.3.

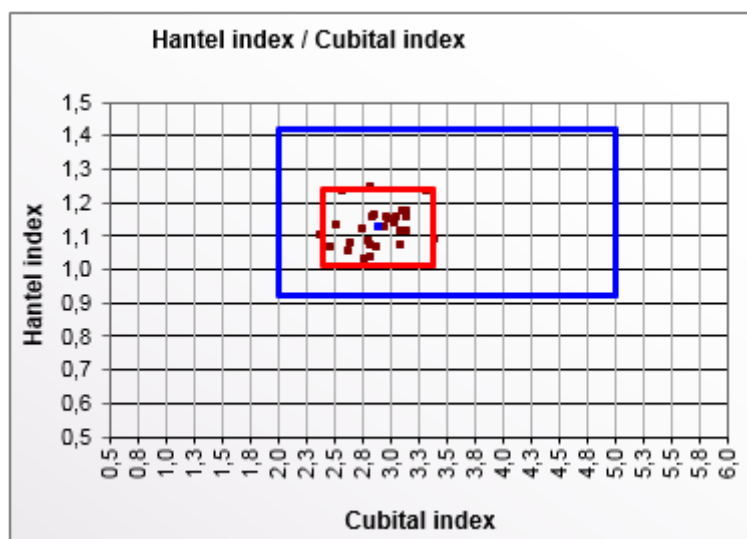


Figure 26 – Distribution of Hi/Ci (breed ranges and confidence intervals for two indices)

6.5. If all possible reasons have been exhausted and this did not lead to an improvement in the evaluation by the program, 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. In the graph below, this rule is not

followed to the left of the "clean line range". The dominant breed in this hybridized colony was identified by the program as "A.m.sossimai". The conclusion of the program is "the uterus of this family is not suitable for reproduction."

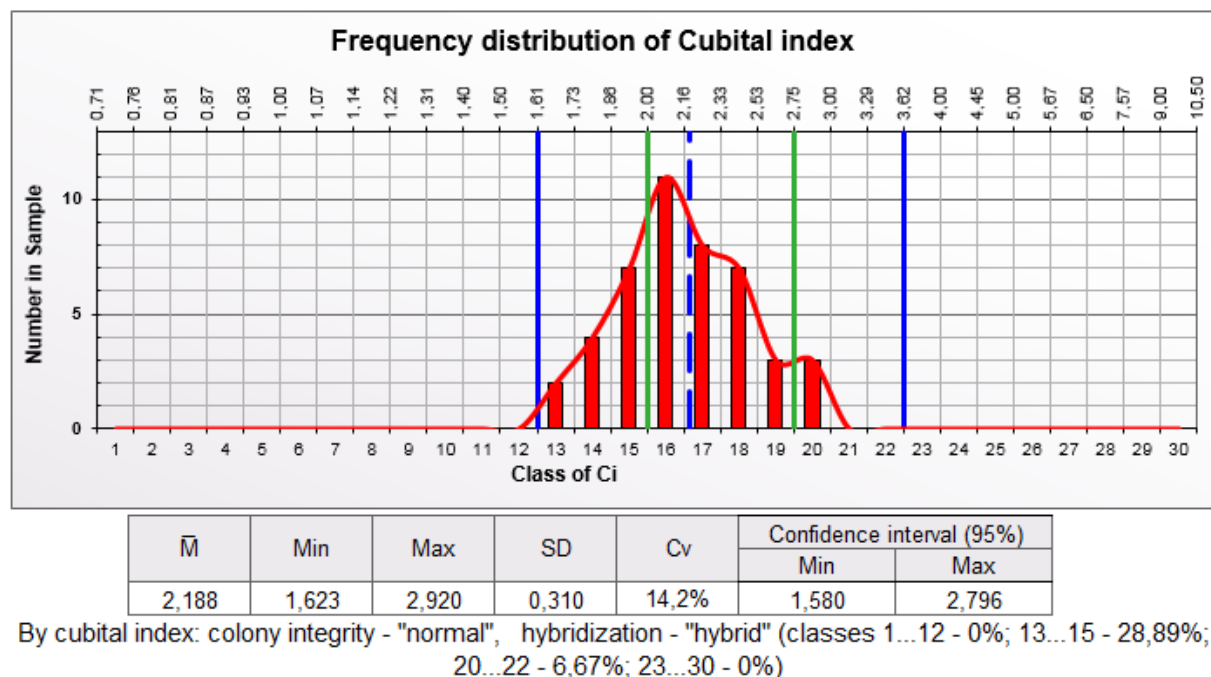


Figure 27 – Variation curve of Cubital index

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**, the **Izmailov index**, and the **Kuzmych 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.

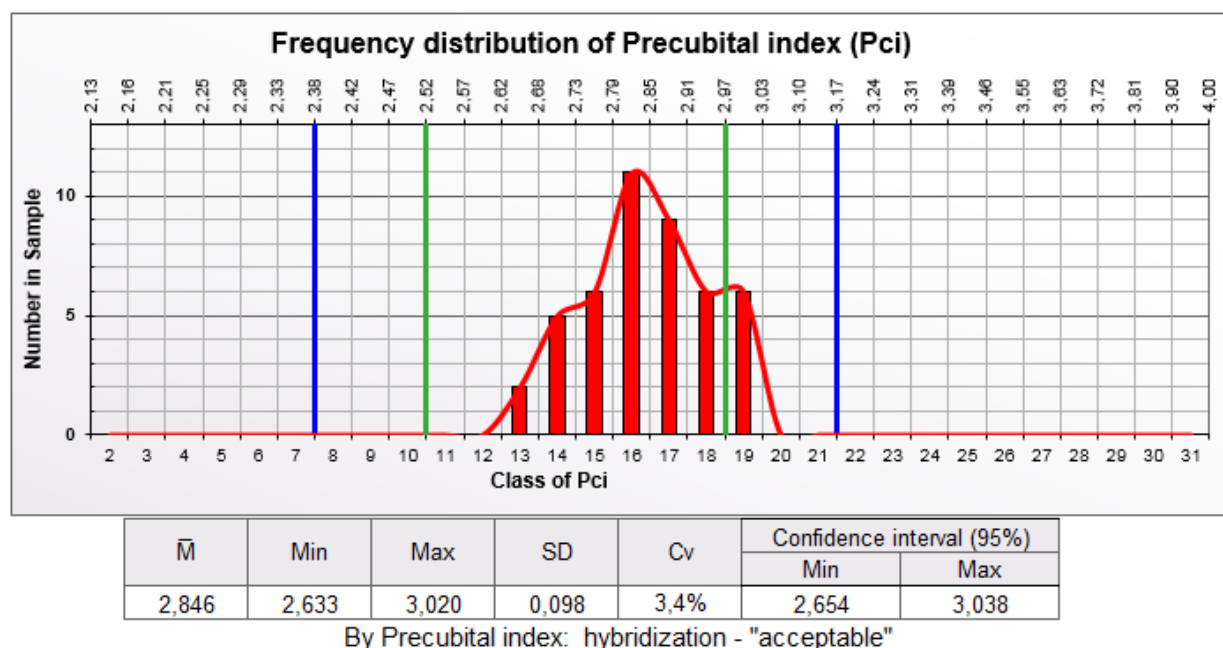


Figure 28 – Variation curve of additional Precubital index

It should be noted here that the above precubital index (Fig. 28), as well as the Kuzmych index, 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 program on the "Appendix 5" sheet.

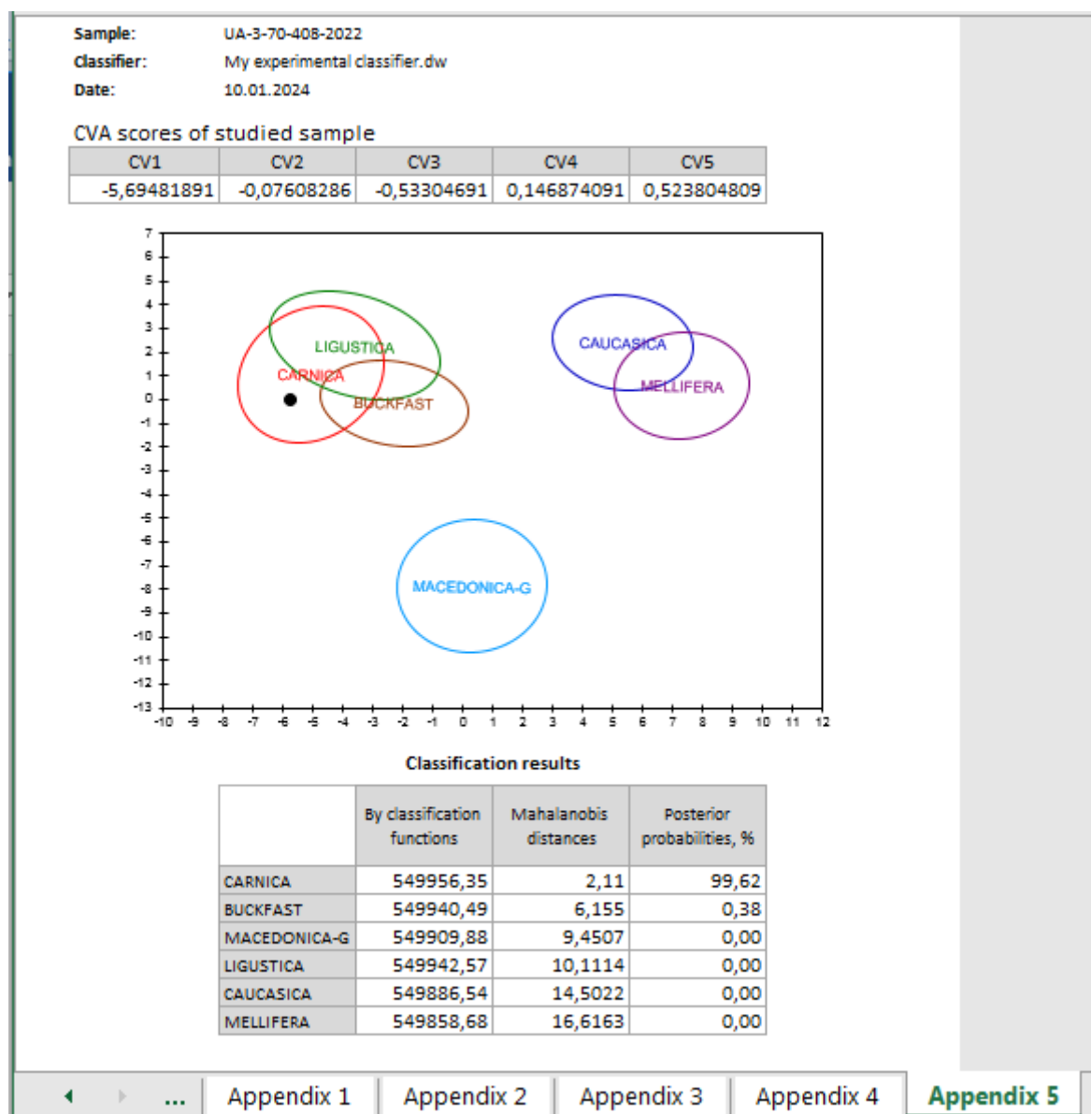


Figure 29 – Geometric morphometry module report

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

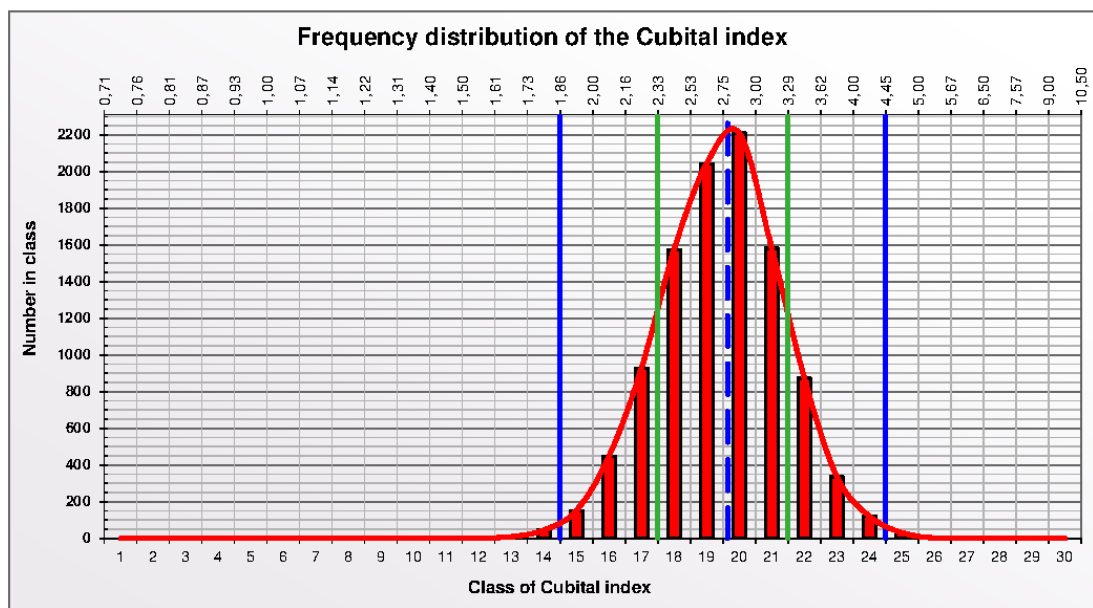
two-dimensional plane. Therefore, the final and comprehensive results of the analysis are in the "Classification results" table, and the graphic part only partially illustrates them.

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.



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 program 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 program will automatically be switched to limited functionality mode. You can get information about the program registration status in the dialog box using the "About" menu command.

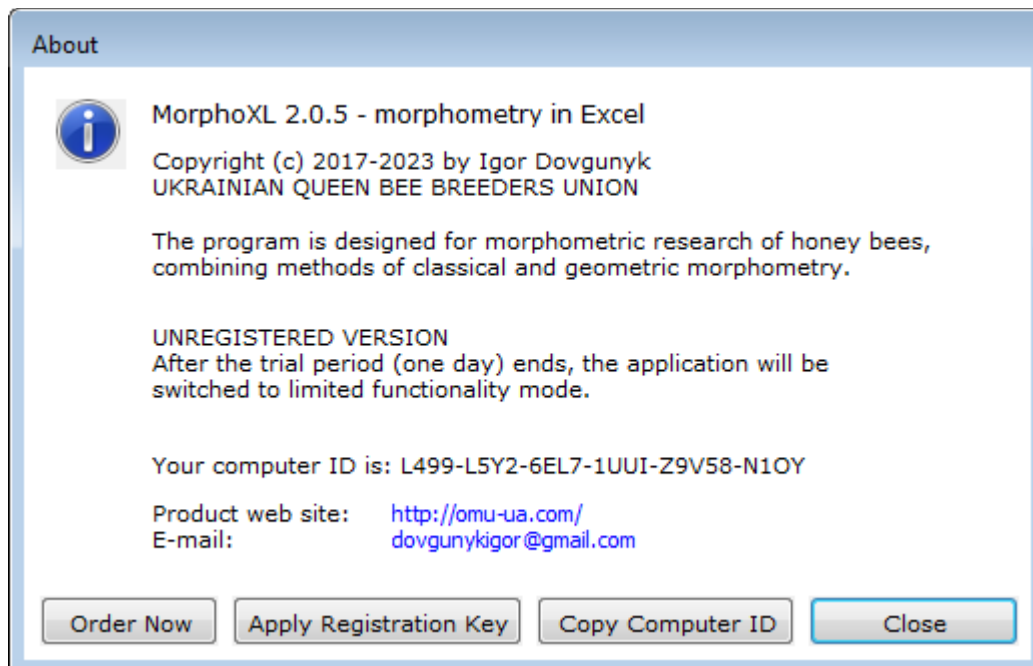


Figure 30 – About dialog box

A registration key is used to register the program. Each individual computer has its own unique identifier "Computer ID". To receive the registration key, you need to send the 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 program on the computer (for example, Microsoft Outlook), then the program 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 program. 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 program will go into full-featured mode.

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Igor Dovgunyk, Lviv, 2017-2023

UNION OF UKRAINIAN QUEEN BEE BREEDERS