Phenotyping avian bill sizes; combining the collection of standardized still images with software to obtain observer-independent measures of avian bill shapes

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Abstract

Avian bill size is a morphological trait with evolutionary and ecological importance. Obtaining large-scale observer-independent measures of bill length and bill depth has proven to be challenging. We developed a device, the Bill Phenotyping Box, that allows taking standardized still images from wild small passerine birds in the field. We combine this with dedicated software that, based on a single human action, determines both bill length (tip to nostril) and depth (at 2/3 of the distance from the tip). We tested for consistency of observations by correlated measurements from two independently taken still images, which was high (r=0.85). We showed that the measurement depends on the angle of the bill position relative to the camera of the device but this error is relatively small compared to the between-observer variation in hand-based measures. We show that these hand-based measures are strongly observer-dependent and that calibrating observers to each other may take at least 50 measurements, which is not feasible in field studies with dozens of observers. We thus have developed a new method that allows large scale observer-independent bill morphological measurement on wild passerines. Both the specifications of the device and of the software is openly available.

Keywords

Bill size, Passerine, Parus major, software, phenotyping

Statements and Declarations

The authors declare no competing interests.

Introduction

Avian bill size is a morphological trait with evolutionary and ecological importance. Across species, bill size and shape are often closely adapted to the food resources a species uses. There is however also ample within-species variation in bill size [1], on which evolution can act. The most well-known example of rapid evolution of bill size in relation to food resource variation is the work on Darwin finches [2]. Despite a large heritable component of bill size, the trait is phenotypically plastic and can thus vary within individuals over the annual cycle. This seasonal variation in bill size is caused by growth and wear of the keratin comprising the bill and is thought to be related to food resources varying over the year [3,4]. Genetic, between individual, variation is reflected in both the keratin and the underlying skeletal elements of the bill.

Other bill functions than acquiring food have also been related to bill morphology. The avian bill plays an important role in acoustic signalling and in body temperature regulation, and therefore in energetics. It is therefore often found to be positively correlated to environmental temperatures, both within and among species [5]. Bill characteristics have also been suggested to play an important role as the first line of defence against harmful ectoparasites [6].

Bill size has been shown to be affected by anthropogenic environmental change such as climate change and urbanization. For instance, northern cardinals' bill size was affected by temperature as birds in warm, dry areas had larger bills than those in cool, dry areas. Over an 85-year period, female cardinals' bill size increased with warming temperatures [7]. Another striking effect of climate change on bill size is found in an avian long-distance migrant, the red knot, which breeds in the high-Artic. Due to the changing climate the red knot's breeding phenology has got out of synchrony with the phenology of the food for their offspring, which as a result have a smaller bill. This in turn leads to problems at the tropical wintering grounds where they can no longer reach their bivalve prey [8]. Bill size in the great tit has gotten longer over time in the UK, potentially in response to supplementary feeding by the general public [9].

To test hypotheses on the evolutionary and ecological importance of bill size, large-scale measurements are needed, and often these need to be taken from birds in the field, for instance when demonstrating fitness consequences of bill size. Under field conditions, often a large number of observers are involved in taking these measurements, making the reduction of between-observer variation a necessity. As there are often many observers, and the number of birds that are measured by more than one observer is usually small, statistical correction for observer differences is difficult, if not impossible. Thus, obtaining large-scale observer-independent measures of bill length and bill depth is needed, but has proven to be challenging. We therefore developed a device, the Bill Phenotyping Box, to take still images of wild passerine bird bills, as well as software to obtain bill length and depth in a standardized way. We describe both the device and software, show that our tools produce repeatable and accurate measurements, and we make a comparison with manual measurements.

Methods

The Bill Phenotyping Box

We developed a device, the Bill Phenotyping Box (BPB) that allows taking standardized still images from wild small passerine birds. The BPB is a wooden box that consists of a camera, a tube to fixate the bird (by folding its wings and gently push the bird into the tube) with its bill clearly visible, a light to illuminate the bill, a metal tab to write the ring number of the bird on, and a ruler for calibration

(Fig. 1). We used a Nikon COOLPIX A100 compact digital camera, but any similar model with a sufficiently short focal distance and wide angle should work.

In short, the procedure is to capture the bird, switch on the light and write the ring number on the metal tab and slide it in the BPB. Then the bird's head is put in the funnel and the bill is carefully positioned. It is important that the bill is at a 90-degree angle to the camera (so the tip and the base at an equal distance to the camera), and the bill is positioned horizontally as much as possible. This minimizes measurement error (see Results). Then a picture is taken and the bird is removed from the BPB. Please see Electronic Appendix 1 for detailed instructions for taking avian bill measurement with the BPB, and Electronic Appendix 2 for construction drawings of the BPB.

The software

We combined the BPB with dedicated software that, based on a single human action, determines both bill length (tip to nostril) and bill depth (at 2/3 of the distance from the tip). We used the nostril following the recommendation of Borras et al. [10] who state that "All of this highly recommends that, especially in granivorous birds, the length of bill should be measured from nostril".

A screen shot of the software is presented as Fig. 2 and Electronic Appendix 3 contains an extensive description of the steps taken by the software to obtain the bill measurements. In brief, the application consists of three steps. First, based on a random image within a series of images of the bill taken in the same measuring session (i.e. using one BPB equipped with same camera), a range of the ruler is selected to determine the conversion factor for pixel to mm for a particular series of images. Secondly, a broader crop area is defined by the user that should encompass all bills of a series of images. Lastly, for each individual image the user selects the position of the front right edge of the nostril. The application then uses a smaller ROI (Region of interest) bounded on the left by the nostril position for successively conversion to grey-scale, thresholding to separate background from bill, kernels denoising and closing of holes in the selected bill area and lastly a rotation of the final bill area and calculation of the relevant coordinates. The source code for the software can be downloaded here: https://gitlab.com/track_32/beak-phenotyping.

Validation tests

Between observer correlations (experienced and novice) of hand-based measurements. The standard for bill measurements are measures obtained manually, using callipers. To determine the between observer correlation and the time it took to gain the necessary skills by a novice, two observers, one expert and one novice, measured tip-to-skull bill length in 181 great tits (*Parus major*) over a three-day period. We then calculated a sliding-window correlation between the two observers over time.

Between device correlations We tested the repeatability between two camera box setups by photographing 41 birds in two separate boxes (including the whole process of placing and orienting each individual). We used the rptR package [11] to assess repeatability using a lmm approach assuming a gaussian distribution and 1000 bootstrap rounds.

Possible deviations based on 3D model

Using a digital 3D-scan of a bill from *P. major* obtained from the mark-my-bird project (https://www.markmybird.org/gallery/parus_major/1136) we manipulated the rotation of the bill around all three axes in the software Blender (version 3.6.1). As rotational origin we defined a point in the centre of the plane when cut vertically at the nostril. The projected bill length was then measured from a point on the bill surface approximating the nostril to the tip using the measure tool in Blender. Measures were obtained separately for two rotational angles around the z-axis and then for a range of angles around the x-axis (in 13 steps from 25° to the left to 25° to the right) and around the y-axis (in five steps from 10° forward to 10° back).

In order to investigate the prevalence of these deviations in the field we tested 100 bill images taken from the top and 100 taken from the side. The top images were measured for rotation around the y-axis and the side on images for rotation around the z-axis. From these measures we plotted the distribution of rotational angles.

<u>Phenotyping bills in the wild</u>

We used the BPB to phenotype great tits' bills in the field. Images were collected in five areas where the Netherlands Institute of Ecology (NIOO-KNAW) has run long-term nest box studies (Hoge Veluwe, Vlieland, Oosterhout, Liesbos and Westerheide, all in the Netherlands), over four years (2018-2021). Parents were captured as part of our long-term field studies at the time of breeding, all were adult birds. Over these areas and years, about 30 observers were collecting the still images using the BPB. The pictures were later analysed using the software, which was done by two of us (JR & BvL). In total we obtained phenotypes of 2041 birds.

Results

In the calibration between observers, we found that the hand-based measurements are strongly observer dependent and that calibrating observers to each other requires over a hundred measurements (Fig. 3), which is not feasible in field studies with dozens of observers.

In our test for consistency of observations we measured two independently taken still images of the same bird and used the software to phenotype the bill. We found that the measurements were highly repeatable ($R = 0.801$, CI = [0.66,0.89], P (LRT) < 0.01, Fig. 4) which shows that the BPB and the software leads to reliable bill phenotypes. Bill depth on the other hand has a lower repeatability ($R=0.606$, CI = [0.36, 0.76], P(LRT) < 0.01).

We tested how the precision of the measurement depended on the position of the bill relative to the camera. The results show that when taking a picture, the position of the bill was important for the measurement but this error was relatively small compared to the between-observer variation in hand-based measurements (Fig. 5). Of the three axes along which the bill could be rotated, the angle to the camera affected the accuracy the most, so the bill should be at a 90-degree to the camera (so the tip and the base at an equal distance to the camera).

In our measurements with the BPB in the wild we phenotyped 2041 individuals, resulting in a normal distribution of bill size (Fig. 7). The loss of images due to low quality was about 15% (373 individuals out of 2414). 50 individuals could not be measured as one observer photographed the bill from the top instead of side-on. Other main reasons for loss are absence of a visible nostril (due to the bill not protruding far enough out of the tube or being covered by feathers) and no satisfactory rotational view. On rarer occasions the image was out of focus or the photo could not be assigned to an individual bird due to issues with the ring number. In our view, this percentage can be brought down significantly if there can be a more intense training for the fieldworkers than we were able to organise.

Discussion

We thus have developed a new device, the Bill Phenotype Box (BPB), with associated software that allows large scale observer-independent bill morphological measurement on wild passerines. Both the specifications of the BPB and the software are made openly available. We show that the potential error in the measurements is small compared to hand-based bill measurements as there is large between observer variation in such measurements. In our test of such a between observer correlation we used one very experienced and one novice (a classical training set-up). The initial correlation between two inexperienced observers is likely to be much lower and will take many more measurements to reach a stable and high correlation. Obviously, this repeatability would be higher between two trained observers (after the initial training) but this is rarely the case in the way field monitoring of birds is carried out. And in cases where just a single observer monitors a population for multiple years hand measurements are likely to have an even higher repeatability. There is relatively little literature on the repeatability of avian morphological measurements between observers. What is often reported is the within individual repeatability, where repeatability is usually around 0.90 [3,12,13].

The current 15% loss of observations is too high, and indeed manual observations are much less likely to be lost. However, during this pilot we were also in the process of determining how to instruct users and which aspects of using the camera box to emphasize. It is now clear that taking multiple images with slight variation of rotational angle is paramount. Making sure the nostril is clearly visible is also indispensable and future instructions will take this into account. On the other hand, errors in field data due to spelling mistakes in ring numbers and observed measures are unavoidable despite best efforts and affect both manual and camera-based observations. On advantage of the photographs is that a certain level of detective work can resolve some of the issues.

Currently the software works well for obtaining bill length from great tit bill pictures. Obtaining depth is configured for the great tit as used in this paper where the bill depth is taken at 2/3 of the distance from the nostril to the tip. This might not be suitable for other, more thick-billed species. They can currently be measured using the manual adjustment options available in the software. For a more flexible use, the placement of the depth line can be adjusted by users in the open-source code of the software.

Taking images with a standardized device, such as the BPB, will also contribute to the comparability between studies. Firstly, because using the same device and the same software will also greatly reduce the variation between studies, but secondly, it also opens up the possibility to share the raw data (i.e. the images taken) instead of measurements only. These images from different studies can then be merged and analysed by a single observer, again reducing between study variation. This might save time and effort and will also contribute to Open Science. Another advantage of sharing photographs is that more detailed shape analysis can be done.

We therefore encourage researchers working on bill morphology in the field to make use of the BPB, which can be build using the technical drawings in Electronic Appendix 2. It will help obtaining observer independent phenotyping and this way contribute to the study on the evolutionary and ecological importance of avian bill size.

Acknowledgements

We are grateful of the people at the NIOO-workshop for building the BPB, and especially to Gerben-Jan van der Kamp for the construction drawing, see Electronic Appendix 2. We also would like to thank all the NIOO personnel that used the BPB in the field, on top of their already busy fieldwork.

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Appendices (see below)

1. Detailed instruction for taking avian bill measurement with the Bill Phenotyping Box

2. Construction drawing of the Bill Phenotyping Box

3. Manual for the bill phenotyping application

References

1. Tobias JA *et al.* 2022 AVONET: morphological, ecological and geographical data for all birds. *Ecology Letters* **25**, 581–597. (doi:10.1111/ele.13898)

2. Grant PR, Grant BR. 2006 Evolution of character displacement in Darwin's finches. *Science* **313**, 224–226. (doi:10.1126/science.1128374)

3. Gosler AG. 1987 Pattern and process in the bill morphology of the Great Tit Parus major. *Ibis* **129**, 451–476. (doi:10.1111/j.1474-919X.1987.tb08234.x)

4. Matthysen E. 1989 Seasonal variation in bill morphology of Nuthatches Sitta europaea dietary adaptations or consequences?. *Ardea* **77**, 117–125.

5. Tattersall GJ, Arnaout B, Symonds MRE. 2017 The evolution of the avian bill as a thermoregulatory organ. *Biological Reviews* **92**, 1630–1656. (doi:10.1111/brv.12299)

6. Clayton DH, Moyer BR, Bush SE, Jones TG, Gardiner DW, Rhodes BB, Goller F. 2005 Adaptive significance of avian beak morphology for ectoparasite control. *Proceedings of the Royal Society B: Biological Sciences* **272**, 811–817. (doi:10.1098/rspb.2004.3036)

7. Miller CR, Latimer CE, Zuckerberg B. 2018 Bill size variation in northern cardinals associated with anthropogenic drivers across North America. *Ecology and Evolution* **8**, 4841–4851. (doi:10.1002/ece3.4038)

8. van Gils JA *et al.* 2016 Body shrinkage due to Arctic warming reduces red knot fitness in tropical wintering range. *Science* **352**, 819–821. (doi:10.1126/science.aad6351)

9. Bosse M *et al.* 2017 Recent natural selection causes adaptive evolution of an avian polygenic trait. *Science* **358**, 365–368. (doi:10.1126/science.aal3298)

10. Borras A, Pascual J, Senar. J. C. 2000 What do different bill measures measure and what is the best method to use in granivorous birds? *Journal of Field Ornithology* **71**, 606–611. (doi:10.1648/0273-8570-71.4.606)

11. Stoffel MA, Nakagawa S, Schielzeth H. 2017 rptR: repeatability estimation and variance decomposition by generalized linear mixed-effects models. *Methods in Ecology and Evolution* **8**, 1639–1644. (doi:10.1111/2041-210X.12797)

12. Yezerinac SM, Lougheed SC, Handford P. 1992 Measurement error and morphometric studies: statistical power and observer experience. *Systematic Biology* **41**, 471–482. (doi:10.2307/2992588)

13. Subasinghe K, Symonds MRE, Vidal-García M, Bonnet T, Prober SM, Williams KJ, Gardner JL. 2021 Repeatability and Validity of Phenotypic Trait Measurements in Birds. *Evolutionary Biology* **48**, 100–114. (doi:10.1007/s11692-020-09527-5)

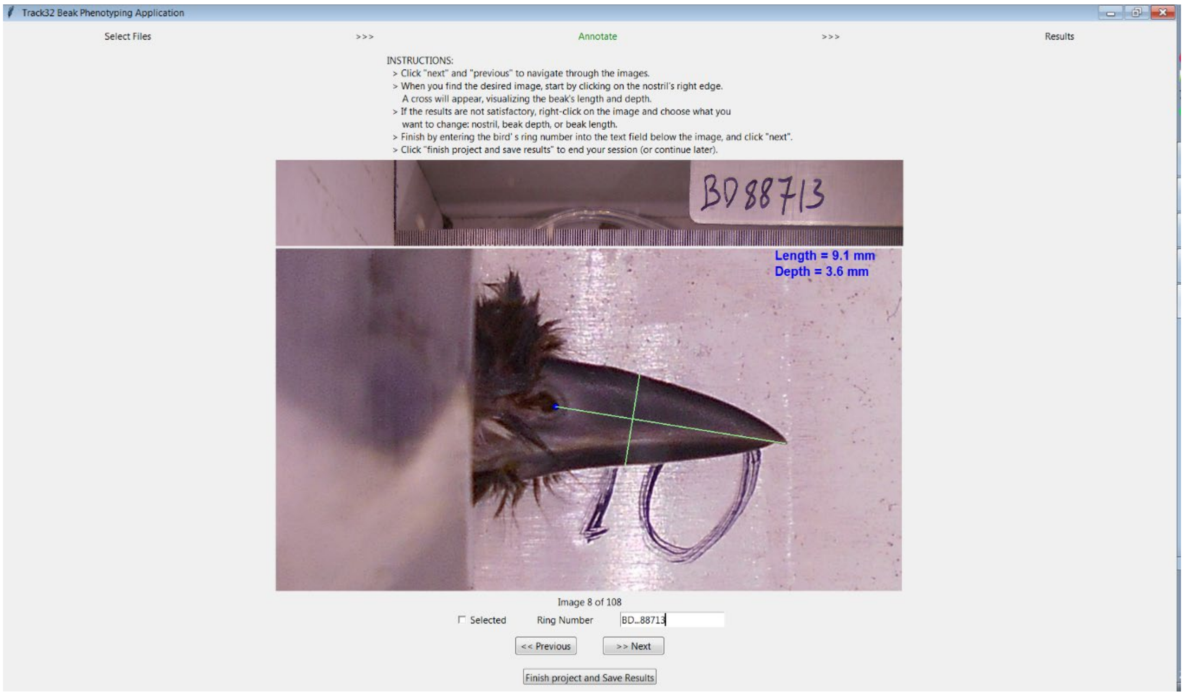
Figure 1a

The Bill Phenotype Box (BPB): outside view with camera and on the bottom left the funnel where the bird's head needs to be inserted.

Figure 1b

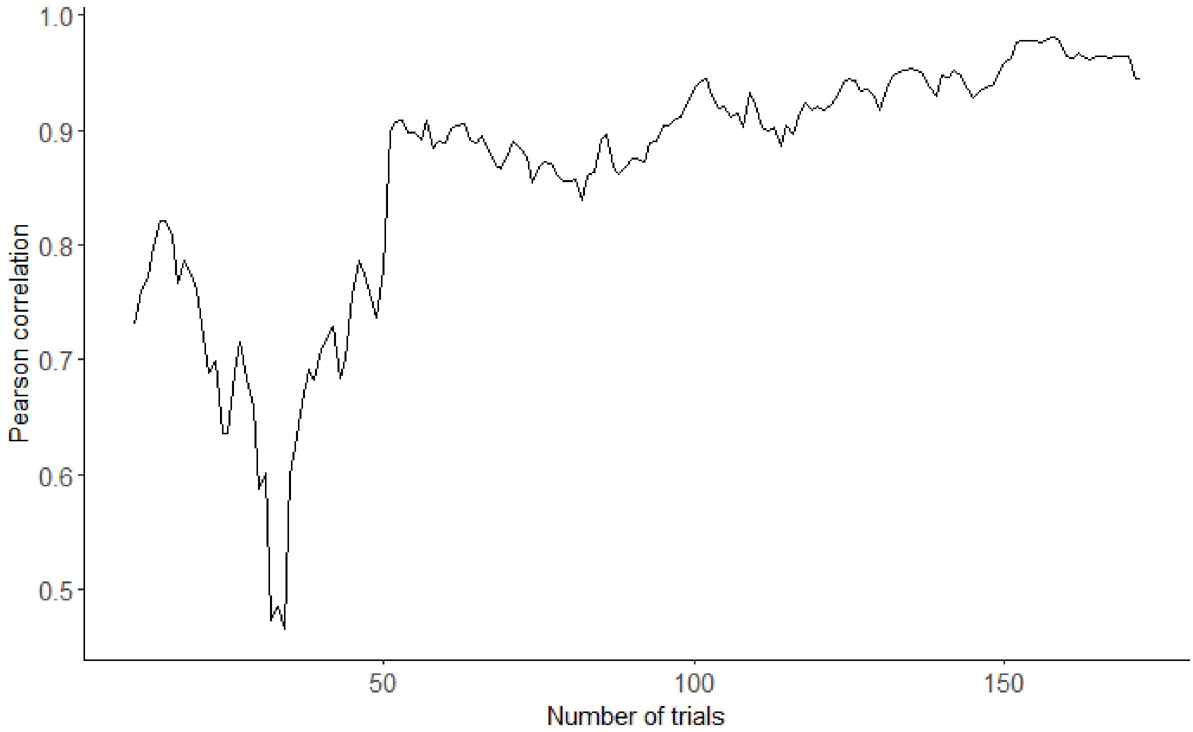
The Bill Phenotype Box (BPB): inside view with a great tit in the funnel. Note the position of the bill, the metal branch with the ring number (BD46594) and the ruler. The batteries are for the illumination.

Figure 2



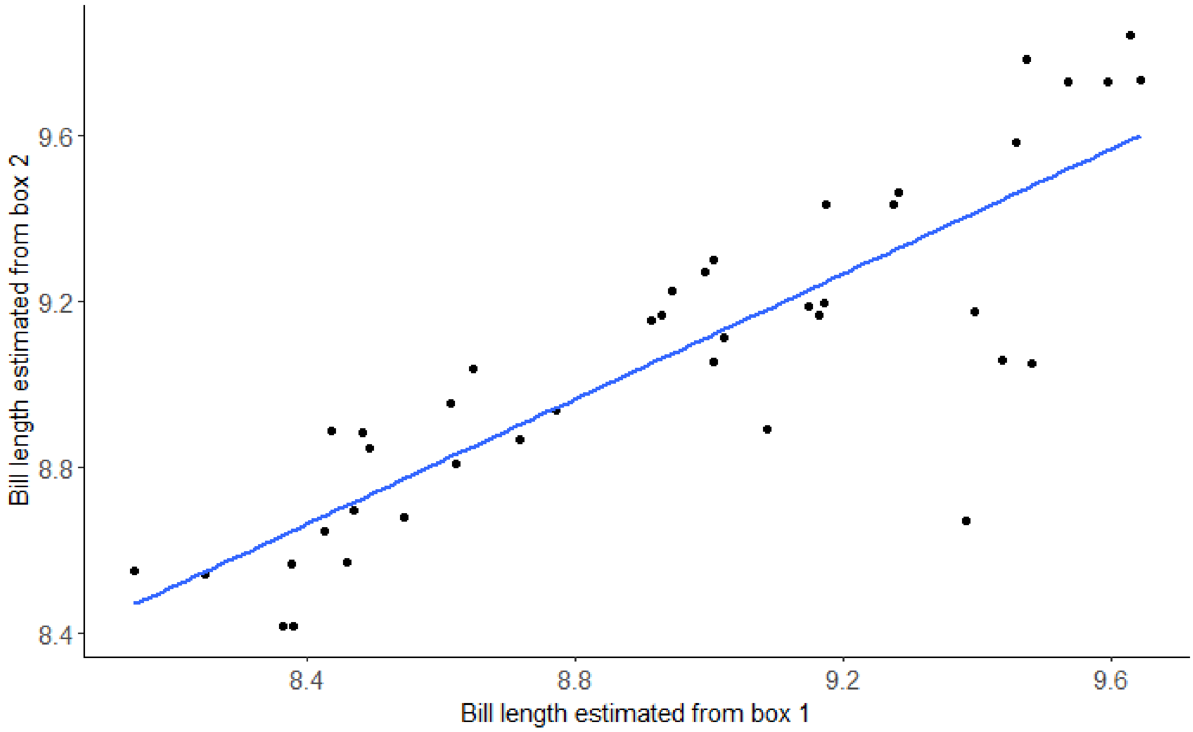
Screen shot of the software. The position of the nostril needs to be indicated after which the software calculated the bill length (horizontal green line) and the bill depth (vertical green line). See Electronic Appendix 3 for a detailed manual.

Figure 3



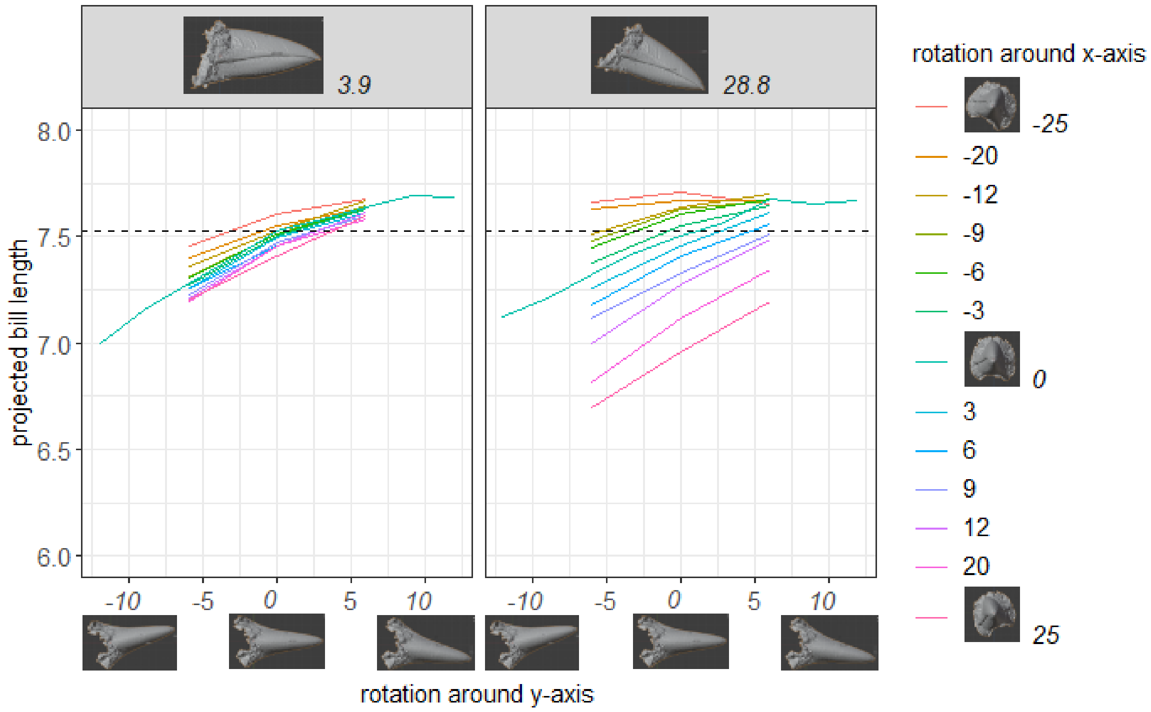
The sliding window (width=20) correlation between the manual bill length (tip-to-skull) measurements of the same 182 great tits by two observers (one very experienced and one novice, i.e. a classical training set-up) over the number of trials.

Figure 4



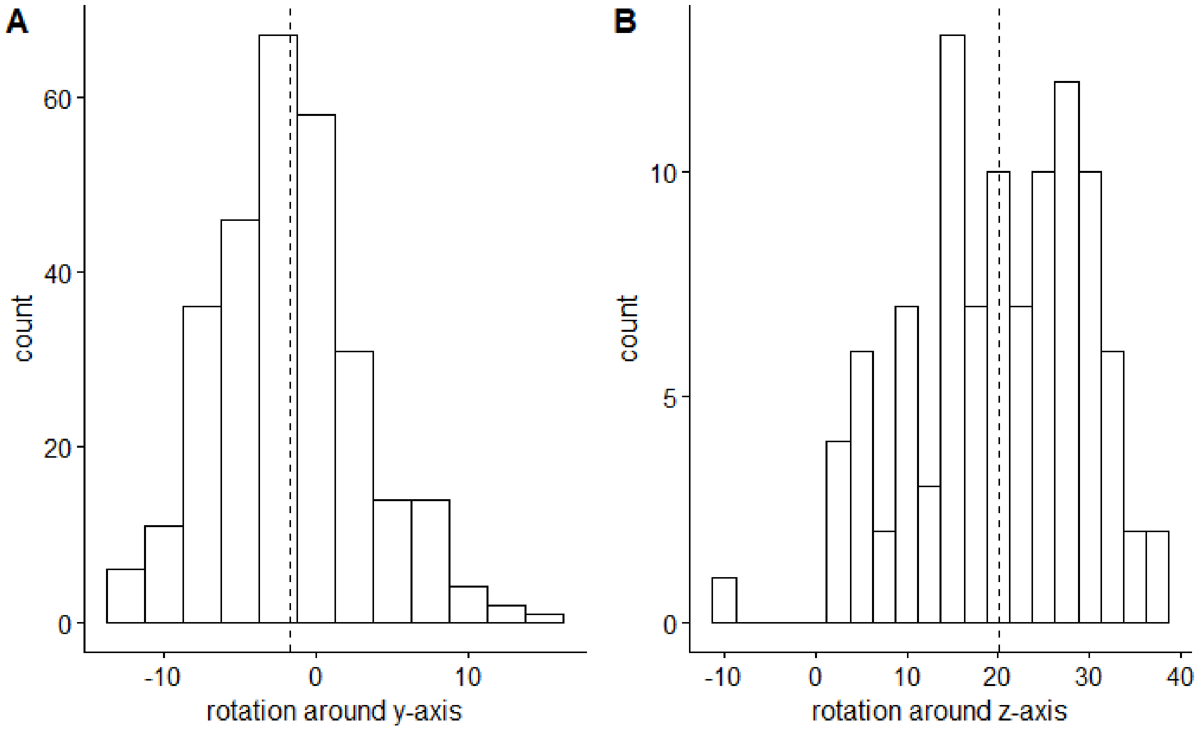
Pairwise comparison of the bill length of a great tit as estimated from two independently acquired still images, taken with the BPB and phenotyped with the software. The blue line shows lm(y ~ x) (r = 0.85). Differences are due to both between box differences and differences between orientation of the bill in the box, with the latter a more likely driver of differences.

Figure 5



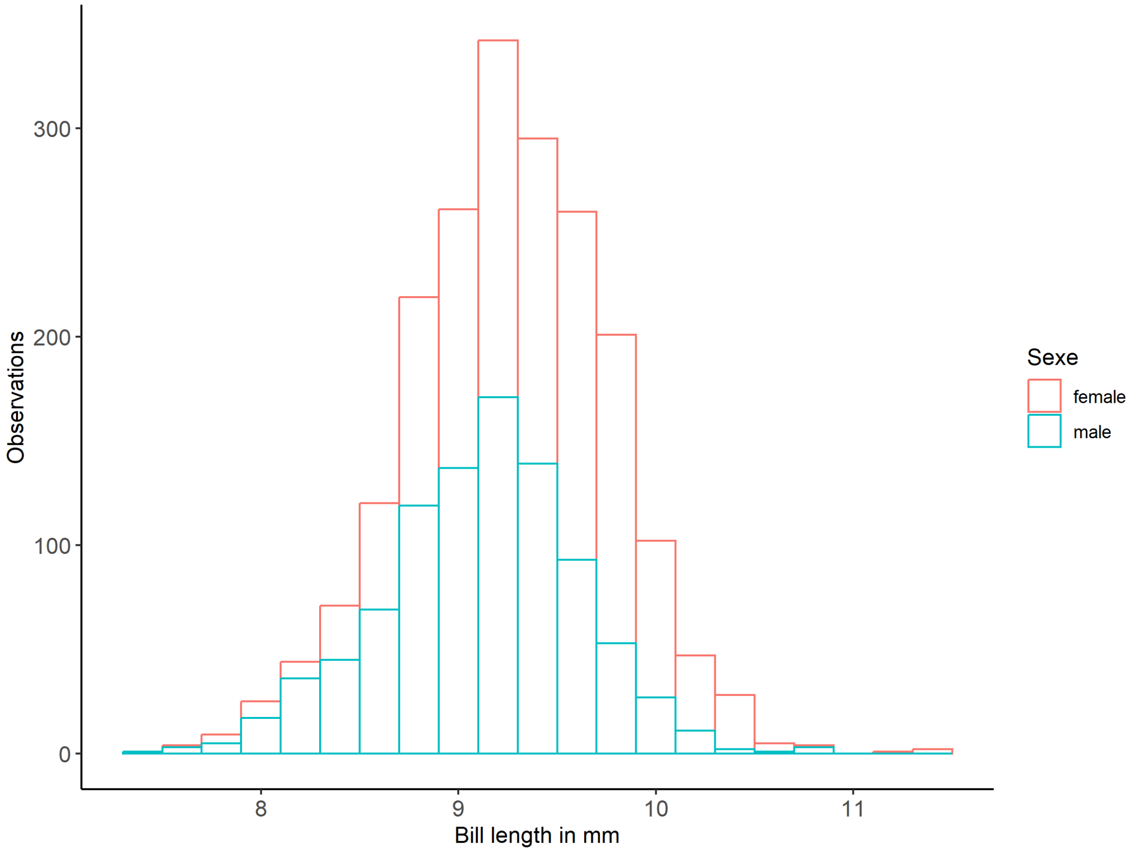
The precision of the measurement dependent on the position of the bill relative to the camera. The two panels show measures of projected bill length for a rotation of 3.9 (left) and 28.8 (right) around the z-axis. Each panel shows a series of measurements for 13 different rotational angles around the x-axis. Each series consists of measures at three different rotational angles around the y-axis, with the exception of the series for zero rotation around the x-axis which has been expanded to nine different angles around y. The dashed line represents the optimal, correct, projected bill length for zero degrees rotation around all three axes.

Figure 6



Observed rotational angles from images. A: 100 images of bills photographed from the top (binwidth=2.5 degrees) and measured for rotation around y-axis. B: Random selection of 100 images of bills photographed from the side (binwidth=2.5 degrees) and measured for rotation around the z-axis.

Figure 7



Histogram of the bill length of 2041 great tits as phenotyped with the BPB and the software in the wild. Data from five great tit study areas in the Netherlands over a four-year period. In red female and in blue male great tits (frequencies are stacked).

Appendix 1

~~Electronic Appendix 1~~

Detailed instruction for taking avian bill measurement with the Bill Measurement Box

Preparation

1. Check the camera (Nikon COOLPIX A100) has enough battery life and a memory card.
2. Place the camera in the mount. Close the lid of the box securely.
3. Verify that the autofocus area is set correctly (indicated by [ ] on the screen, if necessary adjust using manual focus settings in the menu), check that date and time are displayed correctly on the screen.

For each bird

4. Make sure to note the ring number of the bird on the stick and slide into the box.
5. Switch the light on.
6. Put the bird in the clear tube, bill sticking out the small hole:
   Be careful that the bill is facing forward, sometimes the bill points downwards. Enticing the bird to bite/attack an object in the small hole can help. Make sure the shoulders/wings go in and leave the legs out. If the legs are inside the tube, the bird can get back out (Figure 1)
   Don't push the bird too far in and beware of the eyes touching the tube.
7. Push the feathers up and away from the nostril using a wet finger or cotton swab. Make sure the front edge of the nostril is clearly visible (this is the measuring reference point!) (Figure 2 a and b)
8. Place the tube with the bird into the hole on the side of the box so that the bill is in the autofocus area of the camera (indicated by [ ] in the camera display) (Figure 3). Make sure there is no gap between the white background and the edge of the tube.
9. Orient the bird to face the camera side on with the right side of the bill towards the camera. You can use the white cheeks and the bill to orient the bird. (Figure 4)
10. Before each shot make sure the camera focusses the image by pressing the shutter half way, the [ ] should turn green. If the camera cannot focus the [ ] turns red. In that case try to push the end of the tube slightly downward/away from the camera and try again.
11. Take 4 pictures with in each shot the bill rotated slightly compared to the previous one:
    Determining the perfect side position from the camera view can be tricky so taking a series of images with varying rotation allows us to select the most optimal for measurement.

After

12. Switch the light off if there are long breaks between measurements, the camera switches off by itself.
13. Secure the camera and tube before transport.
14. Remember to keep batteries charged and have new memory cards available.

Figure 1: View of tube with bird from the outside of the box

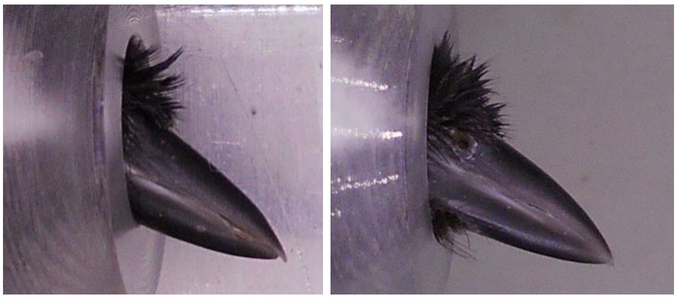


Figure 2: a) NOT GOOD: obscured nostril edge b) GOOD: clearly visible nostril edge

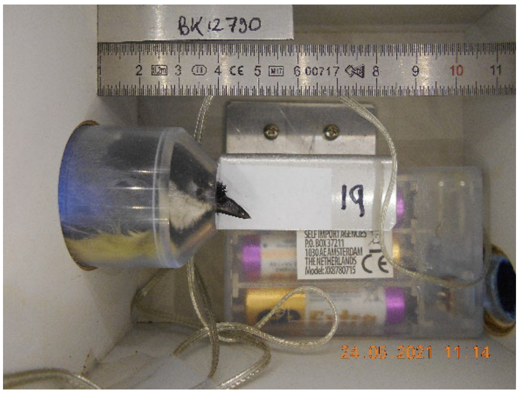


Figure 3: View of the bird in the tube on the inside of the box.

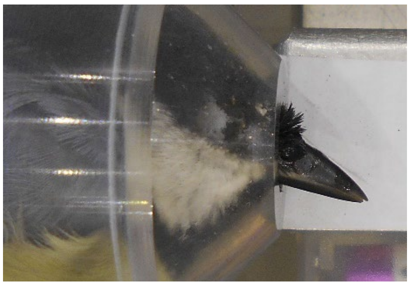
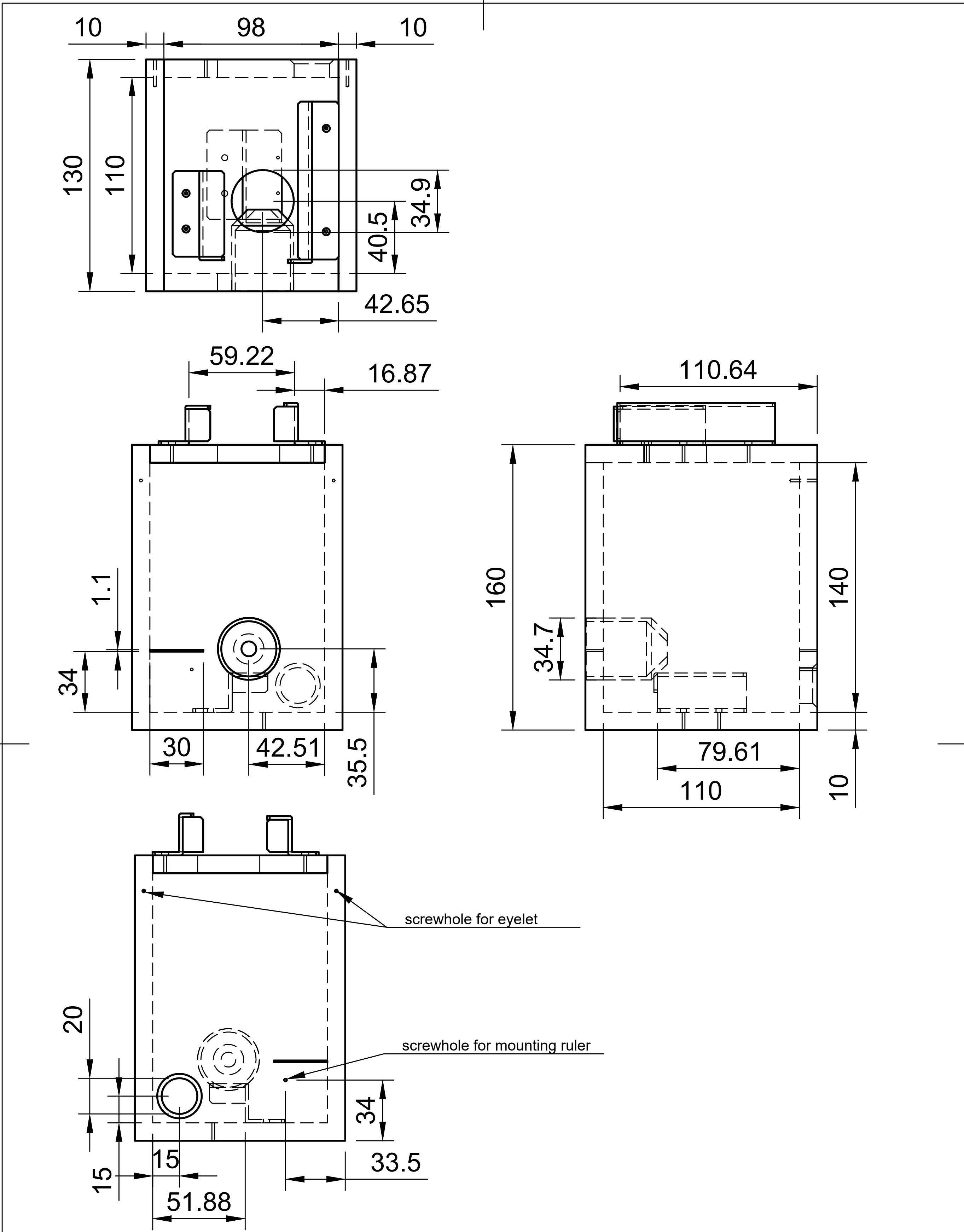
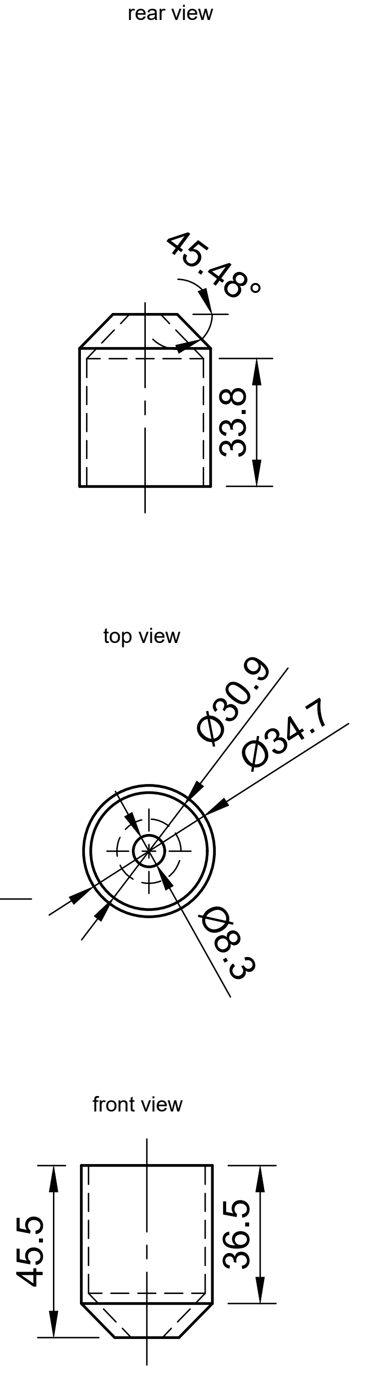


Figure 4: Use the white cheek to initially orient the bird

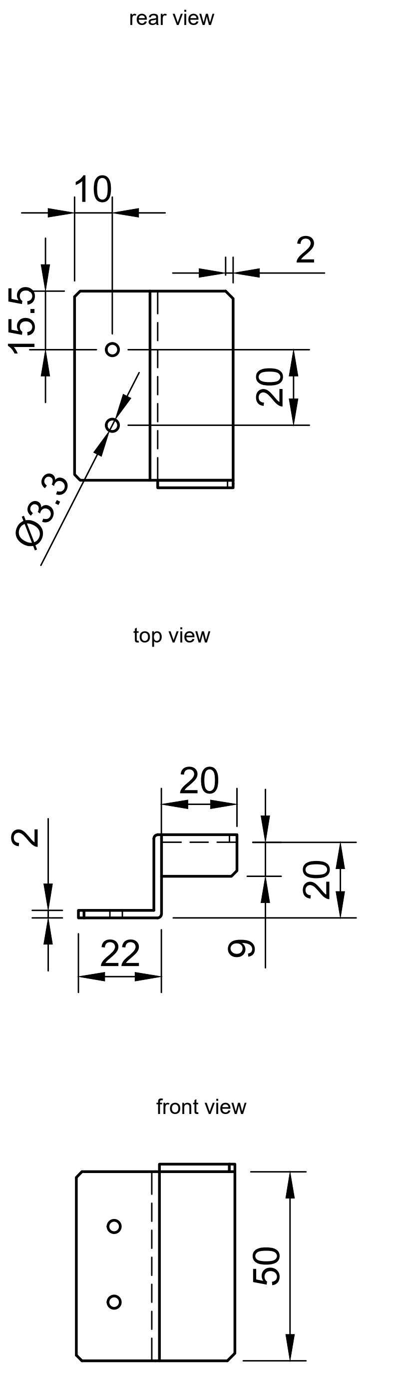
Appendix 2



Dept.	Technical reference	Created by Gerben-Jan van de Kamp 2-8-2023	Approved by		
Box with internal measures -. measurements holes -. measurements for camera placement and battery pack lighting Material box: waterproof plywood, 10mm thick		Document type	Document status		
		Title 23-201 bill length measuring box	DWG No.		
			Rev.	Date of issue	Sheet 1/1



Funnel bird head



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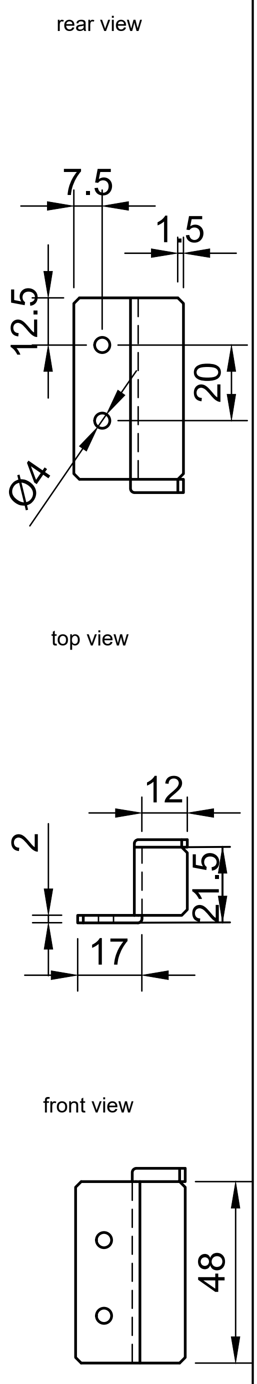

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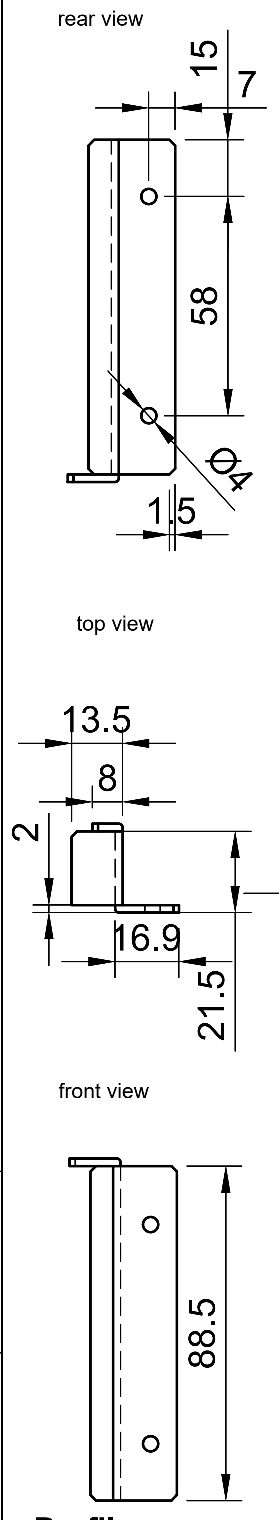


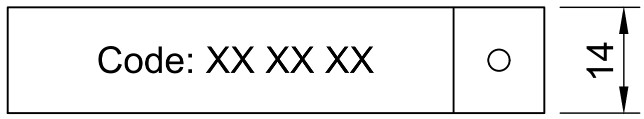
Profile camera Right

Dept.	Technical reference	Created by	Approved by
		Gerben-Jan van de Kamp 2-8-2023	

Materials Funnel: Perspex Battery pack mount: 2mm AL Camera profiles: 2mm AL	Document type	Document status
	Title 23-222 bill length measuring box mounting parts	DWG No.
		Rev. | Date of issue | Sheet 1/1

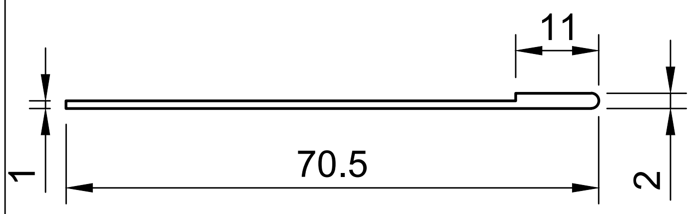
Ring number strip

top view



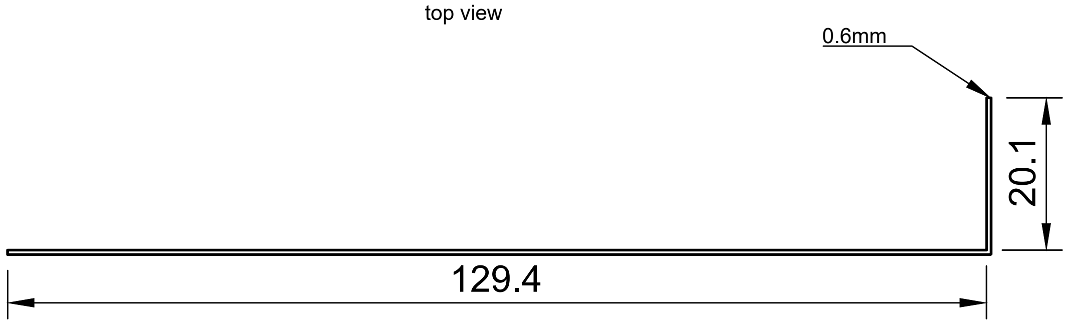
hole diameter
3.0mm

front view



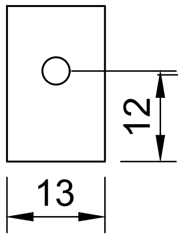
Ruler

top view

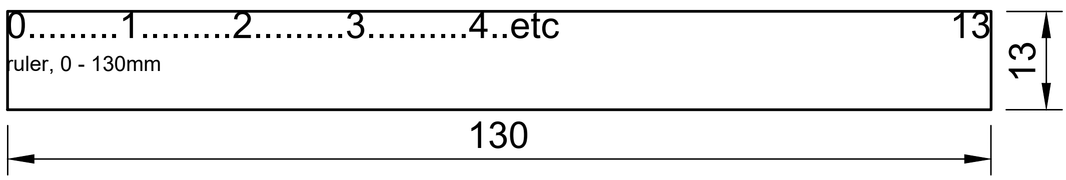


side view

hole diameter 3.0mm



front view



Dept.

Technical reference

Created by
Gerben-Jan van de Kamp 3-8-2023

Approved by

Materials
Ring number strip: AL, 1mm
Ruler: Stainless steel , type nr.
00717

Document type

Document status

Title
bill length measuring box, ruler and ring number strip

DWG No.

Rev.

Date of issue

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1/1

Appendix 3

Manual for the beak phenotyping application

Contents

1 Introduction

In 2019, Track32 supplied NIOO-KNAW with an application they can use to perform phenotyping of bird beaks, in particular measure the length and width of the beak in millimetres. This document explains the different steps involved in this phenotyping application. This document only deals with the steps involved in processing one image. It does not talk about the other features of the application like image folder/project management, different prompts to the user or how the results of the application are saved.

The input of the image processing pipeline is an image and the output is the length and width of the beak in the image. Following are the steps in the phenotyping application to generate the output from the input image.

2 Load image and select region of interest

For each bird image, the beak only forms a small part of the image. The beak part on the image contains a steel plate in its background. This steel plate provides a consistent background to perform segmentation of the beak and hence it is our region of interest. Since the perspective of this steel plate can vary slightly per dataset, user must select the region of interest at every image folder that is loaded into the application. Figure 1 shows an example selection of region of interest in the 'Select Region of Interest' pop-up window from the home Page of phenotyping application. An example of resulting cropped ROI image as shown in the 'Annotate' section of the application is shown in Figure 2.

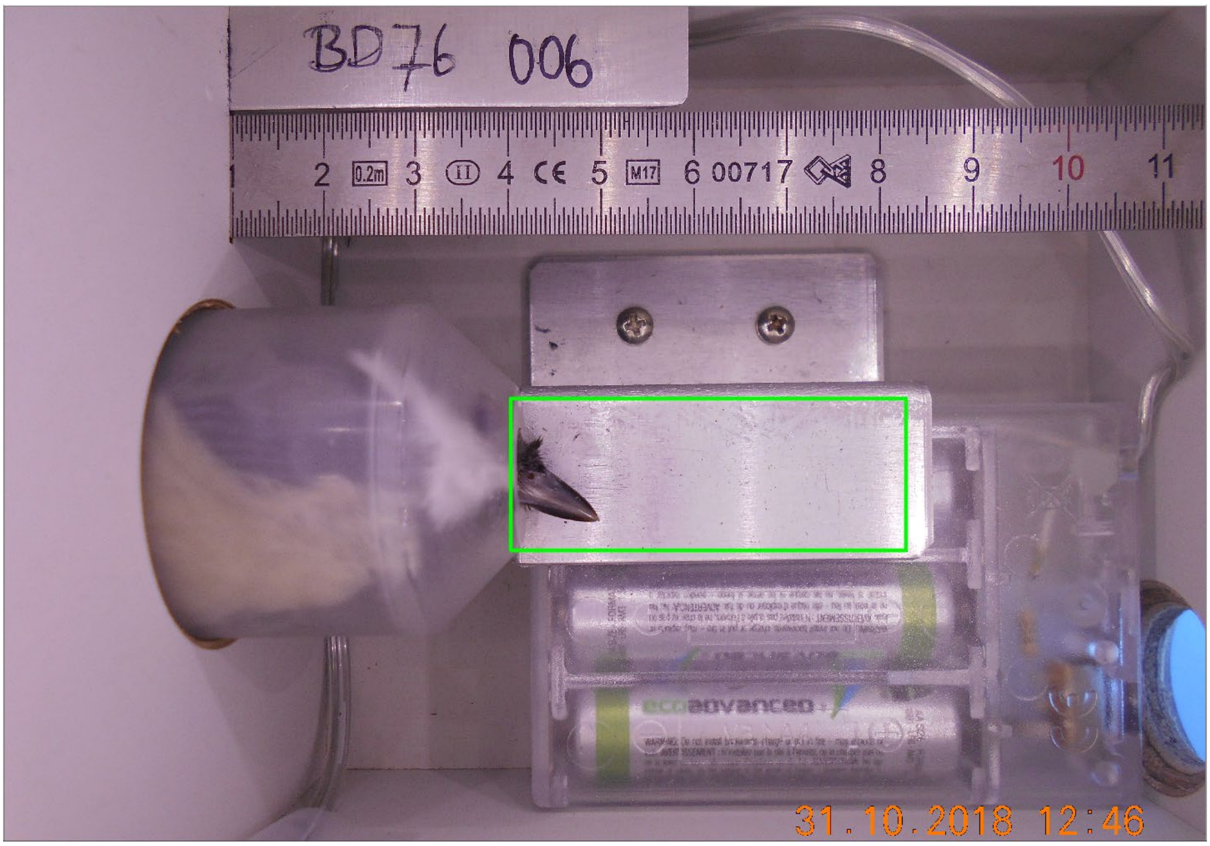

Figure 1: Example region of interest selection in phenotyping application

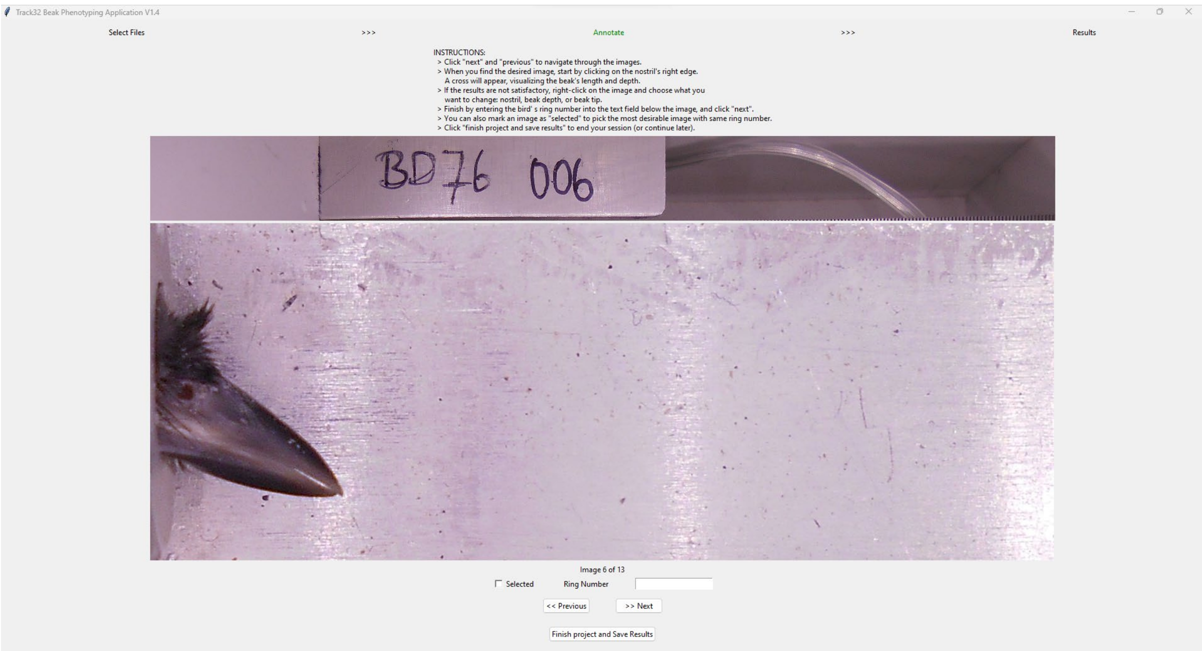

Figure 2: Image after selecting region of interest

3 Find conversion factor from pixels to mm

The output beak widths and lengths calculated in an image is measured in pixels. This needs to be converted to milli meters. The home page offers an option for the user to update this value by measuring it directly from the images. In each bird image, a metallic scale visible. We use that to calibrate the image, to find the pixel to mm conversion factor. This is done by selecting two points on the scale at a known distance (for e.g., 5 cm away from each other) and use that distance in pixels to calculate the conversion factor. An example of this from the phenotyping application's 'Measure factor to convert pixel distances to mm' window is shown in Figure 3. For most images, we see that this conversion factor is 0.025 milli meters for each pixel and hence set as default value in the application. Calibration is not required to be done every image. However, expecting some slight changes in perspective per data collection, the phenotyping application has a feature built in to perform this calibration.

Figure 3: The green lines are selected by user, 5 cm apart and used to find the pixels to mm conversion factor

4 Get user input of nostril coordinates

In the ‘Annotate’ section of the application, the region of interest of the beak image will be shown to user. User starts the image processing steps by clicking on the nostril's right edge as shown by the red dot in Figure 4.

Figure 4: Nostril selected (in red) on a beak region of interest

5 Calculate length and width of beak

With the region of interest selected and the nostril selected, we can calculate the length and width of the beak. The application follows a series of image processing steps to process the input RGB region of interest to yield length and width of beak

a) Crop the ROI image from nostril

From the region of interest, anything left to the nostril coordinate is ignored for calculations. A nostril cropped image from the user selected in Figure 4 is shown in Figure 5.

Figure 5: Region of interest image, cropped from nostril

b) Convert image to Gray

Region of interest image after cropping shown in Figure 5 is converted to a grey image using [OpenCV's cvtColor() function](). Resulting image is a single channel 8-bit image as shown Figure 6.

Figure 6: Grey image from Cropped RGB image

c) Perform Otsu Thresholding on grey image

[Otsu thresholding]() is used to separate the foreground and background pixels. Otsu algorithm estimates a thresholding value automatically which results in separating the pixels into foreground and background. Resulting binary mask image is shown in Figure 7. Foreground pixels are represented with a value of 1 and shown in white. Background pixels are represented with a value of 0 and shown in black.

Figure 7: Resulting binary image after Otsu thresholding

d) Define a kernel and perform morphological operations

To remove the smaller noise and to generate connected components in the foreground, a series of morphological operations were conducted. Three iterations of erosion and two iterations of dilation were performed to remove the noise in threshold image. For further information, refer [OpenCV documentation for morphological operations](). Kernel was defined as a 5x5 matrix of ones (trial and error). The results of the denoised binary image are shown in Figure 8.

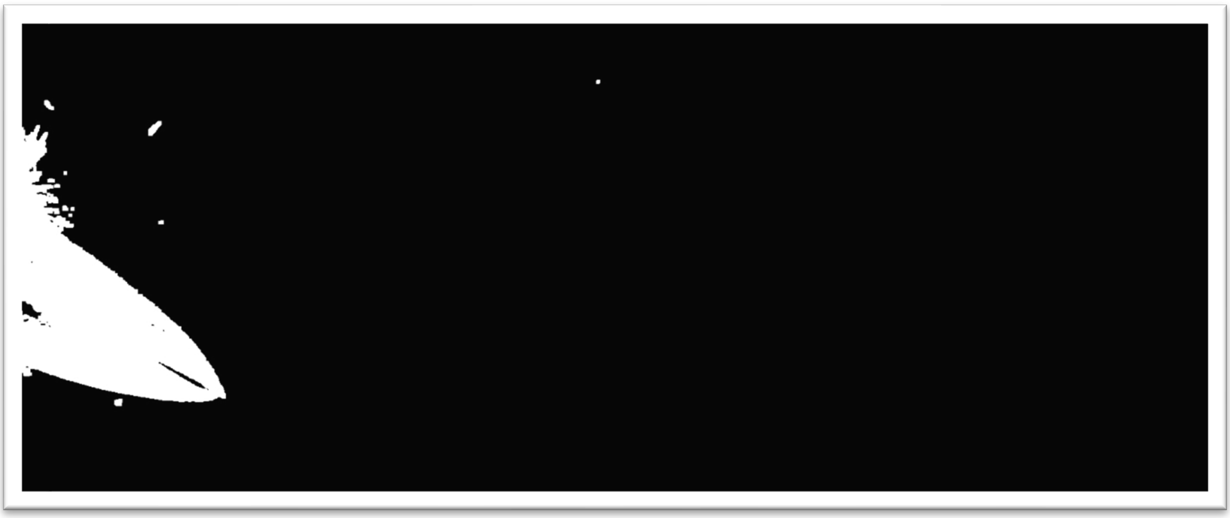

Figure 8: Resulting image after morphological operations

e) Get connected components and select object with max area

From the input binary image, we select the biggest connected region using [OpenCV functions](). From the extracted connected regions, the largest one is filtered out and the smaller components from background which got identified as the foreground were removed. Resulting image is shown in Figure 9.

Figure 9: Output after extracting the largest connected component

f) Define structuring element and fill holes in the selected region

Output of connected components represents the region where the beak is present in an image. As an additional step to improve the quality of this region, we perform a closing operation to fill any holes present in the region. A kernel is used for closing operations. For this application, a 20x20 matrix defined as an ellipse (Figure 10) worked best after trial and error.

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```

Figure 10: Illustration of the kernel we used

Using this kernel, a closing operation is performed on the connected component and the resulting image is trimmed to get the beak region cropped out in the binary image as shown in Figure 11.

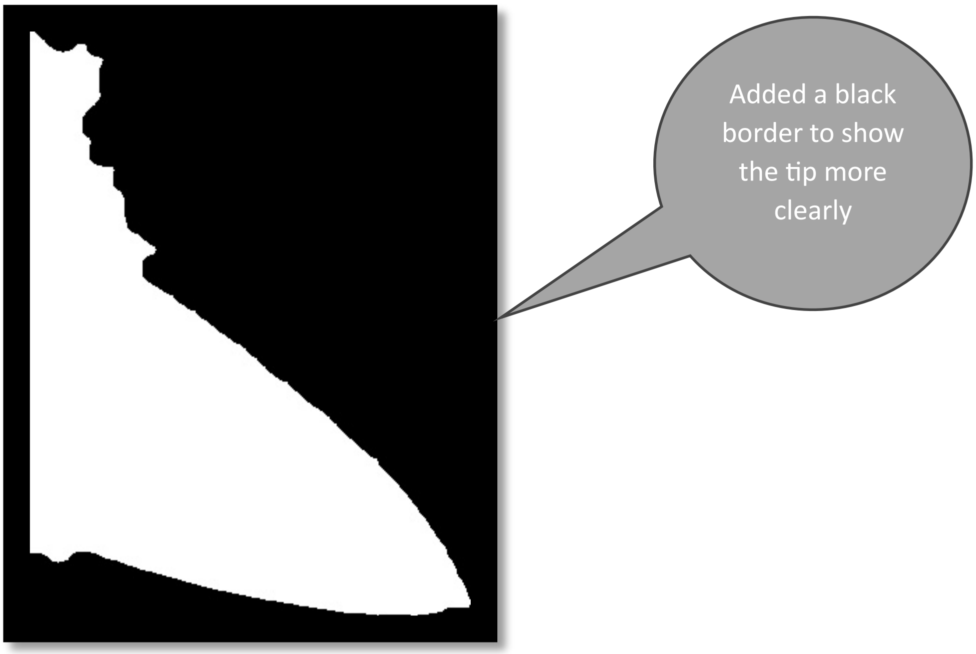

Figure 11: Result after closing holes and trimming

g) Rotate the image to extract beak dimensions

Once the image is trimmed in the previous step, we can see in Figure 11 that the tip of the beak can be easily estimated from the last column of the pixels in the image. With the tip of the beak and the user inputted nostril coordinates, the length of the beak can be estimated.

The width measurement is taken at a specific point along the beak's length. We have chosen to measure the width at a location that is two-thirds (2/3) of the beak length from the tip of the beak.

Using the input nostril coordinates and the tip of the beak, the image is rotated where the length of the beak is horizontal to the image, width of the beak becoming the vertical. This step makes the calculation of width and length of the beak easier. Result after rotating is shown in Figure 12.

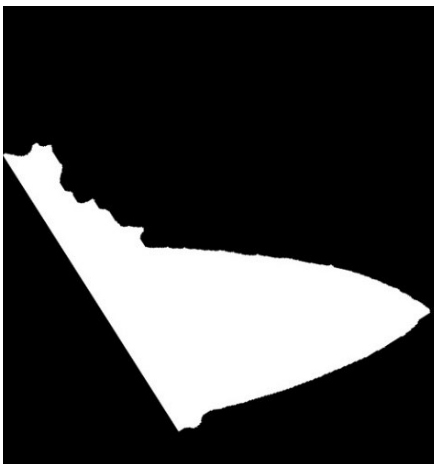

Figure 12: Binary image after applying the rotation

Rotating the image makes the calculation of width and the coordinates a lot easier. Using the two thirds point of beak horizontally, and drawing a vertical line on this point, we can estimate the length of beak as the number of pixels on the vertical line where value is equal to 1.

Finally, we have the length and width of the beak in pixels along with image coordinates where the length and widths are calculated from.

6 Display results to user and additionally save results to disk

Every cropping and rotation performed on the image beak were kept on track. Using this information, we created a transformation matrix to map the estimated beak coordinates back to the cropped image coordinates. Using the pixels to mm conversion ratio, we also calculate what the beak dimensions are in millimetres. Both this information is then shown to the user as shown in Figure 13.

Figure 13: Output of calculated beak length and width