

HIP 1.1

High-sensitive Innominate Processing

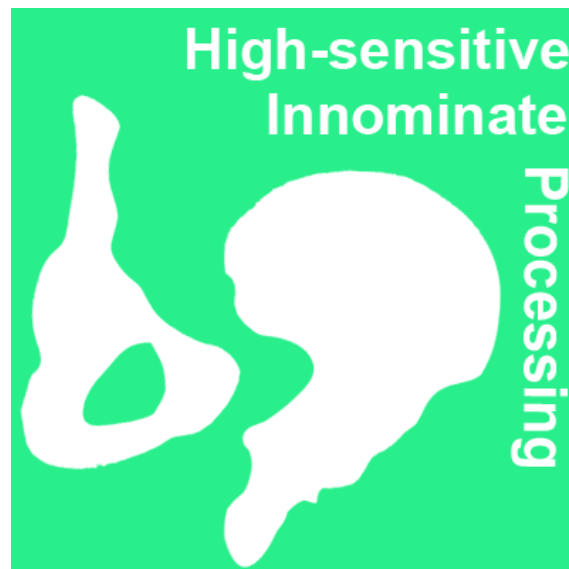
User Manual

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About the program

HIP 1.1 program is the second functional version of the HIP computer program designed for morphometric estimation of the sex of an unknown skeletal finding based on the pelvic bone. The authors of the program are Miroslav Králík , Petra Urbanová , Ondřej Klíma , Tereza Mikešová, Martina Wagenknechtová, and Jana Jungerová. The program works with standardized 2D images of bones from a desktop scanner and allows the case to be classified into a group according to sex using methods of traditional and geometric morphometry. One-dimensional and multi-dimensional statistics computing procedures and graphical procedures are based on the *R* software, distributed under the GNU (GPL) license, and its extending libraries from various authors.

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License

The program is freely available and is distributed under the GPLv3 license. The official license text is available at <http://gnu.org/licenses/gpl.html>.

Citation

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Technical and software specifications

HIP 1.1 is designed for Windows operating system, versions Vista and higher. The program was not tested on lower versions of the Windows operating system

(Windows XP, NT, 98, 95) and probably will not work properly. The program was written in C++ and R [2], and created in QtToolkit application, version 4.8.2, the user interface was created in QtDesigner. It uses Bioconductor R, version 2.15.1. Rtools were used to compile Qt libraries and the entire application, Rtp, Rinside, and examples from QtDensity (under the GPL licence) were used for compiling Qt and R. Qt itself is licensed under LGPL. During the creation of the program, the computational and graphic functions of the following packages of the program R [18] were used: *base* [18], *stats* [18], *graphics* [18], *MASS* [24], *shapes* [7], *klaR* [25], and *Momocs* [4]. These packages also use functions of other packages: *scatterplot3d* [11], *rgl* [1], *methods* [18], *sp* [18], *ReadImages* [12], *ade4* [6], *spdep* [3], *boot* [5], *Matrix* [2], *lattice* [20], *nlme* [15], *maptools* [10], *foreign* [17], *grid* [18], *deldir* [21], *coda* [16], and *splines* [18].

Development of versions

Program HIP 0.3 was finalized in 2012. The software included only one reference sample (Athens) and contained an additional modul for sex estimation by the human clavicle.

Program HIP 1.0 was finalized in 2015. It was intended for the hip bone only (clavicle was omitted) and two additional reference samples were included (Prague and Brno).

Program HIP 1.1 was finalized in 2017. Some new computational routines were added and some bugs were fixed.

Please send your inquiries, comments and suggestions to the address <mailto:mirekkralik@seznam.cz> (Miroslav Králík).

1 User Manual


1.1 Starting the program

The program is freely available for download at the website of the Department of Anthropology MU:

<http://www.sci.muni.cz/lamorfa/veda-a-vyzkum#projekty>

and also at the site:

<https://sourceforge.net/projects/hip-project/>

Click the *Download* link to download the ZIP file to your computer and unzip it. To start the program, double-click the *HIP.exe* file icon  in the *bin* folder. The shortcut (command script) of this *HIP.cmd* file is also located at the top level of directories and can be seen immediately after unpacking and opening the downloaded folder.

1.2 General features of the program

The use of the program consists of preparation/recording of a suitable standardized 2D image of the pelvic bone (synonyms: hip bone, innominate bone). The pelvic bone is evaluated from two standardized views (*Pubic view* and *Iliac view*). In the current version 1.1, it is possible to evaluate separately each pelvic bone view or combine both pelvic bones views in one analysis. The program supports sexual diagnosis of an unknown case using traditional morphometry – distances/measurements (linear distance between measurement points, or interlandmark distances), both single dimensional visualization and comparison of a selected interlandmark distance, as well as multidimensional *Linear Discriminant Analysis (LDA)* of interlandmark distances. In addition to traditional morphometry, the program also offers geometric morphometry, namely the *Generalized Procrustes Analysis (GPA)* and *Linear Discriminant Analysis (LDA)* of *Procrustes shape coordinates*, i.e., sex estimation purely on the basis of shape, without the influence of size variables.

1.3 Reference skeletal collections

Sex estimation of an unknown case is based on a comparison with documented reference bone collections, that is, the bones of people whose sex in life was documented and is known with certainty. *Version HIP 1.1* contains data from three documented collections of pelvic bones:

- (A) Pelvic bones come from the collection deposited at the Institute of Anatomy, First Faculty of Medicine, Charles University in Prague. The collection includes pelvic bones of 100 individuals, 99 individuals are represented by both pelvic bones, one skeleton is represented only by the right pelvic bone. The collection contains pelvic bones of 54 males and 46 females. The skeletal remains are autopsy material. The majority of individuals (90 individuals) are part of the so-called Pachner Collection - skeletons macerated at the Czech anatomical institute during the 1930s (1933–1936), 8 females represent skeletons macerated at the German anatomical institute in 1884–1902, in two cases the pelvis bones belong to individuals of unknown origin. The whole population is assumed to originate from the

lower socioeconomic strata. In most cases, a complete pelvis (assembled from both pelvic bones and the sacrum) was available, isolated pelvic bones were present in 7 individuals. The condition of the skeletal material was varied and included abrasion of the bone surface, fractures and breakage of pelvic bone parts, especially at the junction areas of the individual pelvic bones (the pubic bone near *facies symphysealis* and the area of *spinae iliacae posteriores*). (Note: All 100 individuals were selected into the database, of which 98 individuals have both bones, 1 individual (male P_058) only the bone of the left side, 1 individual (female P_092) only the bone of the right side.)

- (B) Bones from the autopsy collection from the *Department of Anatomy, Faculty of Medicine, Masaryk University*. From the Brno bone collection which originated from the autopsies of the dead human bodies used in the education of medics, there were 29 pelvic bones available, of which 27 are represented by the two pelvic bones, the two skeletons are represented only by the right pelvic bone. This collection includes pelvic bones of 23 males and 6 females. In all cases, the pelvic bones were isolated (i.e. they did not form the pelvis with the other pelvic bone and the sacrum). In this collection, the pelvic bones were preserved in an excellent condition, there were no breaks or abrasions of the bone surface. (Note: The complete collection has more individuals, but some were excluded because of an unknown sex (NA), in one case (female B_089) there was probably a pathology (rickets?), 27 individuals both bones, 1 individual (female B_029) right bone, 1 individual (male B_235) right bone.
- (C) Bones from the collection of *The University of Athens Human Skeletal Reference Collection, National and Kapodistrian University of Athens, Greece*. The pelvic bones originate from *The University of Athens Human Skeletal Reference Collection*, stored at the Department of Animal and Human Physiology, Athens, Greece. The collection contains documented skeletal remains of 225 individuals that were obtained by exhuming graves from several cemeteries in the area of Athens. The group contains skeletal remains of people who died between 1960 and 1996, aged between early childhood and old age (Eliopoulos et al., 2007). Individuals were predominantly from lower or middle socio-economic backgrounds. After the exclusion of children, visibly pathological and strongly fragmentary cases, the studied collection of pelvic bones includes a total of 186 individuals. 171 individuals are represented by both pelvic bones, in 5 individuals the pelvic bone is present only on the right side and in 10 skeletons only on the left side. This collection includes pelvic bones of 102 males and 84 females. All pelvic bones were also isolated and their condition was good. The most common were abrasions or fractures of *facies symphysealis*, *spina ischiadica*, and *spinae iliacae posteriores*. (Note: 171 individuals have both bones, 5 individuals (female A_018, female A_119, male A_126, female A_188, male A_200) have the right bone only, 10 individuals (male A_078, female A_095, male A_107, male A_140, female A_144, female A_167, male A_177, female A_190, male A_192, male A_209) have the left bone only).

In sum, the program contains reference data from three documented bone

collections: 198 pelvic bones from the collection of the Institute of Anatomy, First Faculty of Medicine, Charles University, 56 pelvic bones from the collection of the Faculty of Medicine, Masaryk University, and 357 pelvic bones from The University of Athens Human Skeletal Reference Collection. In total, there are 611 pelvic bones from 315 individuals (179 males, 136 females).

Due to the side differences of the pelvic bone, the program compares an unknown case primarily with the part of the reference collection of bones coming from the same side of the body. However, the opposite side can be also chosen.

1.4 Scanning process

The starting-point of the evaluation is scanning the bone in standardized positions using the CanoScan 4400F 2D desktop scanner. It is a scanner with a horizontal glass platen of a format slightly larger than A4. The desktop scanner provides fast, high-quality recording of images of formations with relatively flat surface. However, it is necessary to standardize the images:

- (a) *Pubic view*: place the pelvis on the outer surface of the pubic bone so that the flat part of *ossis pubis* adheres best to the glass platen. Place the bone in the centre of the platen so that it is recorded whole and orient it so that the most rearward point on the *tuber ischiadicum* and the most backward protruding point on the edge of the bone in the area of the hip joint (*acetabulum*) are on a line parallel to the longitudinal axis of the scanner (corresponding to the direction of movement of the lamp). The lamp moves from the cranial (*crista iliaca*) to the caudal region of the pelvic bone (*tuber ischiadicum*), not vice versa. In this position the bone does not stay by itself, it should be held or weighted down during scanning (e.g. with a large amount of plasticine).
- (b) *Iliac view*: place the pelvic bone on the outer surface of the hip bone (the wing) on the scanner platen so that the flat part of the wing of the hip bone is as close as possible to the glass plane. Place the entire bone in the centre of the platen in order to record it whole and orient it so that the most rearward/medial point (i.e. on the side facing the sacrum) on the *tuber ischiadicum* and the most rearward point on the edge of the bone in the area of *spina iliaca posterior superior* are on a line parallel to the longitudinal axis of the scanner (corresponding to the direction of movement of the lamp). The lamp moves from the cranial (*crista iliaca*) to the caudal region of the pelvic bone (*tuber ischiadicum*), not vice versa. In this position the bone usually stays by itself and does not need holding while being scanned.

The bones of the reference collection were scanned into full-color TIF files, 100% in size, resolution 150 dpi in both directions. The program also loads JPG and PNG files.

Because this resolution has been used with reference cases and has been set as optimal for compromise between image details and file size, we recommend it to be used. But this is not a requirement; the program also works with images with other resolutions if scanning 100% in size is retained.

1.5 Important technical note

The 2D desktop scanner was chosen because it is now affordable for almost everyone. This is related to our commitment to free use of the new methodology and software developed under the GPL license. This would be very limited if the methodology required expensive equipment to record three-dimensional morphology such as a laser scanner or a 3D digitizer.

However, the use of a desktop scanner has its limitations. Different desktop scanners provide almost identical images of the configuration on the scanner plane, but the three-dimensional formations over the platen (beyond its plane) they convert into 2D image slightly differently. Therefore, as with any other recording device, it is always needed to calibrate images. Due to this, we have recorded all existing cases with the *Canon CanoScan 4400F* scanner. For other scanners, the data needs to be adjusted. However, an automatic calibration of the scanner is not yet part of this program. Therefore, do not use HIP 1.1 results based on images from recording devices other than this particular desktop scanner (*Canon CanoScan 4400F*) as conclusive.

We are working on creating a calibration feature for other scanners and cameras, and we expect it to be included in the program in one of the later versions.

2 Procedure

2.1 Image loading

After starting the program, chose from the **Pubic view** or **Iliac view** in the upper tabs. After selecting e.g. **Iliac view**, chose **Load image** in the left sidebar and load from the folder on the computer the corresponding standardized image of the pelvic bone viewing the outer surface of the bone (Figure 1). The image opens and the name and type of the graphic file appears in the upper left column in a cell labelled **File name**. At the same time, the program automatically retrieves and displays the image resolution in both directions (**Horizontal DPI**, **Vertical DPI**). Image resolution is used to calibrate the size. Note: Upper tabs can be moved freely and their order can be changed according to individual need.

2.2 Selecting the side

In the cell labelled **Body side** in the left menu, it is necessary to *manually* specify whether the bone is from the right or left side. If it is the bone from the right side (default setting), nothing changes. If the bone is from the left side, change *right* to *left* and the image flips – a bone from the left side will look like a bone from the right side. However, the data will show that it is the bone from the left side. We created this step because of the asymmetry of sight, the same osteometric points may be evaluated differently, depending on whether they are on the right or left side.

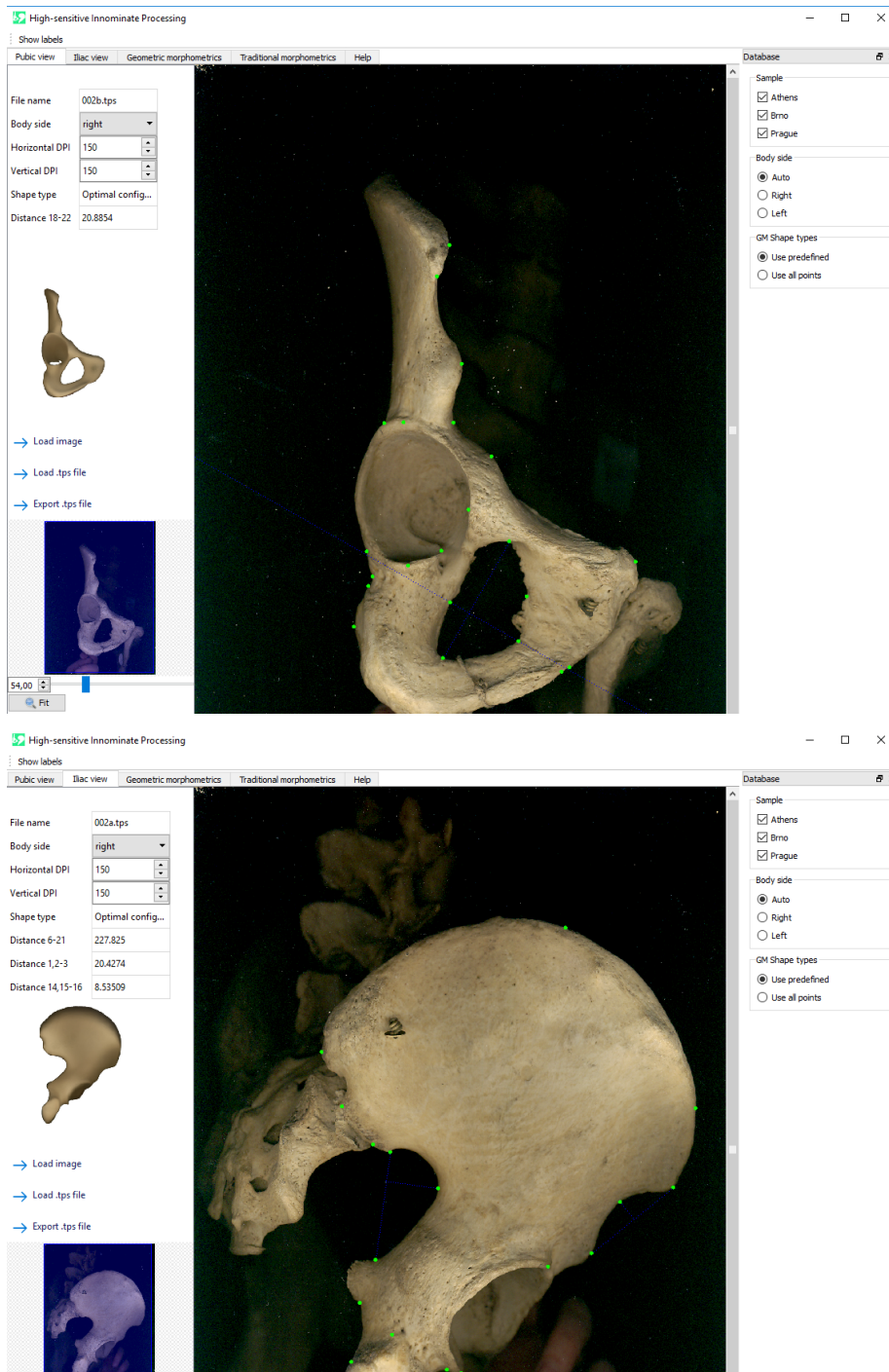


Figure 1: Screenshot of the program window in the *Pubic view* digitization tab (top) and *Iliac view* (bottom). In the left menu there are control functions, measurements are made in the main window.

2.3 Opening the TPS file

In addition to loading the image, it is possible to load data by Load `.tps` file directly in the TPS format (see *Example* bellow), which is commonly used by J. Rohl's morphometric programs and is the output of his *tpsDig* 2D digitizer [19]. If the corresponding image is linked to the data in the TPS file, it opens directly when the TPS file is loaded if it is in the same folder. However, the data must in the number and sequence correspond to the osteometric points defined below.

For example, in the same folder we have image *001b.tif* on which the pelvis is in the *Pubis view*, and a TPS file *001b.tps*, which contains the coordinates of 24 defined osteometric points from this image. The TPS file is a regular text file with a `*.tps` ending, containing the Cartesian coordinates (x, y) of the osteometric points of the view displayed in a list, in the first row is their number (here LM=24) and the last line is refers to the respective image (here IMAGE=001b.tif):

```
LM=24
974.00000 560.00000
621.00000 768.00000
519.00000 854.00000
552.00000 944.00000
484.00000 1054.00000
529.00000 1218.00000
390.00000 826.00000
443.00000 832.00000
451.00000 537.00000
534.00000 564.00000
601.00000 668.00000
367.00000 576.00000
400.00000 504.00000
385.00000 477.00000
346.00000 418.00000
519.00000 220.00000
584.00000 244.00000
815.00000 349.00000
888.00000 368.00000
693.00000 619.00000
583.00000 369.00000
724.00000 414.00000
743.00000 474.00000
566.00000 486.00000
IMAGE=001b.tif
```

2.4 Saving measured data

We also chose the modified TPS format to store digitized data. Clicking **Export .tps file** can save measured/digitized data in TPS format to a folder on your computer so you can return to it later. If the image was digitized directly (i.e., we did not open the TPS file, but the TIF file) and the data were stored in

another folder, the image is saved automatically with them.

The program differs from the *tpsDig* digitizer [19] by storing also an incomplete set of points (for example, when some points cannot be measured due to bone damage). In this case, the NaN code will be written instead of the missing point in both coordinates.

Additionally, the TPS file stored in the HIP program contains an indication of whether it is a bone from the right or the left side. A TPS file of a bone form, the right side with only three points can look like this (*pubic view*):

```
LM=24
974.00000 560.00000
NaN NaN
NaN NaN
NaN NaN
NaN NaN
NaN NaN
NaN NaN
443.00000 832.00000
NaN NaN
NaN NaN
NaN NaN
NaN NaN
NaN NaN
NaN NaN
NaN NaN
519.00000 220.00000
NaN NaN
NaN NaN
NaN NaN
NaN NaN
NaN NaN
NaN NaN
NaN NaN
IMAGE=001b.tif
ID=0
SIDE=right
```

2.5 Example

If you do not have your own bone images, the program contains a number of already measured examples in the *examples* folder, divided by individual views. It is possible to directly upload data in the TPS format of already measured bones using the `Load .tps file` and then work with them. To test digitalization of points, however, it is better to use the `Load image` to open an unprocessed TIF image of the bone (Warning: Opening the correct TPS belonging to the relevant view is checked by the program so that e.g. *iliac view* data cannot be opened in the *pubic view* analysis but it does not check it while opening an image of a bone; therefore, it is necessary to make sure to open the correct image in the corresponding view).

2.6 Enhancing/zooming in on a view

You can adjust the image to the window size by clicking the **Fit** button. You can zoom in or out by dragging the slider above this button or with a mouse wheel (if the mouse pointer is in the slider area, you can also move it with the mouse wheel). While zoomed in, you can move the sliders at the edges of the main window or by moving the blue square in the bottom left view. When measuring, always use an appropriate zoom to place the osteometric points on the image as accurately as possible.

2.7 Measuring – digitalization of points

Measurement on an image means the manual placement of the cursor to the position of the relevant osteometric point given by the definition and recording the Cartesian coordinates (x, y) of that point. These coordinates are the basis for both traditional morphometry and geometric morphometry. When measuring, place the arrowhead of the cursor to the position of the relevant osteometric point and click the right mouse button. The following dialogue appears (Figure 2):

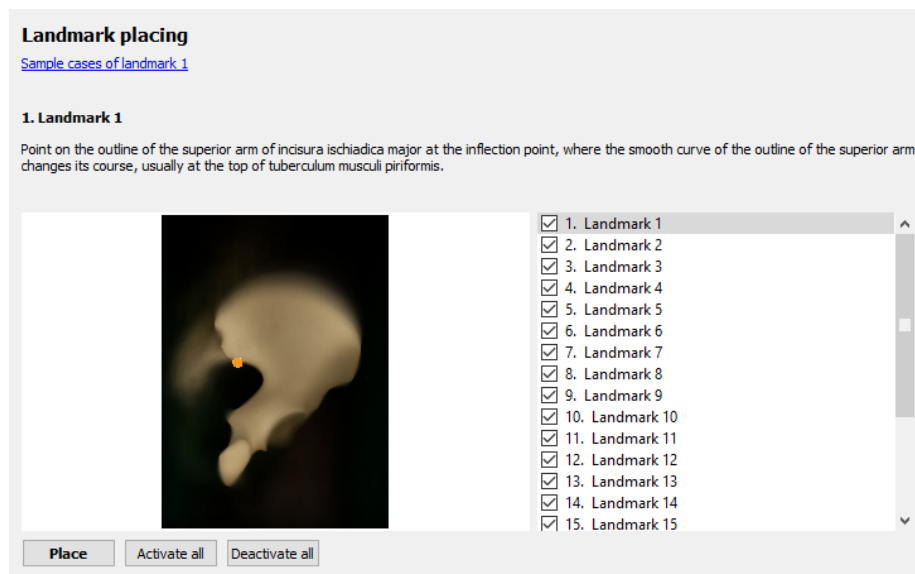


Figure 2: Dialogue of osteometric points digitalization on a pelvic bone in *Pubic view*.

By clicking on the given point, you can see its position on the bone schema and its definition at the same time.

If you click outside of the dialogue window, you will return to the loaded bone image. It is necessary first to **thoroughly study the definitions** of all osteometric points. If you are not sure whether you correctly understand a definition of a point, click on **Sample cases of landmark #** and in the web browser some examples of the positions of the point on real bones or other explanatory diagrams will be displayed. Definitions of osteometric points are given at the end of this manual.

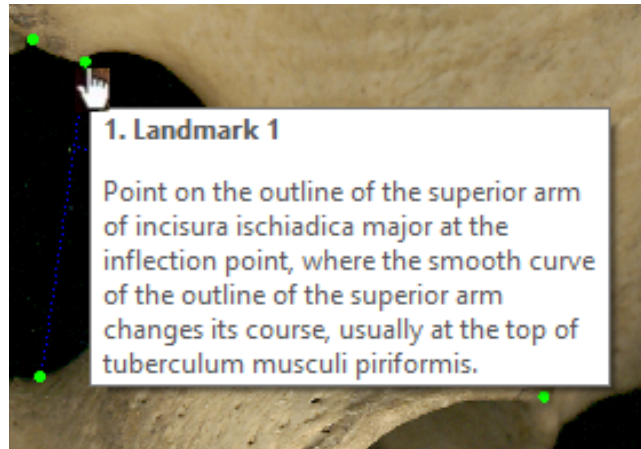


Figure 3: Numerical label and definition of point 1 in *Iliac view* in the frame made by the cursor.

Double-clicking on a point in the list returns to the bone image where the digitized point is shown as a light green dot with the appropriate number. The point can be moved by the mouse to correct its position at any time. Numerical labels of osteometric points can be switched on/off by the **Show labels** button at the top left.

If you place the cursor over an osteometric point in the image, the cursor changes into a hand, the number of the point and its definition are displayed in a frame (Figure 3). If you right-click the point, a dialogue (Figure 4) will appear, which will allow us to manipulate its position with the numerical value of its x,y coordinates. Click the **Remove** button to delete the point if it is entered incorrectly. However, we can only deactivate the point by clicking the **Deactivate** button if, due to uncertainty about its position or bone preservation in the given place, we only want to exclude it from the current analysis and compare the results obtained with and without it. The deactivated point changes colour to blue. Be sure to always activate all deactivated points before completing the analysis; otherwise the program saves their coordinates as NaN, that is, as if the point was missing.

When digitizing the points defined by perpendiculars, HIP 1.1 makes it easier to find their position by automatically displaying blue perpendiculars (blue dotted lines). The program can also determine the smallest/greatest distance between selected points, the displayed distances are in units after calibration (i.e. in millimetres). This is how it is automatically calculated in *Iliac view*: length of a perpendicular from point 3 to the line connecting points 1 and 2, length of a perpendicular from point 16 to the line connecting points 14 and 15, line connecting points 6 and 21 (length of the pelvic bone); in *Pubic view*: distance of points 18 and 22 (width of the lower branch of the pubic bone).

The measurements are similar in both pelvic bone views, the differences are only in the number of defined points and preferred configurations.

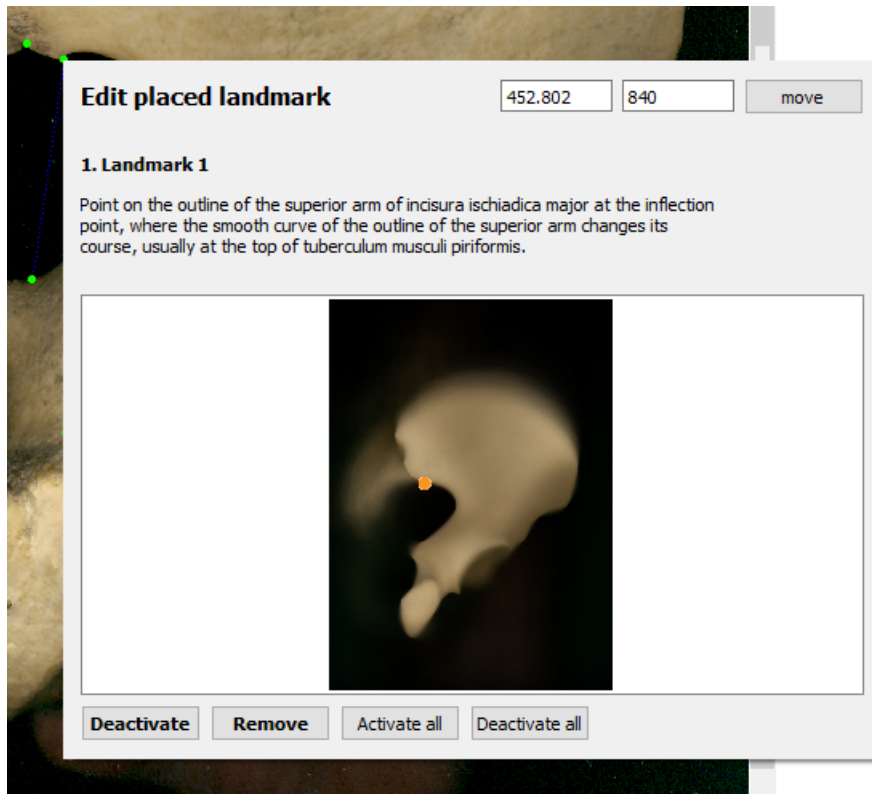


Figure 4: The dialogue for manipulation with an osteometric point after clicking on it.

2.8 Control of point configuration

In digitalization, in the left column in a cell labelled **GM Shape types** is shown the sufficiency of bone coverage with points in terms of the *application of geometric morphometry* (does not concern traditional morphometry). Based on the morphometric analysis, we have selected several configurations of osteometric points (described in the Appendix of this manual) for each view, which sufficiently describe the shape of the bone and are not redundant. Thus, there is information on which of the preferred configurations of a given bone is available (e.g. Optimal configuration). Without fulfilling the so-called minimum defined configuration, no further analysis will take place and the dialogue will indicate that the analysis cannot be performed. With insufficient number of points, the posterior probability often does not reach value 95% or higher and the results of the sex diagnosis cannot be considered reliable. Definitions of the preferred configurations (the only ones allowed in this version of the program) are also listed at the end of the manual for each of the views.

2.9 Sex diagnosis

Once the digitalization is complete, we proceed to sex estimation. In this step, the program offers several options.

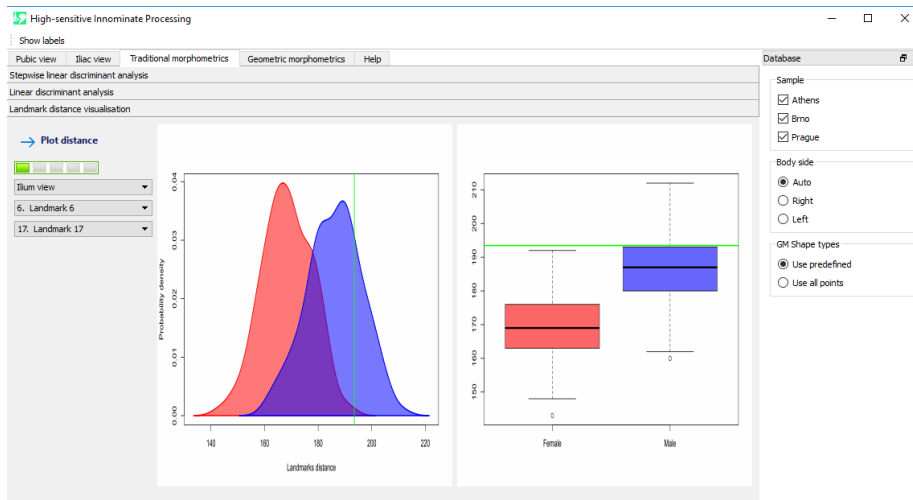


Figure 5: Landmark distance visualization tab from the Traditional morphometry tab after displaying the difference in the 1-3 dimension in *Iliac view*.

2.10 Selection of reference samples

First, it is necessary to choose which comparative/reference samples to compare the case against. In the right menu/**Database** tab, select one, two or all three reference files (Athens, Brno, Prague) in the **Sample** field. Next, in the **Body side** field, choose which side of the body the reference samples should come from (Auto – according to the side of the assessed bone, Right, Left).

2.11 Traditional morphometry

Now select the top **Traditional morphometry** tab. From the coordinates of the osteometric points, all available dimensions (distance between all pairs of measured points) are automatically calculated and converted to millimetres based on the resolution of the images. In the left column we can then choose from several tabs.

2.12 Visualization of individual dimensions

At the bottom left of this tab is the **Landmark distance visualization** tab, which allows you to graphically display the distribution of any dimension. Select a view that you want to evaluate (*Iliac view* or *Pubic view*), select the two points whose distance interests you and click on **Plot distance**. Using the probability density plot and the box and whisker plot, where females are always red and males blue in colour, the sex differences in the selected dimension in the reference file are shown. The green line shows the location of the unknown case. Just by displaying the individual dimensions, it is possible to detect cases extremely masculine or extremely feminine. However, most individuals are in the overlapping zone of male and female values.

2.13 Linear discriminant analysis

The group sorted according to sex can include an unknown case based on a multi-dimensional combination using Linear *Discriminant Analysis - LDA* [9]. The method searches in a multidimensional space for a combination of variables that maximizes differences between groups (in our case between the sexes). The *LDA* program calculates the *lda* function of the *MASS* package [24], the prediction of the unknown case sex is performed using the *predict* function from the *MASS* package [24].

Select the middle tab of the traditional morphometry labelled **Linear discriminant analysis**. Start by choosing in the left column a view you want to evaluate (*Both views together*, *Pubis view*, *Ilium view*). If you select *Both views together*, the calculation will be based on the combined data of both views. Then you can choose from three options: (a) use all dimensions, (b) exclude some of all dimensions (i.e., negative selection), or (c) select only some (i.e. positive selection) from all dimensions.

- a) To calculate discriminant analysis from all available dimensions, select **Use all distances**. However, due to the circumstances described below, this is quite inappropriate. You must always carefully choose the dimensions for discrimination.
- b) If you want to calculate discriminant analysis without some dimensions, select the option **Avoid selected distances**. This option automatically disallows the calculation of discriminant analysis from the dimensions that the authors of the HIP program determined to be inappropriate. Their list is provided in an appendix at the end of this manual. These are dimensions based on pairs of points that vary greatly in the reference sample(s), often approach zero, and at the same time are very small in relation to the total size of the bone. This leads to a high degree of accidental effects (due to an inaccuracy of measurement, occurrence of pathologies, damage to the surface of the bone, etc.) in the process of discrimination. The program blocks all (except for justified exceptions) dimensions whose values (or part of the values) are lower than 10 mm. When this option is selected, the list of the “prohibited” dimensions appears in red at the top right. If there is a reason to add to these inappropriate dimensions more to exclude from the analysis, select the two points that define the rejected dimension in the windows named **Distances on ...view** (... is the name of the view) and click the **Add to list** button on the right. The dimension is added to the blocked dimensions list and will be black. However, it can be unblocked anytime by selecting it and pressing the **Remove from blocked** button.
- c) If you want to calculate discriminant analysis only from selected dimensions, select **Use only selected distances**. Choose the appropriate dimensions either from experience or by viewing individual dimensions and their degree of dimorphism in the **Landmark distance visualization** tab. In the **Distances on ...view** (... is the name of the view) windows, select the chosen dimensions and add them to the list by clicking the **Add to list** button.

Hence, the data can be selected for analysis at two levels, the first level is

the *activation and deactivation of the points* in the measurement, the second level is the *blocking or the selection of the dimensions* before the analysis.

Once the dimensions to enter the analysis are set, you can proceed to the Linear Discriminant Analysis itself. Click the **Run analysis** button. The program selects from the reference collection database all the dimensions that we have allowed and which are currently available from the evaluated case. The LDA will run on these dates, the program calculates a new discriminant function, and then uses it to include an unknown case. During the analysis (while the program calculates) a green progress bar indicator moves below the analysis start button.

Several result tables are displayed after the analysis is complete:

- A) The table on the left shows the **Prior probability**, i.e. the original probability of the case classification by sex even without an analysis that is set to the proportion of the given sex in the reference sample (selection). Posterior probability indicates the likelihood of correct case classification and sex estimation. In the sex with a higher probability, the cells are highlighted in yellow. Posterior probability values of 0.95 and higher are generally considered as practically sure to be allocated in the appropriate class. In addition, there is a classification table that shows the total number of males and females in the sample (**Cases**) and the number (**Misclassified**) and percentage (**Misclassified [%]**) of incorrectly classified males and females.
- B) On the right there is a table of selected results of discriminant analysis and classification. The value of a discriminant score for the unknown case (**Value of disc. function**) is displayed. Additionally, there are the total percentage of **Accuracy** of discrimination of reference cases, the total number of **Misclassified** cases regardless of sex, the **Cut-off** point, and the total number of **Cases** and **Variables** in the analysis.
- C) The coefficients of discriminant function (LD1) for each dimension, the mean of each of the dimensions used (**Male mean**, **Female mean**) and the respective value of the unknown case variable (**Case**) are given in the table below (**Coefficients of discriminant function**).
- D) The *probability density plot* (**Probability density**) and the *box and whisker plot* of the discriminant score values of the reference cases (women in red, men in blue), and a green line indicating the value of the discriminant score of the analysed case are displayed below (similarly to individual dimensions). The graphs are created by the function *density* of the *stats* package [18] and the functions *plot*, *lines*, and *polygon* of the *graphics* package [18].

2.14 Stepwise linear discriminant analysis

The top tab of traditional morphometry is the **Stepwise linear discriminant analysis**. In the left column, select the view you want to evaluate (*Views combined*, *Pubic view*, *Iliac view*).

Since discriminatory analysis is very sensitive to the ratio of file size to number of discriminant variables, it is generally recommended that the ratio of the number of individuals and the number of variables be at least 5:1 (5 cases per variable) or higher [13]. It is therefore appropriate to include only a few

of the most effective variables (in this case, dimensions) in the model. This is the purpose of Stepwise Linear Discriminant Analysis (Stepwise LDA). The program uses functions from the *MASS* [24] and *klaR* [25] packages, in particular the *stepclass* and *lda* functions. Prediction is again performed by the function *predict* from the *MASS* package [24].

Afterwards, proceed as described above with the *LDA*. Choose the dimensions from which the analysis is to be calculated. The stepwise procedure is then realized by a default setting of the function *stepclass* using the function *ucpm* from the *klaR* package [25] to calculate the criteria for assigning a variable to the resulting model. The calculation includes a leave-one-out randomization method, i.e. n -fold cross-validation, where n is the number of samples (cases) of the reference collection(s). The number of cases (and thus the number of cross-validated samples) will vary according to the selection of bone views and, above all, the selection of reference collections. One case is randomly selected from the reference collection and a discriminant function is created on the rest of the collection (with $n-1$ number of cases). The pre-selected case is then used as validation (test) and the discriminant function is used on it. The whole process is repeated n -times. Details of the preconfigured stepwise procedure are described in the manual of the *klaR* package [25].

Click on the **Run analysis** button. Due to the stepwise procedure and verifying, the calculation is time consuming and may take from a few tens of seconds to several tens of minutes depending on the number of individuals and dimensions. The ongoing calculation is again indicated by the movement of the green progress bar below the start button of the analysis. Completion of the calculation is indicated by the window **Stepwise LDA has finished**. The program lists results in the same structure as the classic LDA (Figure 6).

In order to reach a compromise between the calculation speed and the estimation reliability, the preferred method of working with the program (according to the authors of the program) is to use a classical **Linear Discriminant Analysis** on a small number of selected dimensions. We recommend that you deliberately choose 3 well-discriminating dimensions in each view and use these six for the analysis. If it is not successful (posterior probability does not reach 0.95 or higher), choose a few other dimensions and perform the discriminant analysis again. Given the growth patterns of the pelvic bone, it is the right choice (if you have both views available) to select a few dimensions from each of the two views [14]. A certain aid for choosing the dimensions can be the selection of dimensions in the picture (Figure 7). If there is a complete set of points from both views (and therefore all possible dimensions), some of these dimensions (Figure 7) was part of a set of selected dimensions in most of the many thousands of stepwise discriminant analyses performed. They can therefore be considered the most reliable and be used deliberately. If these dimensions are not available (due to the lack of clarity or absence of landmarks), it is advisable to choose dimensions similar to them in direction and position.

2.15 Geometric morphometry

An alternative to traditional morphometry is evaluation using *geometric morphometry* (GM). GM procedures are most often based on data in the form of Cartesian coordinates of *landmarks* and have the advantage that we can evaluate the shape and size of objects separately [23, 22].

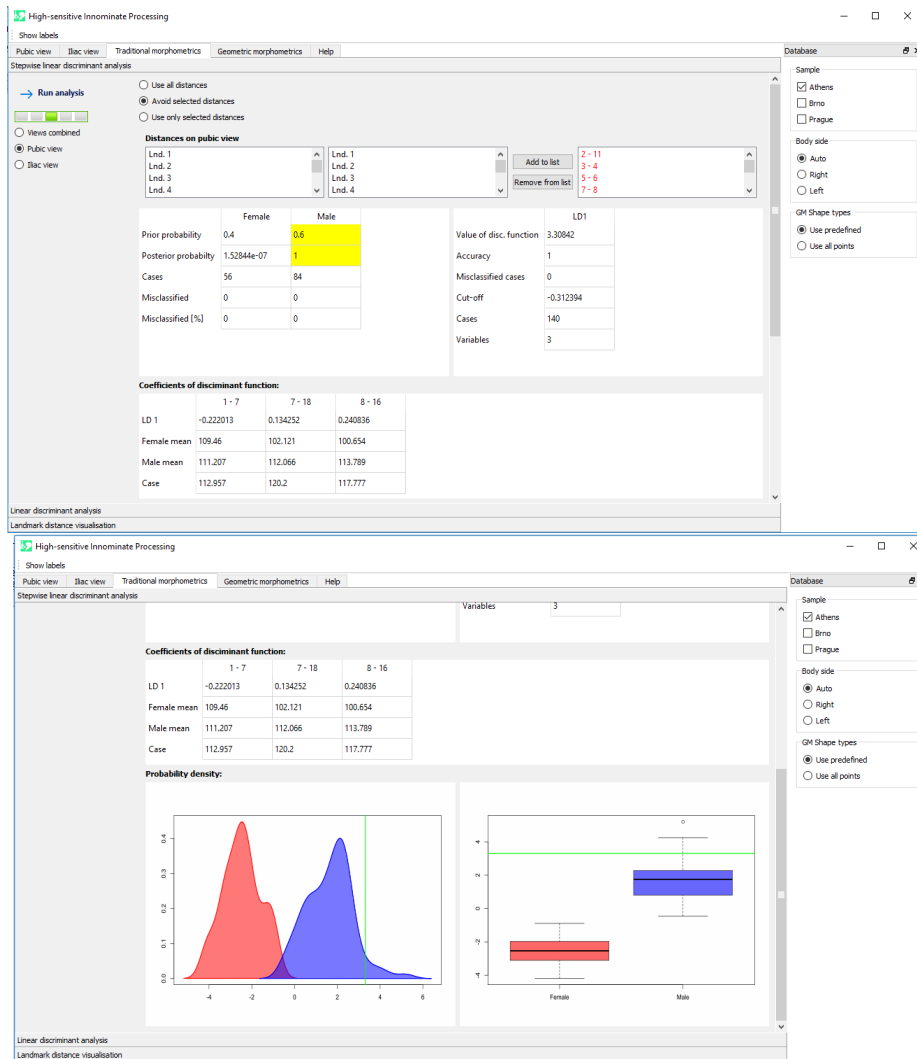


Figure 6: Top and bottom part of the results in the Stepwise linear discriminant analysis from the Traditional morphometry tab after calculating the discrimination in *Iliac view*.

Select the **Geometric morphometry** tab at the top, and in the left column choose the appropriate view (*Pubic view analysis*, *Iliac view analysis*, *Views combined*). If you select both views together, the LDA calculation will be based on the combined data of both pelvic bone views after each has gone through an individual superposition (see below).

At the beginning of the procedure, the program performs *a checking of the digitalized point configuration*. As mentioned above, the appropriate configurations were designed to adequately represent the shape being evaluated and at the same time not contain redundant points – from a group of differently defined points that lie close to each other and strongly correlate, always only one is

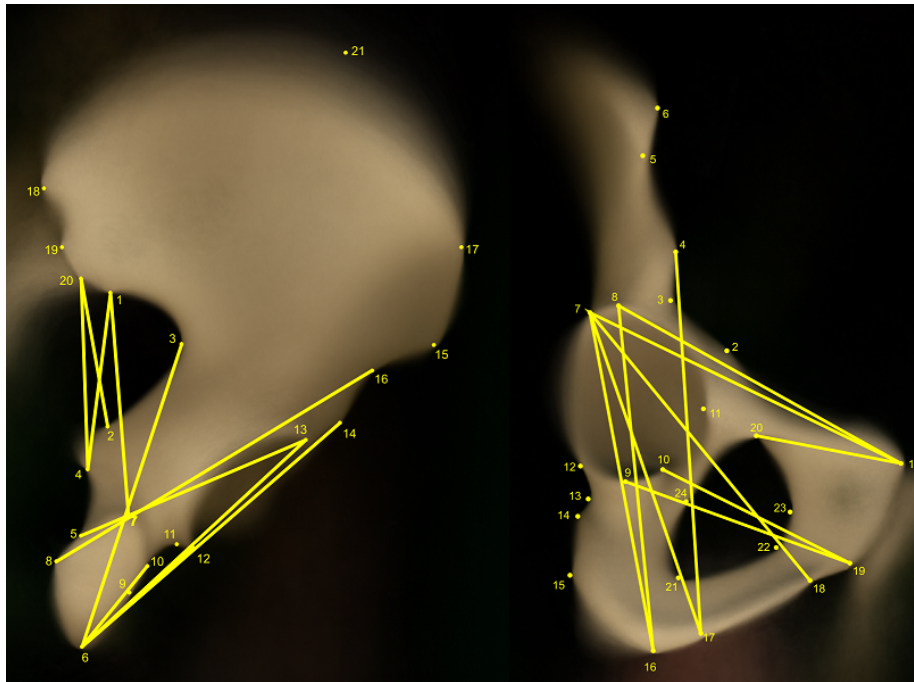


Figure 7: Dimensions of both pelvic bone views, which most often appear in the results of `Stepwise discriminant analysis`.

used (for more details see the *Recommended Configurations for geometric morphometry* at the end of this manual). For Pubic view, *Optimal Configuration* (13 points), *Reduced Configuration* (9 points), and *Minimal Configuration* (7 points) are defined. For Iliac view, *Optimal Configuration* (13 points), *Reduced Configuration A* (11 points), *Reduced Configuration B* (10 points), *Reduced Configuration C* (8 points), and *Minimal Configuration* (5 points) are defined. The program automatically checks which configurations are available. Then it calculates using the one which has the largest number of points. If a configuration has an extra point compared to a lower configuration but the combination of the points is not higher, the excess point will not be used in the calculation.

Selected point configurations are superimposed by *Generalized Procrustes Analysis* (GPA) [8]. Firstly, the program calculates the centroid size (CS), which is the square root of the sum of squared distances of a set of landmarks from their centroid (CS can only be compared between configurations of the same points). Secondly, a standardization of the configurations being compared is performed by removing the differences in the centroid size (CS = 1 in all cases), in the position and rotation of all configurations. The configurations are superimposed on each other so that the sum of squares of point distances within the clusters of individual points is minimal. Finally, after the superposition, only the shape differences between the configurations remain in the data – new so-called *Procrustes shape coordinates*. The size of the original configurations is stored separately as CS. For the GPA calculation, the program uses the *procOPA* function from the *shapes* package [7].

Procrustes shape coordinates as shape indicators and centroid size CS as a

size indicator are then used as input variables for *Linear Discriminant Analysis* by sex. Its procedure has already been described above. When analysing, it is possible to choose whether you want to discriminate purely based on the shape (Procrustes shape coordinates), which is the default setting, or to combine the coordinates with the centroid size. In this case, check the **Include centroid size** box and the centroid size will be included among the *LDA* variables.

Click on the **Run analysis** button. The results of sex discrimination (Figure 8, 9, 10) have a similar structure to that of traditional morphometry, including graphs, as described above. In addition, this analysis shows graphically the shape of the unknown case in a superposition with the average shape (consensus) for males and females. The case being evaluated is represented by a green line, females are in red and males in blue. The representation is complemented by a deformation grid *tps* (thin plate spline) that represents how a plastic square grid would deform, if it was covered by the points configuration of the unknown case, the points were attached to the grid, and then moved to the average female and male configuration. The largest shape changes are shown by thickening (darkening) or by diluting (lightening) of the grid. The grid density and shape change amplification to highlight the graphical visualization of the difference can be adjusted to the left by changing the **Grid size** and **Amplification** values before the calculation begins. The *tps* grid is calculated using the *tps.grid* function from the *Momocs* package [4]. The graph is complemented with a point graph of Procrustes shape coordinates in the same colours (males blue, females red, the unknown case green).

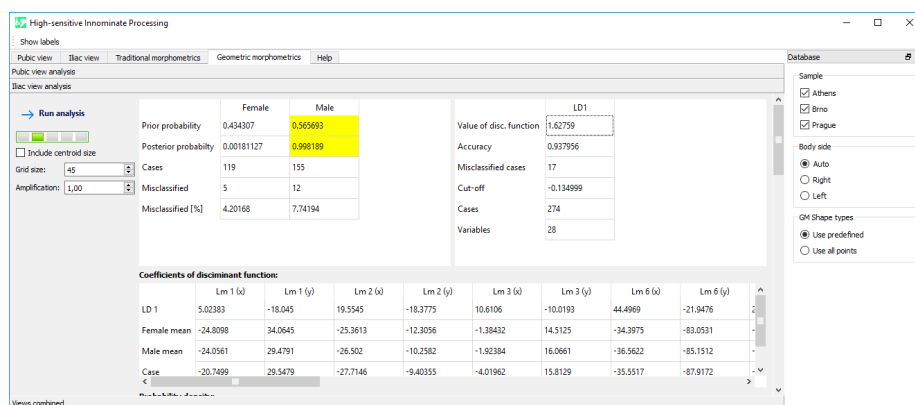


Figure 8: Top results in Iliac view analysis from the Geometric morphometry tab after calculating discrimination in *Iliac view*.

2.16 Results export

All results are automatically saved in the program directory in the *outs* folder in a separate subfolder, named by time (year-month-day hour-minute-second) when the analysis took place (e.g. 2018-09-18 17-45-19). The results are saved in the *HTML* format which can be viewed in any internet browser, data can be simply selected and copied for further use. Images are also stored separately in the *PNG* format.

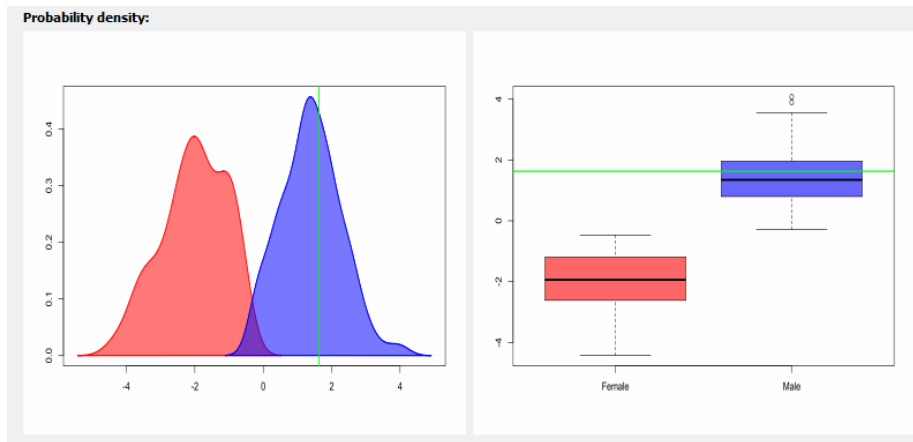


Figure 9: Graphs of the discriminant score in Iliac view analysis from the Geometric morphometry tab after calculating discrimination in *Iliac view*.

2.17 Coming soon

In the next version, we'd like to extend the program with the following functions:

- calibration of deformation for different types of table scanners or cameras
- descriptive statistics of input variables (coordinates, dimensions)
- integrated testing of the assumptions of parametric methods
- inclusion of other indicators of prediction and reliability of discrimination
- inclusion of other classification procedures (e.g. logistic regression)
- new reference samples to support greater versatility and external reliability of the methodology
- adding dimensions measured directly on the bones to the calculations of the traditional morphometry.

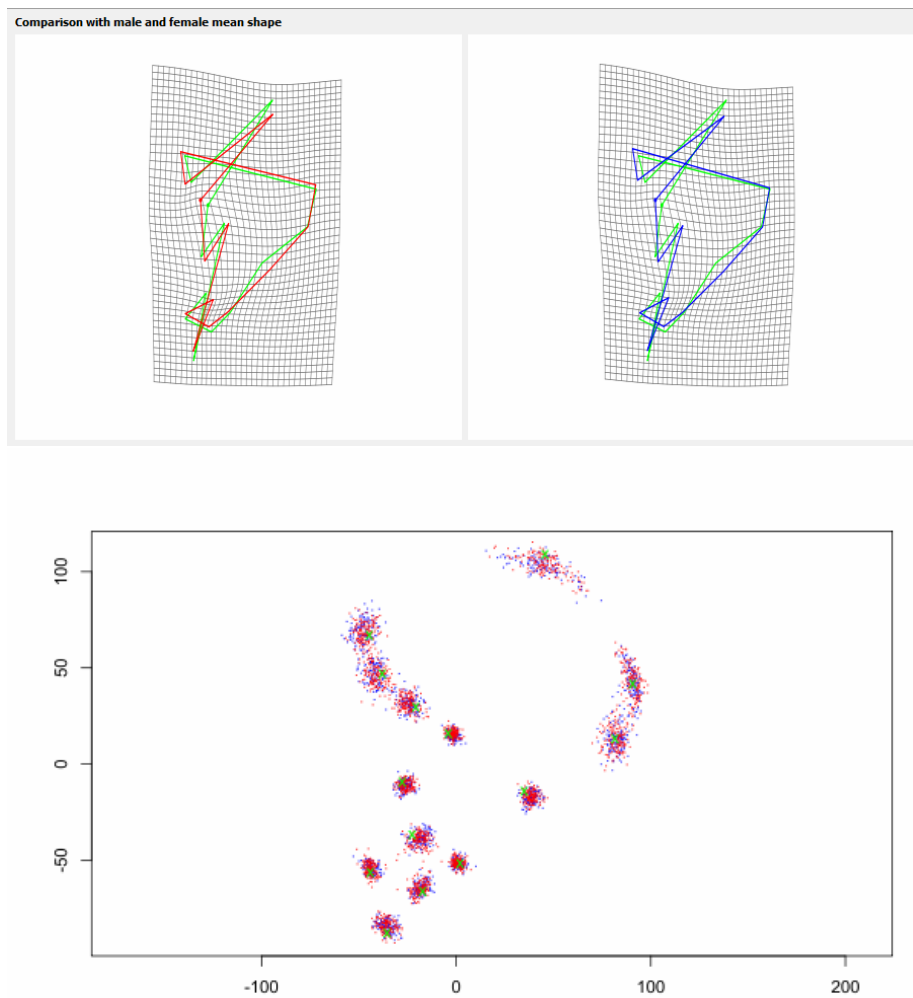


Figure 10: Deformation grids in Iliac view analysis from the **Geometric morphometry** tab after calculating discrimination in *Iliac view* (above) and superimposition of the reference collection (blue points – males, red points – females) with the analysed case (green crosses).

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Appendices

A Osteometric landmarks definitions

A.1 Pubic view

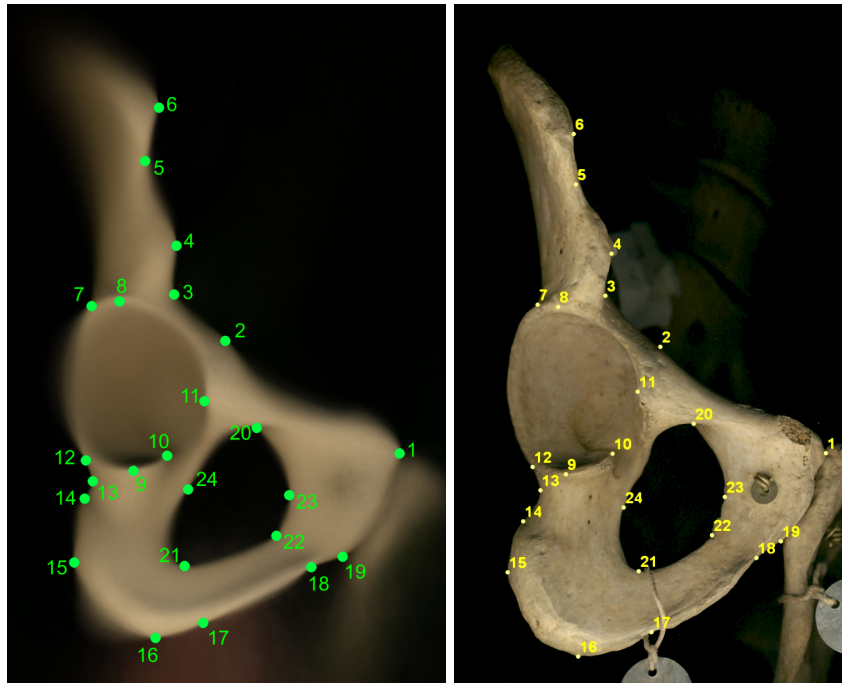


Figure 11: Overview of landmark positions in *Pubic view* on consensus shape (left) and on real-life example (right).

Landmark definitions

- Landmark 1: Point on the outline of facies symphysialis where its anterior edge refracts in the superior edge, or where the anterior and posterior edges meet at the top of facies symphysialis.
- Landmark 2: The most protruding point on the outline of eminentia iliopubica considering the main course of the upper outline of the bone; this point corresponds approximately to the point where the obliterated/fused suture between os pubis and os ilium meets the upper outline of the bone.
- Landmark 3: Point on the edge of os ilium below spina iliaca anterior inferior (where the body of the bone becomes the wing), placed in the deepest notch.
- Landmark 4: The most medially protruding point on the outline of spina iliaca anterior inferior considering the main course of the anterior edge of the bone.

- Landmark 5: Point below spina iliaca anterior superior where labium internum and labium externum cristae iliacaee meet and crista iliaca ends.
- Landmark 6: The most medially protruding point on the outline of crista iliaca.
- Landmark 7: Point where the lateral outline of ala ossis ilii touches the superior margin of acetabulum.
- Landmark 8: The uppermost point on the superior edge of acetabulum placed on the top of this edge (i.e. the point corresponding to the fine edge between articular surface of acetabulum and external bone surface, often corresponds to the most external position).
- Landmark 9: The lowest point on the inferior edge of acetabulum placed on the top of the acetabular rim (i.e. the point corresponding to the fine edge between articular surface of acetabulum and external bone surface, often corresponds to the most external position), analogously to the landmark 8 at the opposite side of the acetabulum.
- Landmark 10: The most medially protruding point by facies lunata placed on the edge of acetabulum near incisura acetabuli.
- Landmark 11: The most laterally protruding point by facies lunata placed on the pubic part of acetabulum; regardless of whether the point lies on the edge of os pubis or facies lunata itself, in the latter case always on the outer edge of this facet.
- Landmark 12: Point on the lateral outline of os ischii where it touches the inferior edge of acetabulum in incisura ischiadica minor.
- Landmark 13: Point on the outline of os ischii between acetabulum and tuber ischiadicum placed in the deepest notch.
- Landmark 14: Point on the outline of os ischii where it touches the edge of tuber ischiadicum, i.e. by the superior end of tuber ischiadicum in incisura ischiadica minor.
- Landmark 15: The most laterally protruding point on the outline of tuber ischiadicum.
- Landmark 16: The lowest point on the inferior outline of tuber ischiadicum.
- Landmark 17: Point on the medial, resp. anterior termination of tuber ischiadicum placed in a small notch frequently located there.
- Landmark 18: Point on the medial, resp. anterior beginning of crista phallica placed in the deepest notch.
- Landmark 19: Point on the outline of facies symphysialis where its anterior edge refracts in the inferior edge, or where the anterior and posterior edges meet at the bottom of facies symphysialis.

- Landmark 20: Point on the outline of foramen obturatum in sulcus obturatorius placed at the apex of the angle which is formed by the anterior and superior, or posterior (if foramen obturatum is triangular-shaped) edges of foramen obturatum.
- Landmark 21: Point on the outline of foramen obturatum where the inferior and posterior edges of foramen obturatum meet; generally it is the point on the outline of foramen obturatum opposite to landmark 20.
- Landmark 22: Point on the inferior outline of foramen obturatum which is the closest to landmark 18.
- Landmark 23: The most medially lying point on the anterior edge of foramen obturatum.
- Landmark 24: Point on the posterior edge of foramen obturatum placed on the vertical from landmark 22 to the longitudinal axis of foramen obturatum given by landmarks 20 and 21.

A.2 Iliac view

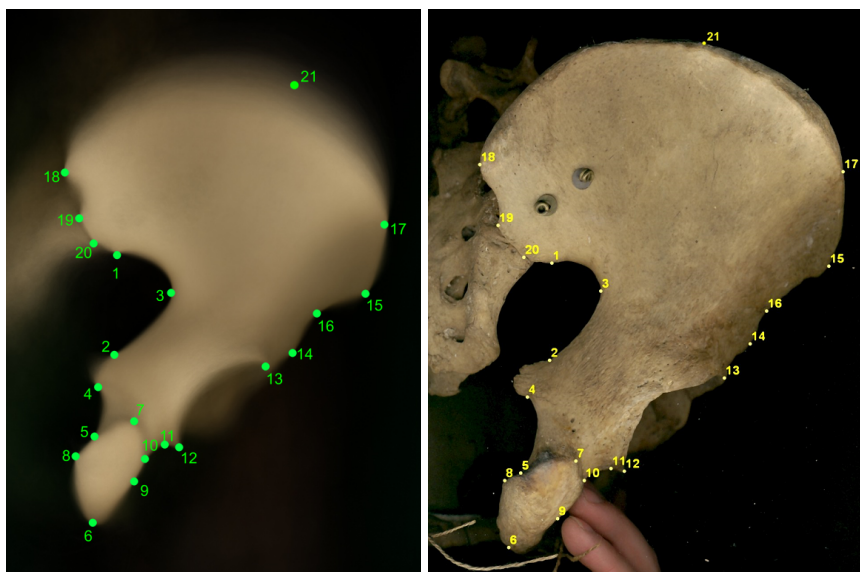


Figure 12: Overview of landmark positions in *Iliac view* on consensus shape (left) and on real-life example (right).

Landmark definitions

- Landmark 1: Point on the outline of the superior arm of incisura ischiadica major at the inflection point, where the smooth curve of the outline of the superior arm changes its course, usually at the top of tuberculum musculi piriformis.

- Landmark 2: Point on the outline of the inferior arm of incisura ischiadica major on the superior base of spina ischiadica.
- Landmark 3: The lowest point placed of the outline of incisura ischiadica major which is the furthest from the line connecting landmarks 1 and 2.
- Landmark 4: Point on the inferior base of spina ischiadica, placed on the outline of incisura ischiadica minor where the opposed facing edges of bone (upper edge corresponding to the lower outline of spina ischiadica, the lower edge representing outline of incisura ischiadica minor) connect in an obtuse angle.
- Landmark 5: Point where the outline of incisura ischiadica minor connects with the medial edge of tuber ischiadicum.
- Landmark 6: Point on tuber ischiadicum lying at the intersection of its inferior edge with its longitudinal axis.
- Landmark 7: Point on tuber ischiadicum lying at the intersection of its superior edge with its longitudinal axis; opposite to landmark 6.
- Landmark 8: Point on the medial outline of tuber ischiadicum which is the furthest from the longitudinal axis of tuber ischiadicum given by landmarks 6 and 7.
- Landmark 9: Point on the lateral outline of tuber ischiadicum placed on the vertical from point 8 to the longitudinal axis of tuber ischiadicum given by landmarks 6 and 7: joining of landmarks 8 and 9 makes the transverse axis of tuber ischiadicum.
- Landmark 10: Point where the edge of os ischii below acetabulum connects the lateral edge of tuber ischiadicum; if the edges do not meet, the point is placed on the outline of tuber ischiadicum where the edges are closest to each other but always above landmark 9.
- Landmark 11: Point on the outline of os ischii between tuber ischiadicum and acetabulum where the opposed facing contours of the bone connect in an obtuse angle.
- Landmark 12: The lowest point on the inferior outline of acetabulum placed on the most external position of the acetabular rim.
- Landmark 13: The highest point of the superior outline of acetabulum placed on the most external position of the acetabular rim (i.e. the point corresponding to the fine edge between articular surface of acetabulum and external bone surface, often corresponds to the most external position on the rim); analogously to the landmark 12 at the opposite side of the acetabulum.
- Landmark 14: The most protruding point on the outline of spina iliaca anterior inferior considering the main course of the upper outline of the bone, which is the furthest from the line passing by the inferior and superior termination (base) of the contour of spina iliaca anterior inferior.

- Landmark 15: The most protruding point on the outline of spina iliaca anterior superior, which is the furthest from the line passing by the inferior and superior termination (base) of the contour of spina iliaca anterior inferior.
- Landmark 16: The deepest point on the outline of os ilium between spina iliaca anterior inferior and spina iliaca anterior superior: i.e. the furthest point from the line connecting landmarks 14 and 15.
- Landmark 17: Point on the anterior edge of os ilium where it meets with the external edge of labium externum cristae iliacae.
- Landmark 18: The most medially (in the image: to the left) protruding point on the outline of spina iliaca posterior superior.
- Landmark 19: The most medially (in the image: to the left) protruding point on the outline of spina iliaca posterior inferior, in the region where the inferior arm of facies auricularis (on the opposite side of the bone and, therefore, not visible on the image) ends.
- Landmark 20: Point on the outline of spina iliaca posterior inferior where the superior arm of incisura ischiadica major ends: on facies pelvina (on the opposite site of the bone not visible in the image) it corresponds to the point where the inferior edge of facies auricularis in its termination point refracts upwards.
- Landmark 21: Point on the outline of crista iliaca which is the furthest from the landmark 6

B A list of rejected dimensions for traditional morphometry

B.1 Pubic view, Iliac view

By default (except for justified instances), we rejected dimensions that had unsuitable features in terms of discrimination. HIP software offers an option to avoid these dimensions from computations of traditional morphometrics. These dimensions are (a) whose values (or only a part of the values) are within a range of less than 10 mm and are therefore more strongly affected by a measurement error, (b) and/or depend on the relationships between landmarks of different types of definitions (e.g., landmarks 9 and 10 in Iliac view) and, as a result, their values vary considerably (from a few cm to zero), and/or (c) the distribution of their values is asymmetrical with many outlying values, (d) and/or the male and female values almost overlap (e.g. dimension 22-24 in Pubic view).

In Pubic view, we have rejected the use of these 20 dimensions for traditional morphometry: 2-11, 3-4, 5-6, 7-8, 9-10, 9-12, 9-13, 12-13, 12-14, 13-14, 13-15, 14-15, 16-17, 16-18, 17-18, 20-23, 21-22, 21-23, 22-23, 22-24.

In Iliac view, we have rejected the use of these 16 dimensions for traditional morphometry: 1-19, 1-20, 2-4, 5-7, 5-8, 7-9, 7-10, 9-10, 9-11, 10-11, 10-12, 11-12, 13-14, 14-16, 15-16, 19-20.

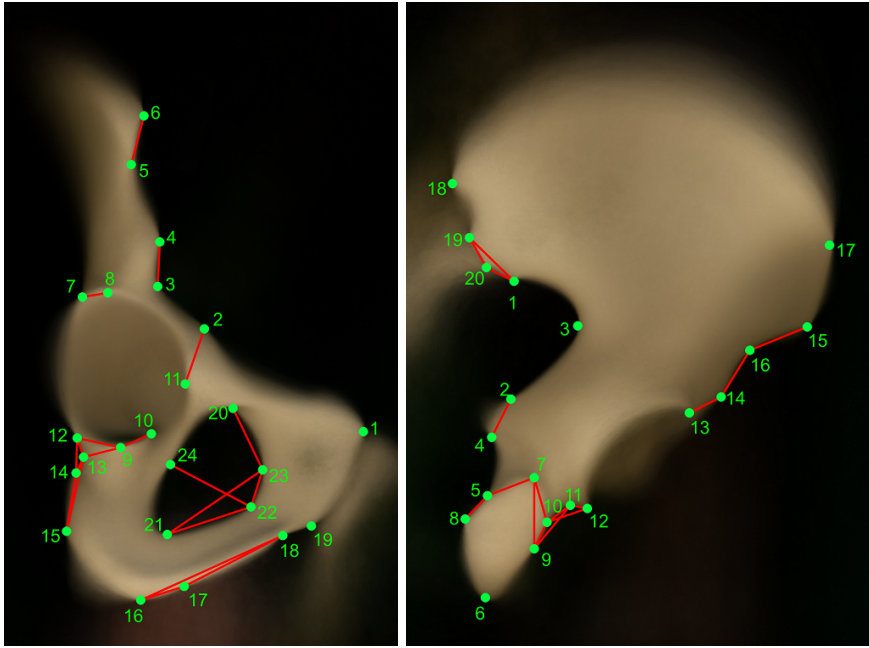


Figure 13: Rejected dimension in Pubic view (left) and Iliac view (right).

C Recommended configurations for geometric morphometry

After digitizing each of the views, the program automatically checks which configuration is available. Then it calculates with the configuration that has the highest number of landmarks present. The process of configuration settings is even more complicated because some points are redundant – there are more of them relatively close together. We have chosen this procedure so that in the case of not being able to locate one of the landmarks (e.g. due to bone damage), a differently defined landmark nearby is available that describes the given part of the bone similarly to the missing one. When selecting a landmark for the appropriate configuration, however, the program selects only one of the closest landmarks – the most appropriate one. In the configurations below, these groups are indicated in brackets. Within the group, the program selects from left to right, if the group is missing a point on the right, it proceeds to the point closest to the left. If there are no landmarks in a group of a given configuration, the program proceeds to a configuration with a lower number of landmarks where this group does not exist.

C.1 Pubic view

Optimal configuration

Optimal configuration is composed of 13 points: 1, 2, 4, 6, 8, 9, 14, 15, 16, 18, 19, 20, 22. If any of these points are not present, some of them may be replaced by another point from their group, but always only one to make the total of 13:

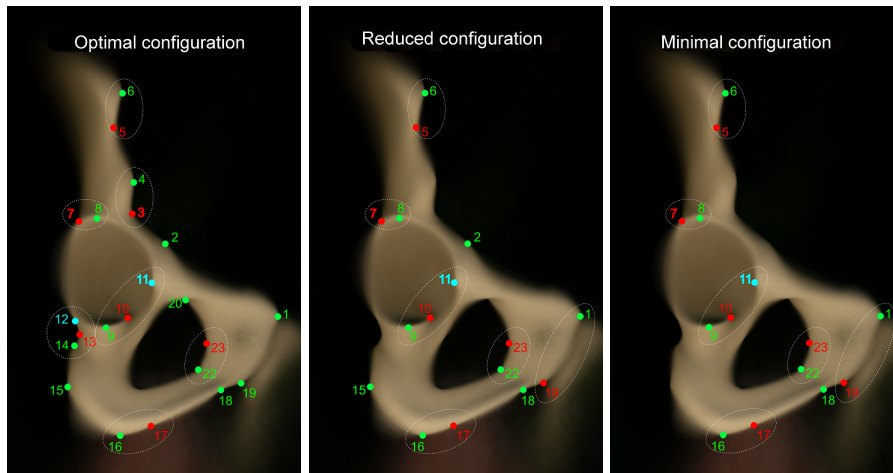


Figure 14: Example of three configurations in Pubic view. The groups of landmarks belonging to each other are surrounded by an ellipse. Only one point is used from each group: priority is green, if it is not present, red is used, if it is not present either, blue is used.

1, 2, (group 4 – 3), (group 6 – 5), (group 8 – 7), (group 9 – 10 – 11), (group 14 – 13 – 12), 15, (group 16 – 17), 18, 19, 20, (group 22 – 23). If the configuration is available, the program calculates with this configuration of 13 points. If it is not possible to create this optimal configuration because of missing points, it proceeds to the reduced configuration.

Reduced configuration

Reduced configuration is composed of 9 points: 1, 2, 6, 8, 9, 15, 16, 18, 22. If any of these points are not present, some of them may be replaced by another point from their group, but always only one to make the total of 9: (group 1 – 19), 2, (group 6 – 5), (group 8 – 7), (group 9 – 10 – 11), 15, (group 16 – 17), 18, (group 22 – 23). If the configuration is available, the program calculates with this configuration of 9 points. If it is not possible to create this reduced configuration because of missing points, it proceeds to the minimal configuration.

Minimal configuration

Minimal configuration is composed of 7 points: 1, 6, 8, 9, 16, 18, 22. If any of these points are not present, some of them may be replaced by another point from their group, but always only one to make the total of 7: (group 1 – 19), (group 6 – 5), (group 8 – 7), (group 9 – 10 – 11), (group 16 – 17), 18, (group 22 – 23). If the configuration is available, the program calculates with this configuration of 7 points. If the configuration is not available, the program announces that analysis by the method of geometric morphometry cannot be performed.

C.2 Iliac view

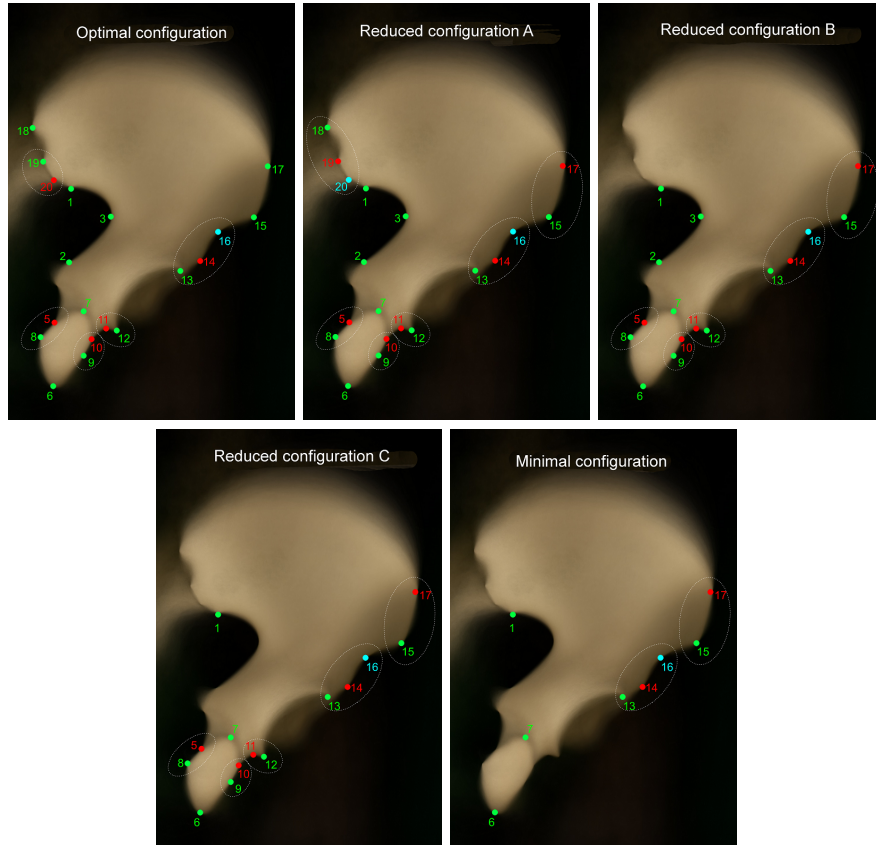


Figure 15: Example of five configurations in Iliac view. The groups of landmarks belonging to each other are surrounded by an ellipse. Only one point is used from each group: priority is green, if it is not present, red is used, if it is not present either, blue is used.

Optimal configuration

Optimal configuration is composed of 13 points: 1, 2, 3, 6, 7, 8, 9, 12, 13, 15, 17, 18, 19. If any of these points are not present, some of them may be replaced by another point from their group, but always only one to make the total of 13: 1, 2, 3, 6, 7, (group 8 – 5), (group 9 – 10), (group 12 – 11), (group 13 – 14 – 16), 15, 17, 18, (group – 20). If the configuration is available, the program calculates with this configuration of 13 points. If it is not possible to create this optimal configuration because of missing points, it proceeds to the reduced configuration A.

Reduced configuration A

Reduced configuration A is composed of 11 points: 1, 2, 3, 6, 7, 8, 9, 12, 13, 15, 18. If any of these points are not present, some of them may be replaced

by another point from their group, but always only one to make the total of 11: 1, 2, 3, 6, 7, (group 8 – 5), (group 9 – 10), (group 12 – 11), (group 13 – 14 – 16), (group 15 – 17), (group 18 – 19 – 20). If the configuration is available, the program calculates with this configuration of 11 points. If it is not possible to create this reduced configuration A because of missing points, it proceeds to the reduced configuration B.

Reduced configuration B

Reduced configuration B is composed of 10 points: 1, 2, 3, 6, 7, 8, 9, 12, 13, 15. If any of these points are not present, some of them may be replaced by another point from their group, but always only one to make the total of 10: 1, 2, 3, 6, 7, (group 8 – 5), (group 9 – 10), (group 12 – 11), (group 13 – 14 – 16), (group 15 – 17). If the configuration is available, the program calculates with this configuration of 10 points. If it is not possible to create this reduced configuration B because of missing points, it proceeds to the reduced configuration C.

Reduced configuration C

Reduced configuration C is composed of 8 points: 1, 6, 7, 8, 9, 12, 13, 15. If any of these points are not present, some of them may be replaced by another point from their group, but always only one to make the total of 8: 1, 6, 7, (group 8 – 5), (group 9 – 10), (group 12 – 11), (group 13 – 14 – 16), (group 15 – 17). If the configuration is available, the program calculates with this configuration of 8 points. If it is not possible to create this reduced configuration C because of missing points, it proceeds to the minimal configuration.

Minimal configuration

Minimal configuration is composed of 5 points: 1, 6, 7, 13, 15. If any of these points are not present, some of them may be replaced by another point from their group, but always only one to make the total of 5: 1, 6, 7, (group 13 – 14 – 16), (group 15 – 17). If the configuration is available, the program calculates with this configuration of 5 points. If the configuration is not available, the program announces that analysis by the method of geometric morphometry cannot be performed.