

1 **Title Page**

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3 **Title: High throughput photogrammetric measurement of**
4 **morphological traits in free-ranging phototropic insects**

5

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27 **Abstract**

28 1) Remote measurement of morphological traits in free-ranging animals is very useful for
29 many studies, but such non-invasive photogrammetric methods are limited to large
30 mammals and have yet to be successfully applied to insects which dominate terrestrial
31 ecosystem diversity and dynamics. Currently, insect traits are measured using collected
32 specimens; the process of collection and maintenance of specimens can impose a heavy
33 and unnecessary cost when such specimens themselves are not needed for the study.

34 2) We propose a rapid, simple, accurate, and semi-automated method for high-throughput
35 morphometric measurements of phototropic insects from shape and size calibrated digital
36 images without having to collect a specimen. The method only requires inexpensive, off-
37 the-shelf, consumer equipment and freely available programming (*R*) and image
38 processing (*ImageMagick*) tools.

39 3) We demonstrate the efficacy of the method using a data set of 3675 images of free-
40 ranging hawkmoths (Lepidoptera: *Sphingidae*) attracted to a light screen. Comparison of
41 trait values from a subset of these images with direct measurements of specimens using a
42 scale showed a high degree of correspondence. We have also identified several error
43 metrics which help in assessing the method in an objective manner.

44 4) Although this method was developed for nocturnal phototropic insects, it can be used for
45 any other (small) animal that can be imaged on a simple graph paper. While this technique
46 will be generally useful for a variety of studies of insect traits, we suggest that it is
47 particularly suited as a commensal on multi-epoch and multi-location population monitoring
48 of insects in the context of climate and land-use change, where repeated sampling

49 obviates the necessity of collecting specimen every time. It will help in accumulating a
50 large amount of reliable trait data on hundreds of thousands of individual insects without
51 an overwhelming expenditure on collection, handling, and maintenance of specimens.

52

53

54 **Keywords: light traps, phototropic insects, morphometric measurements, body size,**
55 **wing area, image-calibration, distortion correction, photogrammetry**

56 **Introduction**

57 Morphological traits of insects, particularly body and wing sizes, have been key ingredients
58 in a variety of studies spanning physiology (e.g. thermoregulation; Parmesan & Yohe,
59 2003; Sheridan & Bickford, 2011), macroecology (e.g. Blackburn & Gaston, 1994; Gillooly
60 et al., 2001), ecogeography (e.g. Shelomi, 2012; Vinarski, 2014) and allometry (e.g. Voje
61 et al., 2014). The need for standardized measures of morphological traits of insects and for
62 large databases has been highlighted by many ecologists (e.g. Chown & Gaston, 2010;
63 Moretti et al., 2017). Morphological measurements are traditionally carried out (only) on
64 collected specimens using calipers or calibrated images from expensive microscopes.
65 Wing measurements require proper mounting/spreading of the specimen (e.g. Moretti et
66 al. 2017). Their measurement for a large number of individuals is the rate-limiting
67 procedure for many investigations.

68

69 Photogrammetry is the method of estimating (free-ranging) subject length attributes such
70 as size or distances from photographs (Baker, 1960). Typically, photogrammetry involves
71 elaborate procedures and expensive equipment (e.g. Mahendiran, Parthiban, Azeez &
72 Nagarajan, 2018) and so has been predominantly used for large taxa (mostly mammals,
73 some birds) with small population sizes and high conservation priority (e.g. Durban &
74 Parsons, 2006; Berger, 2012; Kurita, Suzumura, Kanchi & Hamada, 2012) .

75

76 We have not come across photogrammetry of free-ranging phototropic insects, which are
77 usually skittish and difficult to get to pose at the desired location. The usual practice is to
78 collect all the individuals visiting a light trap. Apart from the challenges of actual
79 measurement, we estimated that the cost of transforming a collected insect into a museum
80 specimen *with long-term utility* costs 1-3 USD even in an inexpensive country like India.
81 This can add substantially to project costs for taxa which arrive at a trap in their (tens of)

82 thousands. Furthermore, the target taxon may be only a small fraction of the total
83 individuals at the trap; the expense of processing unwanted but unavoidable specimens
84 may surpass that for the targets.

85

86 We describe here a rapid procedure of photogrammetry to measure morphological traits of
87 free-ranging phototropic insects without having to collect them. It can be used to measure
88 the lengths of any visible morphological feature including body, wing, antennae, head,
89 abdominal segments, elytra, etc, provided that it is being held parallel to the screen. The
90 use of a screen as a substrate compels most individuals of a particular taxon (e.g. moths)
91 into a similar posture, resulting in the uniformity necessary for automated processing. The
92 procedure requires inexpensive, off-the-shelf, consumer equipment, and very little training
93 and care while imaging the animals. We will also describe heuristics for quantifying errors
94 of measurement and image quality (and hence usability).

95

96 We also suggest a different perspective of the generality of this method, ironically, arising
97 from its particular suitability for moths. Moths are among the most abundant and diverse
98 taxa and one of the most important herbivores and prey species in many ecosystems
99 (New, 1997). They are easy to attract to light screens in large numbers. As insects go, they
100 are relatively easy to identify down to genus from images, and to a lesser extent even to
101 species. A procedure to collect body and wing sizes of large numbers of moths, as a
102 commensal of population monitoring studies while not requiring a large amount of
103 resources for preserving the specimens in a museum, would contribute to a global
104 database and a variety of studies.

105

106

107 **Method & Materials**

108

109 **A. Field Data**

110 This procedure was developed for a study of traits of hawkmoths (*Lepidoptera*: Family
111 *Sphingidae*) in Eaglenest wildlife sanctuary in Arunachal Pradesh, North-east India
112 (Athreya, 2006; Mungee, 2018).

113

114 We attracted moths to a light screen made of a rectangle frame of fabric (1.6 x 1.1 m²)
115 hanging from a portable and light-weight tetrapod (Figure 1a). An ultraviolet actinic lamp
116 (8W T4-BL40) to attract moths and an optical lamp (8W CFC tube) to assist human vision
117 were placed along the upper bar of the tetrapod for maximal effect. The lamps were
118 powered by a 12V lead-acid battery through a DC-to-AC converter. The screen material
119 (Figure 1c) was ordinary shirt fabric with thin checks (rectangular 7.0 x 7.3 mm², for size
120 and shape reference) on a white background (for maximum reflectance), stretched taut
121 across the frame using elastic bands. The entire set up costs less than 250 USD in India at
122 current prices.

123

124 The number of moths and other insects varied from 5 to 3000 individuals each night
125 (Figure 1b), depending on location, lunar phase, weather conditions, etc. We set up 2-8
126 screens at locations separated by 200-1000 m in elevation and 2-30 km along a forest
127 road. The insects were photographed on the screen with cameras ranging from digital
128 SLRs (Nikon D90 + 105 mm macro) to point-and-shoot (Panasonic Lumix DMC-TZ30).

129

130 These screens were manned by a large team of field staff with different levels of technical
131 proficiency and educational backgrounds, resulting in images of variable quality. Most
132 images suffer from a variety of distortions to a greater or lesser degree (Figure 2). We

133 used the rectangular grid on the checked screen as the reference to post-facto calibrate
134 the images for shape and size (Figure 3).

135

136 **B. Image Processing**

137 The post-imaging calibration procedure consists of 3 tasks including de-distortion, error
138 estimation and trait measurement, all of which require the identification of image
139 landmarks. We developed 3 functions in R (version 3.4 or higher; R Core Team, 2013)
140 using the libraries *imager* (v. 0.41.2 or higher, Bache & Wickham, 2007), *magick* (v. 2.0 or
141 higher, Ooms, 2018), *jpeg* (v. 0.1.8 or higher, Urbanek, 2014), *reshape2* (v. 1.4.3 or
142 higher, Wickham, 2007) and *plyr* (v. 1.8.4 or higher, Wickham, 2011); and the image
143 processing software suite *ImageMagick* (v. 6.9 or higher; ImageMagick Studio, L. L. C.,
144 2008). The 3 functions are used to (i) output the pixel coordinates of salient image
145 landmarks, (ii) create and execute command files for dedistorting images, and (iii) estimate
146 error heuristics to determine the quality of the final image. Manual intervention was largely
147 limited to locating the landmarks, which took less than a minute per image.

148

149 The tasks were successfully executed under Linux Ubuntu (18.04), Windows and Mac
150 operating systems.

151

152

```
R>>> DD_landmarks (parameters)
# output pixel coordinates of dedistortion landmarks on the RAW image into an output CSV file

R>>> DD_dedistort (parameters)
# use the previous CSV to dedistort the images to create a montage of the RAW and the
# dedistorted/calibrated (CAL) images

R>>> DD_landmarks (parameters)
# output pixel coordinates of error landmarks on the CAL-RAW montage images

R>>> DD_dderror (parameters)
# use the previous CSV file to calculate the error heuristics

R>>> DD_landmarks (parameters)
# output pixel coordinates of trait landmarks on the CAL images to measure the traits
```

153 **i) Landmarks on the Image**

154 The function ***DD_landmarks*** outputs a user-specified number of landmarks in a CSV¹ file.
155 On execution it launches the selected graphic pane (X11, Quartz, or Windows) and
156 displays all the images in the folder with the specified file tag by turn (Figure 4a).

157

158 The advantage of our landmark procedure over previous implementations is that pixel
159 location is a 2-step process. In the first step the required number of landmarks are
160 approximately located on the full image. Subsequently, the function displays a sequential
161 montage of postage stamps of the zoomed-in, higher contrast 100x100 pixel views of the
162 neighbourhood of the landmarks (Figure 4). The user can now mark the required pixel with
163 greater accuracy.

164

165 **ii) De-distortion**

166 Essentially, this task requires the user to mark two rectangles, ABCD and EFGH, using
167 ***DD_landmarks*** as per the pattern in Figure 5. ABCD should be a *grid-corner rectangle* –
168 i.e. its vertices lie on grid intersections – and it provides both the scale and the shape
169 reference since the basic grid is a rectangle of known size (X: 7.0 mm, and Y: 7.3 mm in
170 our case). The size of ABCD can be fixed in two ways:

171 1. Fix the same at the start (parameters ***markcode*** = “***DD8***”, ***ngridX***, ***ngridY***):

172 Mark ABCD such that AB = CD = ***ngridX***, and BC = DA = ***ngridY*** in units of the grid.

173 2. Mark the size on the fly (parameters ***markcode*** = “***DD12***”, ***ngridX***, ***ngridY***):

174 Mark ABCD of any size, and additionally mark line segments IJ = ***ngridX*** parallel to
175 AB, and KL = ***ngridY*** parallel to BC. The ratios of AB/IJ and BC/KL in pixels yield the
176 size of ABCD.

177 The rectangle EFGH is used to identify the region of interest on the image in both cases.

1 CSV : a text file in the Comma-separated-value format, which can be read by any spreadsheet software like MS-EXCEL or LibreOffice

178

179 Using the recorded location of ABCD on the raw image, the function ***DD_dedistortion***
180 (parameters: ***mmpexXgrid = 7.0 mm, mmpexYgrid = 7.3 mm, pixpermm = 10***) calculates
181 transformed location of ABCD on the calibrated image (requiring that all the angles be 90°,
182 and fixing the scale of the output image using

183
$$AB = CD = (ngridX) \times (mmpexXgrid) \times (pixpermm) \text{ and}$$

184
$$BC = DA = (ngridY) \times (mmpexYgrid) \times (pixpermm)$$

185 The coordinates of ABCD on the raw and the calibrated images are used as parameters of
186 the *ImageMagick* command to dedistort the image. Currently, the ***magick*** library in R does
187 not provide the dedistortion utility. So we create a system command/batch file which can
188 be executed either manually (***fg_imagemagick = FALSE***) or by the R function itself
189 (***fg_imagemagick = TRUE***)

190

191 **iii) De-distortion error estimation**

192 The function ***DD_dderror*** calculates the errors in the calibration process when provided
193 landmarks from ***DD_landmarks*** using one of the two options below:

194 1. parameters ***markcode = "ER8", ngridX, ngridY***

195 ○ On the calibrated image: mark the grid-corner rectangle ABCD with $AB = CD =$
196 ***ngridX*** and $BC = DA =$ ***ngridY*** as shown in Figure 5. EFGH can be any grid
197 corner rectangle which approximately encompasses the subject. The location of
198 A and E should coincide.

199 2. parameters ***markcode = "ER12", ngridX, ngridY***

200 ○ On the calibrated image: mark ABCD and EFGH as for ***ER8***

201 ○ On the raw image: mark grid-corner rectangle IJKL at the locations
202 (approximately) corresponding to EFGH.

203

204 The task calculates the following errors (see Figure 2)

205 1. Pixel location error: Length AE is a measure of human error in marking the desired
206 pixel, since A and E are meant to be marked on the same pixel.

207

208 2. Grid misalignment error: Segments EF and GH are meant to be horizontal, and FG
209 and HE vertical, and their deviations from the same are a measure of the errors in
210 the dedistortion process. A deviation θ results in a fractional length error $(\delta L/L) = (1$
211 $- \cos\theta)$, i.e. a 5.03 inclination of a grid line will cause a linear error of ~1%. Rotation
212 in itself causes no change in lengths, though it can introduce errors if the scale
213 distortions along the X and Y axes are not identical.

214

215 3. Scale error: This is the deviation from the expected scale **pixpermm** (= 0.1 mm per
216 pixel for us) set in the previous task. It is measured by counting the number of
217 pixels between a known number of grid intersections on the calibrated image. If the
218 measured length of AB and EF in pixels are AB_P and EF_P , the number of grids in EF
219 $\equiv EF_G = \text{round}(EF_P/AB_P)$. The expected number of pixels in $EF_{P-EXP} = EF_G \times$
220 **mmpexgrid** \times **pixpermm**. The percentage scale error $\delta S_{EF} = 100 \times (EF_P - EF_{P-}$
221 $EXP)/EF_{P-EXP}$. A similar scale error can be calculated for the other 3 sides.

222

223 4. Perspective distortion:

224 Perspective changes a rectangle into a trapezium, and results in a gradient in scale
225 across the image. It occurs when the plane of the camera is not parallel to the plane
226 (of the subject) in which the measurements are to be made. The quantity below,
227 which is zero for a rotated rectangle or a parallelogram (shear distortion) and non-
228 zero for a trapezium, provides a heuristic estimate of the perspective error:

229

$$\delta_{PersX} = \frac{EF - GH}{\frac{FG + HE}{2}} \quad \& \quad \delta_{PersX} = \frac{FG - HE}{\frac{EF + GH}{2}}$$

230

231

One can also estimate the efficacy of the procedure by comparing the perspective

232

error before and after de-distortion.

233

234

We combined the values for the above 4 errors in the X- and Y-direction to derive a

235

standard deviation for the distribution of the entire sample of moths as an estimate of the

236

accuracy of the procedure.

237

238

5. Any gap between the plane of the insect wings and the gridded cloth reference,

239

even when they are parallel, would over-estimate lengths, since the wings, which

240

are nearer, are projected against the farther screen. The fractional error due to this

241

gap should be equal to the ratio of the wing-screen and camera-wing distances.

242

243

Other distortions (e.g. shear, pin-cushion, etc) can be quantified individually but are not

244

necessary for our purpose. Essentially, the scale error is much larger than the others and

245

we suspect all the others are reflected in it. Images with error values much larger than

246

expected from the distribution for the whole sample were re-processed to eliminate human

247

error. Those which continued to have high errors were removed from subsequent

248

analyses.

249

250 **iv) Measuring traits on the de-distorted image**

251

We measured the following traits on the de-distorted images using the landmark pattern A-

252

B ... C-D-E ... F-G-H shown in Figure 5:

253

- Body-length = AB (length of the segment AB)

- 254 • Thorax-width = CF
- 255 • Wing triangles \equiv C-D-E and F-G-H. Using wing terminology, CD and FG are the
- 256 (right and left) costa, DE and GH are the termen, and EC and HF are the dorsa.
- 257 • Body-volume, modelled as a bi-cone (spindle-shaped)

258
$$BodyVolume = \frac{1}{3} * \Pi \left(\frac{ThoraxWidth}{2} \right)^2 * BodyLength$$

259

- 260 • Wing area, calculated from the lengths of the sides

261
$$WingArea = \sqrt{(s * (s - Costum) * (s - Termen) * (s - Dorsum))}$$

262 where,

263
$$s = \frac{Costum + Termen + Dorsum}{2}$$

- 264 • (Average) Wing-breadth
$$WingBreadth = \frac{2 * WingArea}{Costum}$$
- 265
- 266

267 **v) Curation of trait values**

268 We identified outliers in the distribution of traits (body-length, body-volume, wing-length =

269 costum, and wing-area), either separately for each taxon or for the entire community, as

270 appropriate, in terms of the deviation from the mean in units of the standard deviation.

271 Species with substantial sexual dimorphism should result in a bimodal distribution but we

272 did not encounter any such in our data.

273

274 Wings held at an angle to the gridded screen will acquire distortion when the background

275 grid is de-distorted (Figure 6). This could result in a large difference between the trait

276 values of the left and the right wings. We used the normalised difference between the left

277 and the right wing dimensions to identify poorly positioned moths. Normalising this

278 difference allowed us to combine the data from all species into a single distribution for

279 better estimation of the standard deviation.

$$normalized_{diff} = \frac{(Trait_L - Trait_R)}{\frac{(Trait_L + Trait_R)}{2}}$$

280
281
282
283
284

285 Since we were interested in the relationship between the body-volume and wing-area we
286 also used the residuals from their linear regression to identify and flag outliers. Any pair of
287 related traits of interest may be used in a similar manner. We used the following
288 transformed values for the regression

289
290

$$Trait_{normalized} = \frac{\log 10(Trait_{individual}) - \log 10(Trait_{SpeciesMean})}{stdev[\log 10(Trait_{Species})]}$$

291
292
293

294 **vi) Comparison with specimens measured using calipers**

295 We assessed the accuracy of the method by comparing body lengths from
296 photogrammetric and direct (using a scale of least count 1 mm) measurements for 105
297 specimens that had been both imaged and collected.

298
299

300 **Results**

301 The linear regression between the body lengths from specimens and images, for the 105
302 individuals with both measurements, is shown in Figure 7 (y-intercept = 1.71 ± 1.10 , slope
303 = 0.96 ± 0.02 , $R^2 = 0.94$). The value is consistent with zero y-intercept at 1.6σ . A ratio test
304 of the two, equivalent to forcing a linear regression with y-intercept = 0, yielded a 95%
305 confidence interval for the mean of [0.996, 1.010] against the expected value 1.0 (Table 1),

306 which argues for no bias. Similarly, a difference test yielded 95% C.I. for the mean of [-
307 0.22, +0.41] mm against the expected value 0.0.

308

309 We obtained images and collected 2 middle legs for DNA analysis from 4808 hawkmoth
310 individuals, of which 3675 images had the gridded screen as background. Some of the
311 others had rested on the surrounding vegetation or the tetrapod, while the rest could not
312 be photographed properly due to heavy rains. These images were used to identify the
313 individuals to (morpho)-species and de-distorted in batch mode to measure the traits.
314 Approximately 500 images could be dedistorted during an 8-hour session.

315

316 Since our images were taken from a distance of about 30 cm, and the resting wings are
317 mostly held flush against the screen, or at most a few millimeter away, the error due to the
318 gap between the wing and the screen should be about 1 % (ratio of the two distances).

319

320 The statistics of the various metrics are listed in Table 1.

321

322 The distribution of perspective errors after de-distortion is shown in Figure 8². The second
323 panel in Figure 8 shows the distribution of the difference in the absolute values of the
324 perspective error before and after the procedure. As expected, the values are mostly
325 positive, i.e. the errors reduced, and the numbers fall sharply below zero.

326

327 The distributions of the other three error metrics are shown in Figure 9. The pixel location
328 error SD = 1.76 pixels (= 0.18 mm) corresponds to a fractional length error of 0.35% for a
329 typical hawkmoth body length of 50 mm. The mean axis misalignment SD = 0.°43,
330 correspond to a negligible fractional length error of 0.02%. The scale factor showed SD =

2 In the plots in Figures 8-11, the solid red line represents the mean and the dotted lines on either size are at thrice the standard deviation.

331 2.3%.

332

333 The distribution for the normalized difference between left and right wing-area and wing-
334 costum length are shown in Figure 10. The means of both the distributions are close to
335 zero and SD = 1.6% for wing length, and 2.5% for area. We found that in some cases even
336 though the left-right asymmetry was stark, averaging the two (as is appropriate for an
337 individual) resulted in a value within the species distribution; presumably this effect
338 impacts the two wings in opposite directions without appreciably changing their mean.

339

340 We generated species-wise trait histograms for body volume and wing area, and
341 regression plots for wing area on body volume. Figures 11 and 12 show the distributions
342 for a single species, *Cechetra lineosa*, as an illustration.

343

344 One can define thresholds for selecting “usable” images in several ways. It can be on the
345 basis of deviation from the mean in units of the standard deviation (e.g. reject images
346 whose error estimate was more than 3 x SD from the mean). On the other hand when the
347 errors are very low (e.g. only 0.01% for misalignment error), it is wasteful to eliminate
348 images using this criterion. Alternatively, the accuracy required by the investigation can set
349 the threshold (e.g. 10% fractional length error).

350

351 In the sample of 3675 images presented here only 14 images (0.4%) had scale errors in
352 excess of 10% and 3470 images (94.4%) of the images had an error less than 6.1 % (3 σ
353 after robust clipping).

354

355 **Discussion**

356 We have demonstrated a simple, inexpensive method for obtaining robust morphometric
357 measurements from digital images. Images of free-ranging moths on a light screen
358 provided basic morphometrics without having to allocate considerable resources for the
359 collection, processing and storage of specimens. This method can be applied to other
360 phototropic insect taxa as well (e.g. *Coleoptera*, *Aphidina*, *Diptera*, *Trichoptera*,
361 *Heteroptera* and *Hymenoptera*; see van Grunsven et al., 2014, for attracting phototropic
362 insects) and any visible morphological trait such as body length, thorax width, head width,
363 wing sizes, antennae length etc. The equipment and material necessary for this are all
364 consumer-level, off-the-shelf items which makes the procedure accessible to researchers
365 all across the world, and yet provides measurements accurate to a few per cent for tens of
366 thousands of individuals.

367

368 Photogrammetry is currently limited to large mammals and birds. Previous methods of
369 distortion correction from digital images have relied on additional information such as
370 aerial photographs from multiple angles with multiple cameras (Gerum et al., 2017) or on a
371 checkerboard in the field of view for edge detection and distortion correction (CCTM of
372 MATLAB; Heikkila & Siliven, 1997). In our case, the gridded screen, needed as a resting
373 surface for phototropic insects, provides a dedistortion reference at no extra cost.

374

375 The landmark task in R developed for this work (***DD_landmarks***) differs from previous
376 utilities (e.g. in the R-package Geomorph, Adams & Otàrola-Castillo 2013) by employing a
377 two-step marking procedure for better accuracy in locating pixels.

378

379 The human effort required was only about 1 minute per individual in the field, and in the
380 lab; a person can process about 500 images a day in the lab. Both these tasks can be
381 easily carried out by personnel with little or no academic or technical background. As

382 importantly, the procedure yielded quantifiable errors on images which are available for
383 repeated measurements.

384
385 The comparison of lengths from specimens and images showed that any systematic
386 difference between the two is less than 0.04 mm (half pixel) at 95% confidence level. The
387 overall precision, reflected in the standard deviation of the scale factor, was 2.3%, perhaps
388 arising from the stretchable nature of the fabric and variable humidity conditions. This was
389 adequate for our purpose, especially since intraspecific variation is much larger (e.g.
390 Figure 11), but a less stretchable screen can reduce the error even further.

391
392 The rapid rate of environmental change, from both land-use pattern and climate changes,
393 are expected to trigger substantial responses in many ecosystems. One can directly track
394 these responses by monitoring changes in species communities and in their key
395 morphological traits since individuals interact with and respond to the environment via their
396 traits. Such multi-epoch monitoring would require the sampling of hundreds of thousands
397 of individuals every year, and can benefit from not having to collect tens of thousands of
398 specimens every time. Our method provides a way of accumulating large amounts of
399 reliable trait data at minimal cost, especially in such situations. The target hawkmoths
400 comprised less than 1% of all the moths individuals arriving at the light screen in our study.
401 Having to allocate limited resources to collecting and processing all the moth specimens
402 visiting the light trap would have forced us to scale down the intended study.

403
404 Even in the case of collected specimens, this technique would make expensive
405 instruments like a calibrated microscope unnecessary except for high precision
406 measurements of very small subjects.

407

408 This method is particularly good for nocturnal phototropic insects, and the fact that such
409 taxa form the bulk of biodiversity makes for its wide applicability across the globe. We
410 acknowledge that many morphometric studies may require the collection of full specimens,
411 even in large numbers. The photogrammetric technique presented here obviates the
412 necessity of a large expenditure on specimen collection, processing and maintenance,
413 where it is not essential, and yet yields reliable morphometric data which can be re-
414 examined by others at a later date.

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423

424 **STATEMENT OF AUTHORSHIP:** RA designed the project, MM carried out the
425 computations and image processing; all the rest including collection of field data, analysis,
426 and writing of this manuscript were shared by both. We declare that we have no competing
427 interests.

428

429 **DATA ACCESSIBILITY STATEMENT:** All relevant information in support of the results
430 presented here will be archived in the recommended public repository DRYAD, upon
431 acceptance of the manuscript.

432 1. We have uploaded the data file (as a Libre-office spreadsheet) for the referee.

433 2. We should also be happy to deposit the source code of the three R functions used

434 in the analyses in any public repository recommended by the journal

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Error type	Final Set			Initial Set		
	N	Mean	Stdev		Mean	Stdev
Pixel location (pixel)	2 x 3470	0.33	1.75	2 x 3675	0.33	1.76
Grid rotation (degree)	4 x 3470	0.03	0.42	4 x 3675	0.03	0.43
Perspective (per cent)	4 x 3470	0.02	0.90	4 x 3675	0.02	0.92
Scale (per cent)	2 x 3470	-0.20	2.03	2 x 3675	-0.21	2.35
Specimen – Image length ratio	105	1.003	0.04	105	1.003	0.04
Specimen – Image length difference (mm)	105	0.090	1.63	105	0.090	1.63
Normalised difference (Wing area)	3261	-0.04	2.47	3424	0.04	3.16
Normalised difference (Wing length)	3261	-0.05	1.58	3424	-0.05	1.79

Table 1. Statistics of the error metrics. Each image may yield 1, 2, or 4 independent values of the metric. Robust statistics have been calculated using 3σ clipping.

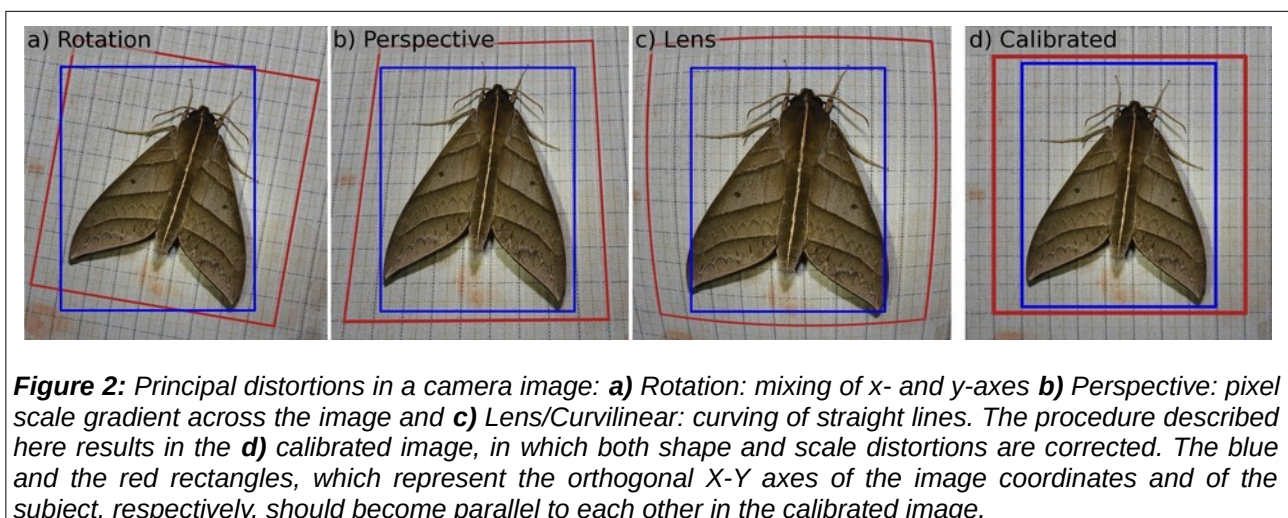
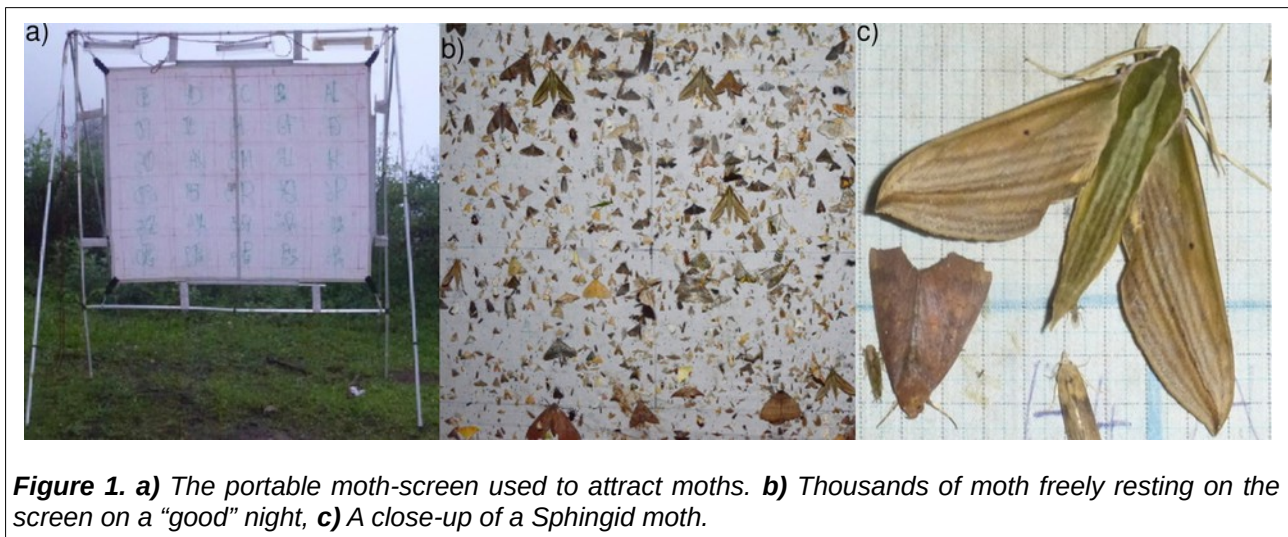




Figure 3. *Top row: Examples of distorted raw images. Bottom row: Final de-distorted/calibrated images.*

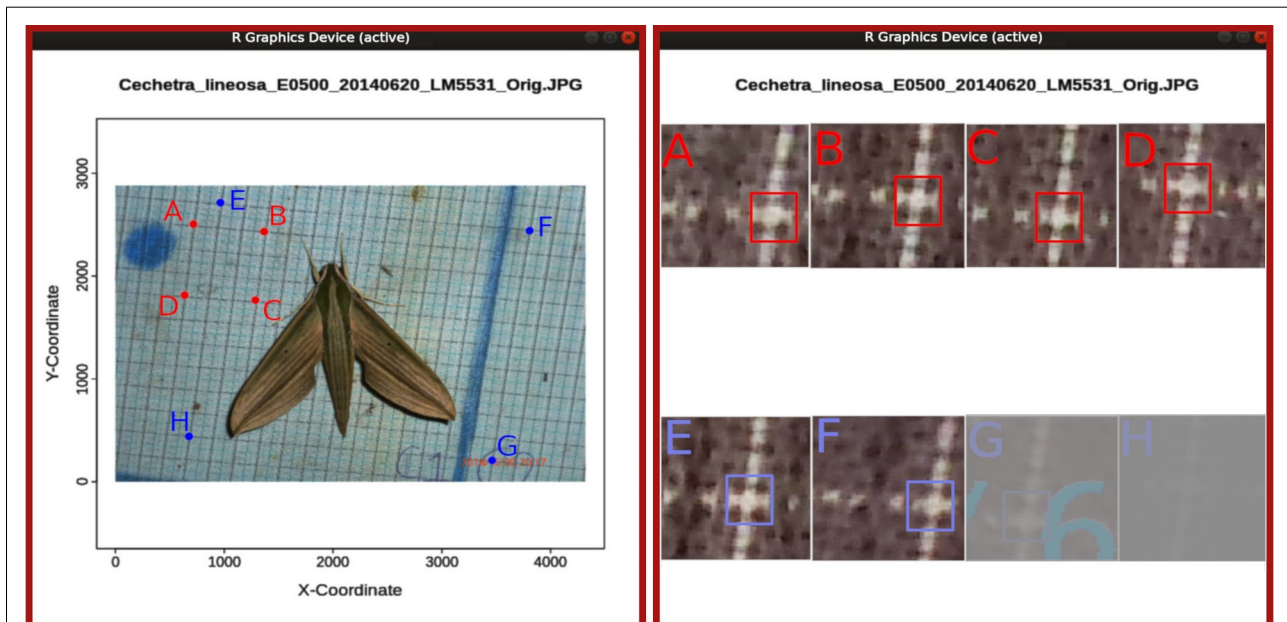
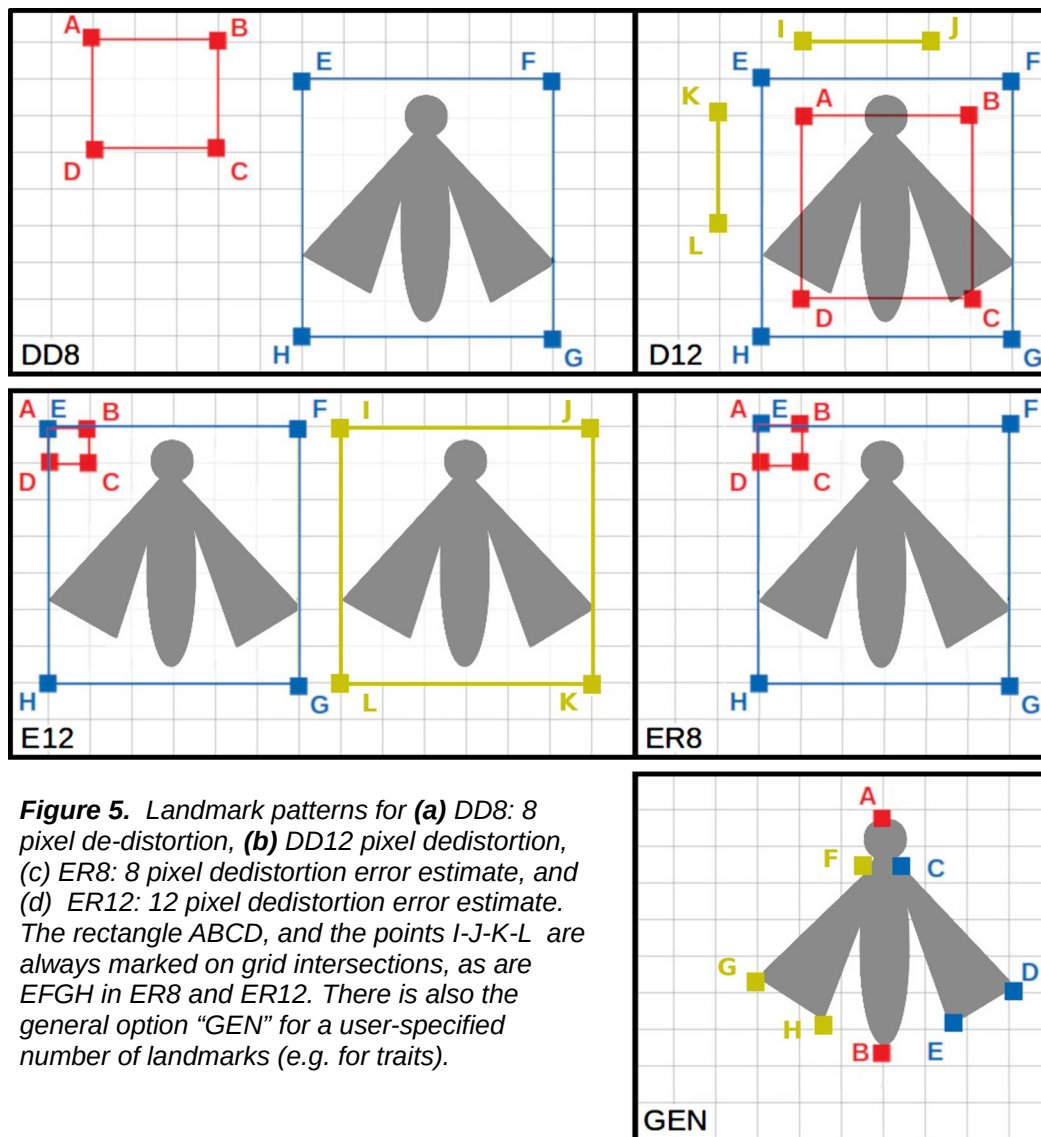


Figure 4: *The Graphical User Interface (GUI) of the two-step Landmark function. Left: First, the image is shown in full for approximately marking the pixels, e.g. A-H, using mouse clicks. Right: Subsequently, high contrast, magnified 100 x 100 pixel postage stamps around the marked locations are displayed in sequence for more accurate selection of the pixels. The panels suggest that the postage stamps A-F have been displayed and marked, and G and H are to follow.*



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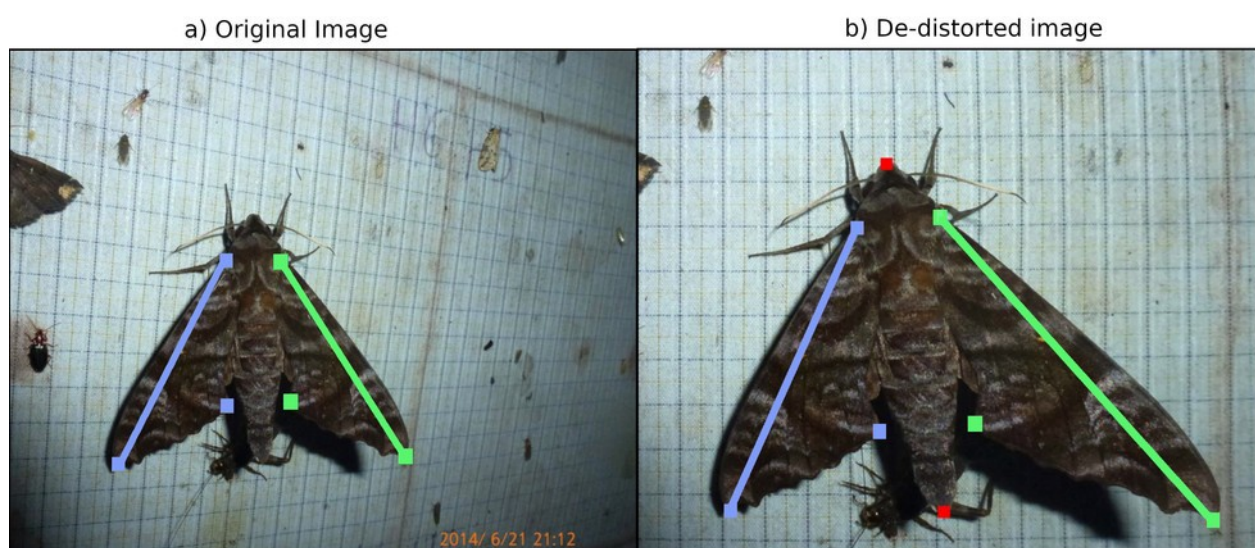


Figure 6. A left-right asymmetry is introduced by the de-distortion procedure when the wing is not parallel to the screen – the shadow under the right wing suggests a large gap. The original image shows a well-positioned moth with symmetrical wings on a screen which is angling away. De-distortion transformed the distorted grids into rectangles and imposed the same, but inappropriate, correction on the subject.

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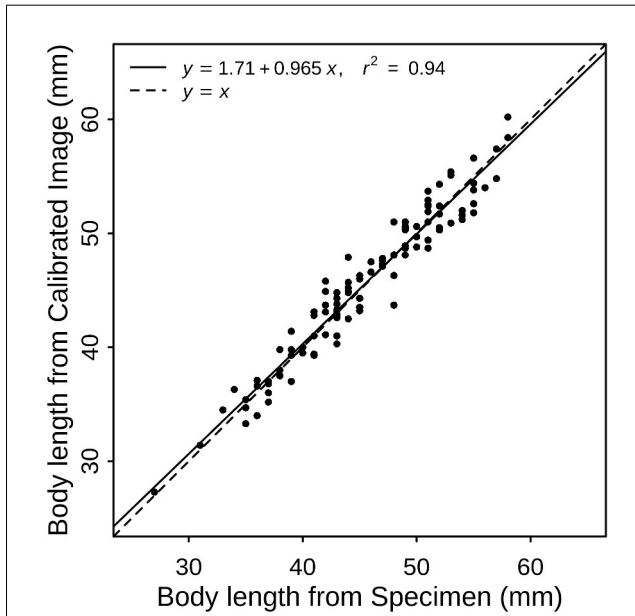


Figure 7. Comparison of body lengths from photogrammetry and direct specimen measurement using a scale.

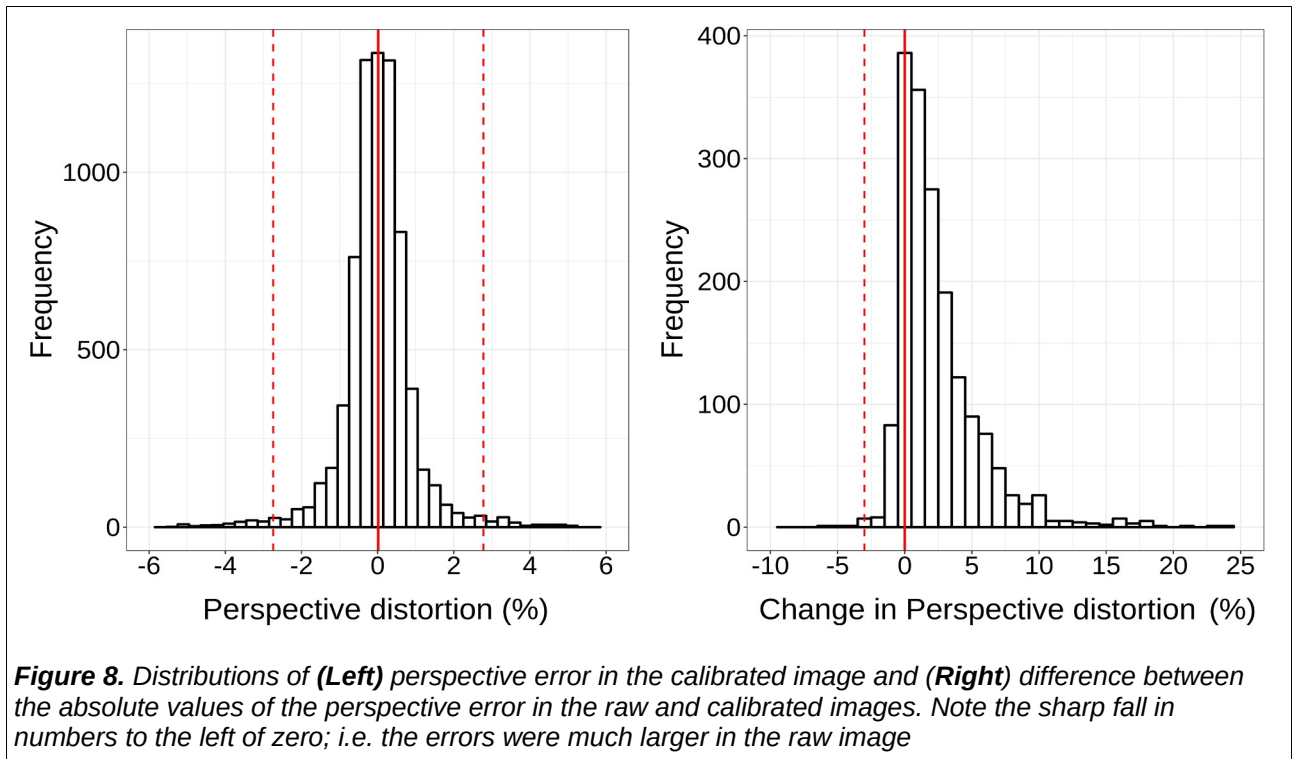


Figure 8. Distributions of (Left) perspective error in the calibrated image and (Right) difference between the absolute values of the perspective error in the raw and calibrated images. Note the sharp fall in numbers to the left of zero; i.e. the errors were much larger in the raw image

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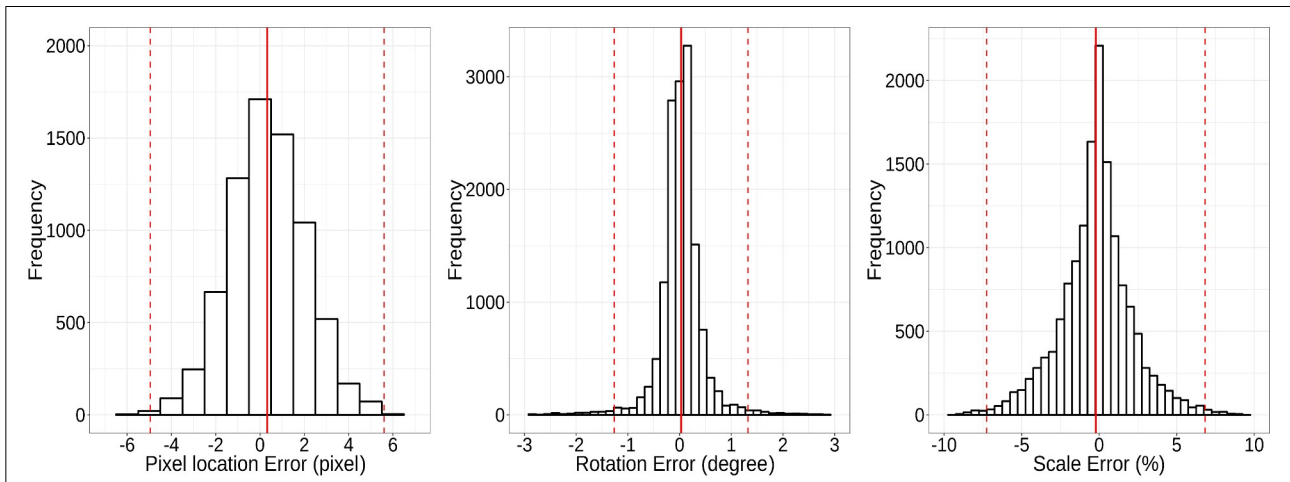


Figure 9. Distributions of dedistortion error-metrics: **(Left)** Error in marking the target pixel, **(Mid)** Misalignment of the grid lines from the vertical and the horizontal, and **(Right)** Scale error, i.e. the deviation from the expected number of pixels along a grid segment

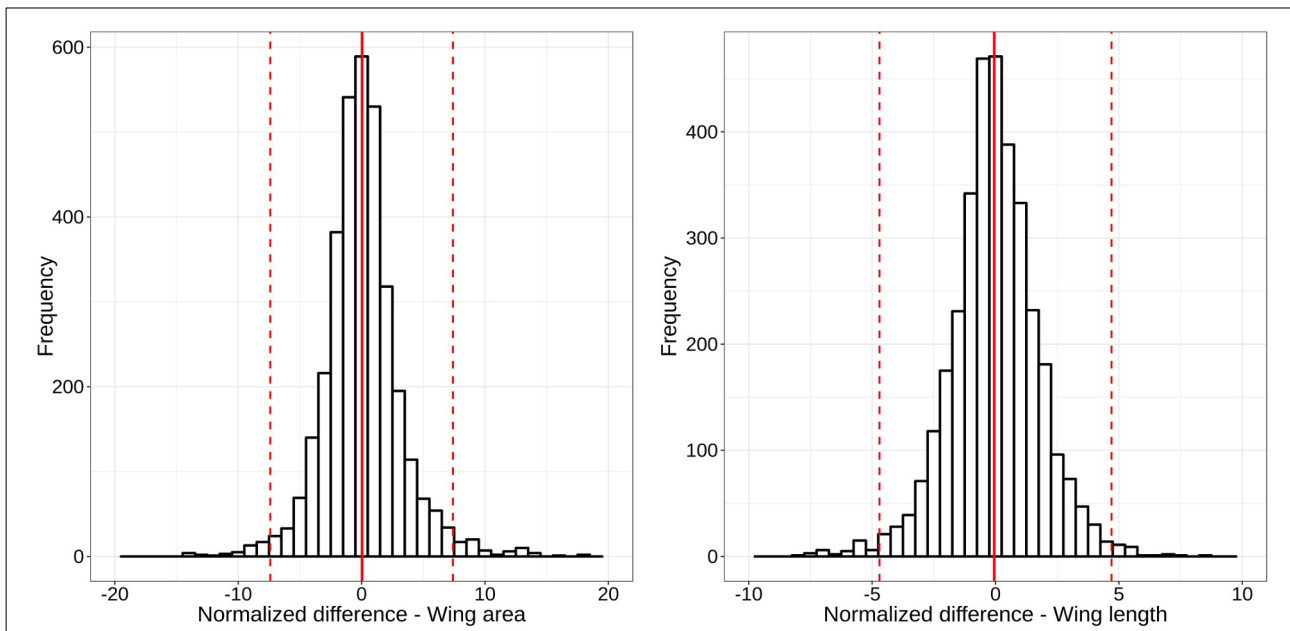


Figure 10. Distributions of the normalized difference between the left and right wings: **(Left)** area, and **(Right)** costum length. The difference is a measure of non-coplanarity between the wing and the screen.

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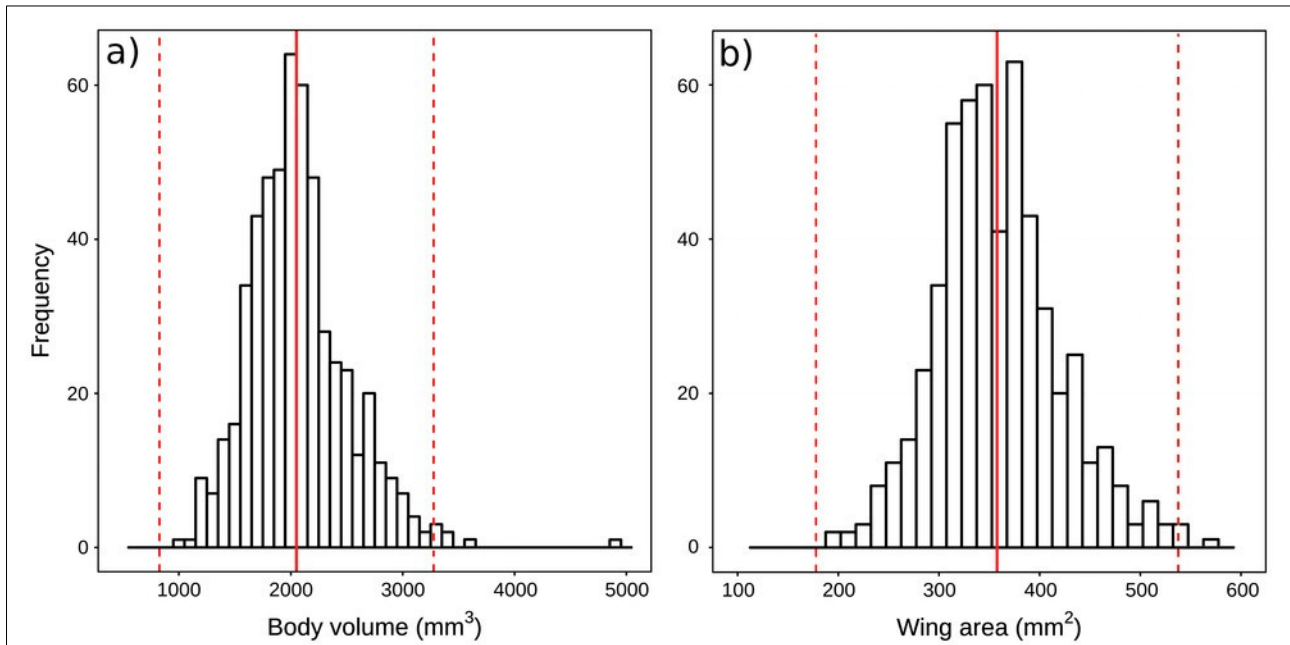


Figure 11. Distributions of body volume (**Left**) and wing area (**Right**) for individuals of the species *Cechetra lineosa*. The red lines represent the means and the 3σ values.

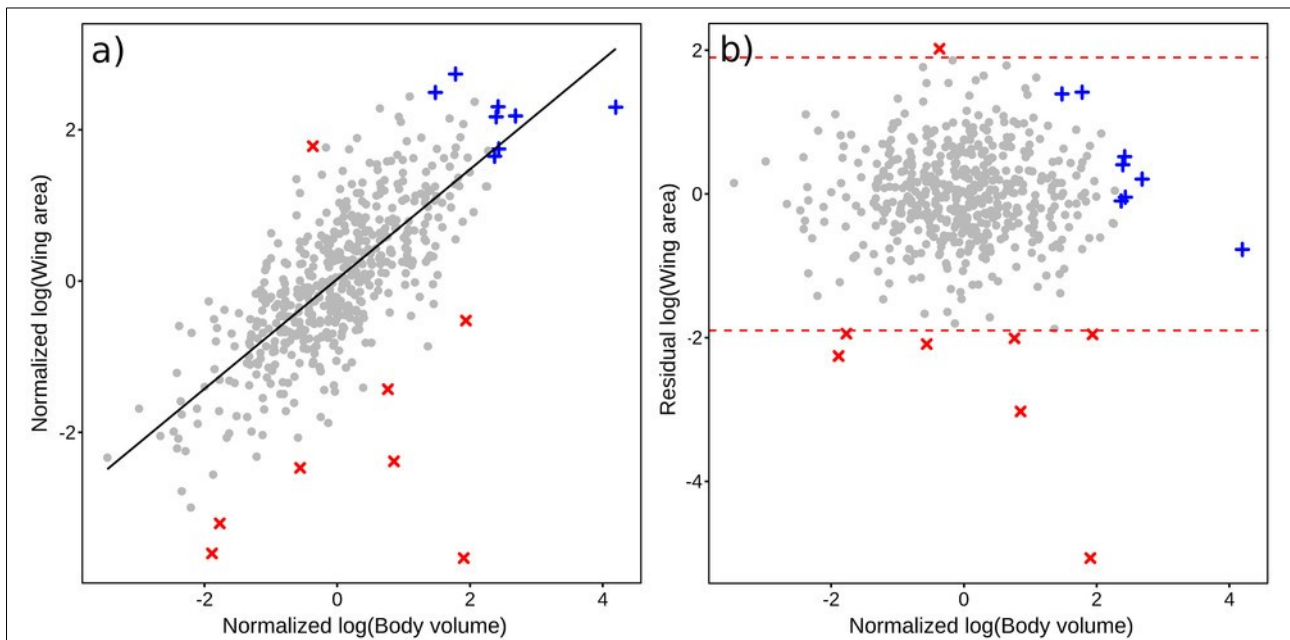


Figure 12. Linear regression of wing-area on body-volume (**Left**) and its residual (**Right**) for *Cechetra lineosa*. Regression outliers are in red and insects with only one wing measurements are in blue. Wing-area and body-volume are expressed as deviation from the species mean in units of the standard deviation.