



Methods

Studying flowers in 3D using photogrammetry

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Summary

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- Flowers are intricate and integrated three-dimensional (3D) structures predominantly studied in 2D due to the difficulty in quantitatively characterising their morphology in 3D. Given the recent development of analytical methods for high-dimensional data, the reconstruction of flower models in three dimensions represents the limiting factor to studying flowers in 3D.
- We developed a floral photogrammetry protocol to reconstruct 3D models of flowers based on images taken with a digital single-lens reflex camera, a turntable and a portable lightbox.
- We demonstrate that photogrammetry allows a rapid and accurate reconstruction of 3D models of flowers from 2D images. It can reconstruct all visible parts of flowers and has the advantage of keeping colour information. We illustrated its use by studying the shape and colour of 18 Gesneriaceae species.
- Photogrammetry is an affordable alternative to micro-computed tomography (micro-CT) that requires minimal investment and equipment, allowing it to be used directly in the field. It has the potential to stimulate research on the evolution and ecology of flowers by providing a simple way to access 3D morphological data from a variety of flower types.

Introduction

Flower shape, size and colour influence the attraction of pollinators, the way pollinators access floral rewards and contingently the exchange of pollen between anthers and stigmas (Faegri & Van Der Pijl, 1979; Fenster *et al.*, 2004; Willmer, 2011). Flower shape is also important in wind-pollinated species (anemophily) as it influences interactions with air flows and so determines efficient pollen release, dispersal and capture (Timerman & Barrett, 2019). Because flowers are three-dimensional (3D) structures that interact with a 3D biotic and abiotic environment for conspecific exchange of pollen, characterising flower shape and colour in 3D is important to promote a comprehensive understanding of flower development and the role of flower shape in the ecology and evolution of species.

Only recently has it become feasible to study the variation of flower shape in 3D due to the development of methods to build 3D flower models. The first reconstruction of flowers in 3D used micro-computed tomography (micro-CT, or HRCT for high-resolution CT) to acquire and visually render digital 3D shape data of both surfaces and internal structures (Stuppy *et al.*, 2003). Micro-computed tomography (micro-CT) helps to visualise minute plant structures and to study their external 3D morphology and internal structures qualitatively and quantitatively. The characterisation and comparison of these 3D flower models using geometric morphometrics (Rohlf & Marcus, 1993)

has opened a vast array of possibilities for the study of flowers in 3D, which was deemed to represent a ‘revolution’ for the study of flowers (van der Niet *et al.*, 2010). Although other 3D modelling techniques are available such as laser-scanning and structured light that record surfaces, micro-CT scanning remains the most common 3D digitization technique applied to plant specimens (Mathys *et al.*, 2013; Davies *et al.*, 2017).

Despite the fact that several studies recently used 3D flower models (Gamisch *et al.*, 2013; Wang *et al.*, 2015; Dellinger *et al.*, 2019; Hsu *et al.*, 2020; Reich *et al.*, 2020; Artuso *et al.*, 2021, 2022), the widespread analysis of 3D flowers has not occurred. Geometric morphometric studies of flowers in 3D are still limited compared with the mass of literature in the fields of anthropology, zoology and paleontology. This could be due in part to the difficulties of using micro-CT on the soft tissues of flowers, even though solutions for optimising HRCT scanning of flowers have been proposed (e.g. Staedler *et al.*, 2013; Dellinger *et al.*, 2019). In addition, and perhaps more importantly, the high cost of micro-CT techniques (Mathys *et al.*, 2013) contributes to reducing their accessibility. Lastly, the fact that flower colour is lost when reconstructing 3D models using X-ray scanning technologies (Mathys *et al.*, 2013) limits the use of this technique for studies interested in colour or colour patterns.

Recently, research based on 3D imagery has evolved rapidly and has received considerable attention (e.g. Katz & Friess, 2014; Cunliffe *et al.*, 2016; Evin *et al.*, 2016; Ströbel *et al.*, 2018;

Christiansen *et al.*, 2019; Florey & Moore, 2019; Giacomini *et al.*, 2019; Igihaut *et al.*, 2019; Medina *et al.*, 2020). A 3D technique of interest is photogrammetry (or structure from motion), which uses a collection of digital images to reconstruct a 3D model (see Linder, 2009; Luhmann *et al.*, 2013). Photogrammetry was originally used to reconstruct models of landscapes, buildings or large objects, but it can also be used for medium (close-range photogrammetry) or small objects (ultra-close-range photogrammetry). In short, photogrammetry begins by taking pictures of an object from all angles, ensuring that all aspects of the object are present in several overlapping photographs. The sets of photographs are then aligned using the relative position of homologous points in the overlapping pictures in a 3D space, and picture information is then used to reconstruct a 3D model with colour (see the [Floral photogrammetry protocol](#) section and Fig. 1 for more detailed information). Although used in many fields of biological sciences, photogrammetry has not yet been applied to the study of flowers.

The objective of this study was to demonstrate the potential of photogrammetry to reconstruct 3D models of flowers to facilitate studies of floral shape and colour. We describe an affordable and portable photogrammetric set-up that could be used in the field and outline a detailed protocol for reconstructing 3D photographic models of flowers of various shapes, colours and sizes. To illustrate the approach, we present an example of application in the study of the shape and colour of flowers from species of the Gesneriaceae.

Floral photogrammetry protocol

Here, we provide a summary of the photogrammetry protocol we developed. The full protocol is available from Github (<https://github.com/plantevolution/photogrammetry-protocol>), and details of the source and costs of materials, tools and software are provided as Supporting Information Table S1. Specific terms in photography, 3D modelling and geometric morphometrics are defined in the glossary (Box 1). Our objective was not to provide a unique and final protocol, but to provide guidelines for users to employ photogrammetry to model 3D flowers and guide them on how to adapt this approach for their own system.

Image acquisition

The first step of photogrammetry involves acquiring photographs encapsulating flower details for later modelling in 3D. This step is perhaps the most important as high-quality images are key to produce high-quality 3D models. We capture images using a digital single-lens reflex (DSLR) camera and a fixed focal-length macro lens. We save images in RAW format using an aperture of F16 (highest field depth without deteriorating the image quality), lowest ISO (e.g. 100) to avoid image noise created by the sensor, and a shutter speed adjusted to allow the appropriate amount of light to reach the camera's sensor to result in a well-exposed image (see Table S2 for a summary of the settings we used).

To facilitate the photo capture of the flower from all directions, we use a turntable and automated remote camera control

(Fig. 1a,b). To help later photo processing and mask the background in the pictures, we recommend using a uniform background. Good lighting conditions are also necessary for optimal picture quality. These conditions can be recreated in the field using a portable lightbox (see Table S1).

The flower to be photographed is fixed at the centre of the turntable using pins or clamps, or could be placed in a tube or a cut pipette tip depending on the structure and stiffness of the flower (see Fig. 1c). A scale should be placed so that it is visible in several photographs to allow scaling of the resulting model.

To capture the entire flower surface and details, we take a 360° series of photographs of flowers placed in normal and inverted positions (e.g. ventrally and dorsally). Typically, 20 photographs per rotation were taken at three different camera heights and angles of c. 0°, 30° and 60° for each side of a flower (see Fig. 1b), for a total of 120 photographs per flower. Depending on the flower's complexity, the number of photographs, camera angles and flower positions can be adjusted to capture all visible floral details. It is also possible to add close-up photographs to enhance the model and reveal concealed and minute parts (e.g. reproductive organs). If using a variable focal lens, it is preferable that the focal length is kept identical for all the pictures, and ideally at the minimum or maximum focal length possible to avoid optical deformations (Agisoft LLC, St Petersburg, Russia).

Colour and exposure calibration

Photographs must be colour-calibrated to adjust the reflectance and colour of an object and allow accurate comparison between flowers (Troscianko & Stevens, 2015). To calibrate multiple photographs with the same parameters, we use DNG (Digital Negative) colour profiles created from an additional RAW photograph of a standardised colour chart, taken for each series of photographs of a flower under the same light conditions and camera parameters. We convert the colour chart in a DNG profile (e.g. using Adobe Digital Negative converter) and standardise the series of photographs corresponding to the colour chart using Adobe LIGHTROOM (Adobe Inc., San Jose, CA, USA), providing an accurate reproduction of the flower colour for subsequent analyses of pigmentation patterns. We also standardise the photo exposure using a 75% grey colour chip from the colour chart. Exposure calibration can also be performed at a later stage directly using the colour file (texture) of the 3D model. From the calibrated RAW photographs, we export JPG images for the model reconstruction (Fig. 1c–e).

3D model reconstruction

The procedure we use to obtain a 3D model from photogrammetry includes photo alignment, which results in a sparse 3D point cloud, surface generation through depth maps calculation, and texture generation using the projection of photographs onto the surface of the model. Our protocol uses the commercial software Agisoft METASHAPE PROFESSIONAL EDITION v.1.7 (Agisoft LLC), but open-source photogrammetry software also exists (see Medina *et al.*, 2020).

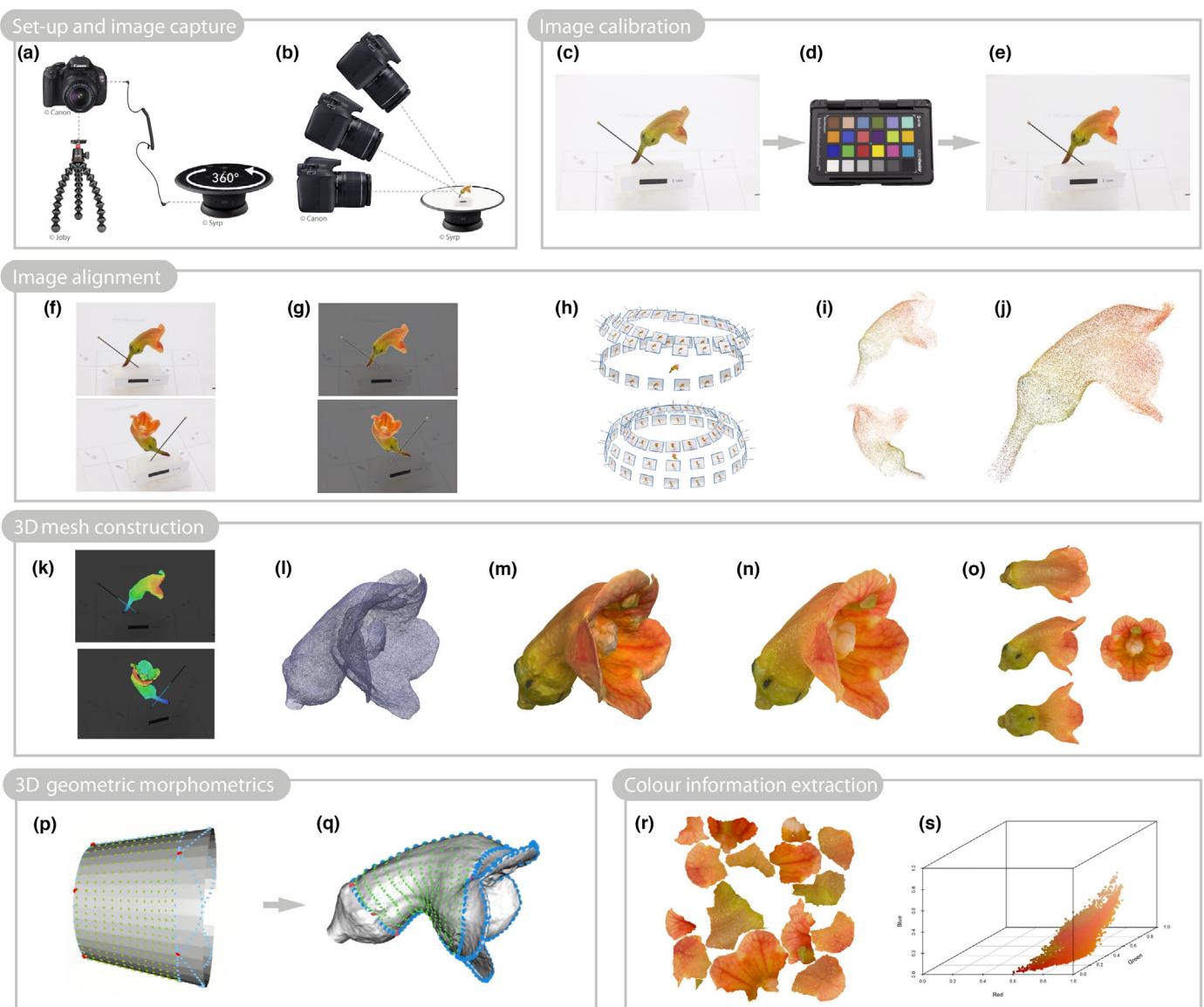


Fig. 1 Graphical workflow of the photogrammetric approach used to study floral morphology and colour in three dimensions (3D). Flowers are attached to a 360° turntable that automatically triggers a camera as the turntable rotates in steps of a few degrees and are photographed using three camera angles for both ventral and dorsal views (a, b). All RAW images (c) are calibrated identically using a colour chart (d) to obtain realistic colour representation of flowers (e). Masks are applied to remove the background (f, g) before aligning the images (h), which results in a tie point cloud of homologous pixels detected in multiple photographs (i). The separate sets of photographs are then aligned and merged to give a unique tie points cloud (j). Using depth maps (k), a 3D mesh is reconstructed (l) and the interpolated colour of the mesh polygons is calculated from images (m). A more realistic 3D model is obtained by building the texture from the original photographs (n), providing a finely detailed and coloured 3D model on the outer and inner surfaces of the flower (o). Landmarks (in red) and semilandmarks are positioned on the flower model for curves (in blue) on the petal margin, petal base, dorsal and ventral corolla curvature, and the base of the sepals, as well as on the simplified truncated cone template. Surface semilandmarks (in green) are automatically applied on flowers according to the template (p, q). The flower texture wrapping the model can be extracted as a 2D representation of the 3D surface (r) and used to analyse and quantify colour variation of the entire flower surface (s).

During photo alignment (also referred as camera alignment), source images are positioned by searching for common points in the photographs (tie points) and using the triangulation of the matching points. The alignment procedure can customarily be performed in a single step, by attributing images from different flower positions to distinct camera groups, or by separating images from different flower positions into different chunks. Treating sets of images separately can be useful for merging

models of different flower parts (e.g. for modelling a complete flower by merging the flower with and without its perianth). Chunks of images can also be treated separately during the alignment procedure when the overlap between pairs of images is not optimal to facilitate the alignment calculation (Fig. 1f–i, showing an example with two chunks of images). Before aligning images, masks can be captured manually or automatically to separate the flower from the background and restrict the searching of

Box 1 Glossary**Photography**

Aperture: Size of the opening of a lens's diaphragm (or generically called shutter) through which light passes, noted f/N .

ISO: Camera sensor sensitivity to light.

Sensor: Part of the camera that detects and transforms light into information to produce an image.

Shutter speed: Time during which the shutter allows light to reach the sensor.

3D Modelling

3D Mesh (3D object): Structural tri-dimensional shape built of polygons along x , y and z axes to represent its height, width and depth.

Depth map: An image in which a colour gradient indicates the distance from the camera.

Edge: A connection between vertices.

Key point: A distinct feature recognised in a single image.

Mask: A delimited region of a photograph that is not the main subject.

Texture (or texture map): 2D object with details of the surface appearance, or information about the colours used to wrap a 3D object.

Tie points: Automatically detected or manually placed 3D points that are matched in multiple images and used to compute their 3D position.

Vertex: A position in a 3D space with three-dimensional x , y and z coordinates.

Vertex colours: Colours applied to each vertex according to the average colours of the corresponding areas on the images source.

Geometric morphometrics

Landmark: Fixed point at a particular position, usually on a distinguishable homologous feature.

Semilandmark: Sliding point between landmarks or other semilandmarks, describing curves or surfaces.

model accordingly. Finally, we build the texture (detailed colour) of the model using the 2D picture's information to generate a realistic visualisation of the flower surface in 3D (Fig. 1n,o). The flower surface mesh can subsequently be used in geometric morphometric applications (Fig. 1p,q), and the 3D textured surface can be exported as a 2D layout of the 3D surface, used in quantification of flower colour using each pixel colour information (Fig. 1r,s).

Performance of the photogrammetry approach

The above protocol was applied to diverse flowers selected to represent a variety of floral forms, colours and complexity. These were taken from species belonging to different Angiosperm families, such as the Fabaceae (*Phaseolus coccineum*), Cactaceae (*Schlumbergera* sp.), Lamiaceae (*Salvia nemorosa*), and 19 Gesneriaceae species from the living collections of the Montreal Botanical Garden, mainly from the genera *Rhytidophyllum* and *Gesneria* (see the [Application example](#) section for more information on these specimens). Flowers from five species modelled by photogrammetry were also scanned using micro-CT to compare models originating from both approaches.

3D flower reconstruction

The overall 3D reconstruction process using photogrammetry took us from 0.5 to 2 h depending on the complexity of the project and the computer resources. The 3D models, the RAW photogrammetry image series to generate these models and the corresponding colour charts and calibrated textures can be accessed from MORPHOSOURCE (<https://www.morphosource.org/>) under the project 'Gesneriaceae of the Montreal Botanical Garden'.

The photogrammetric approach generated models of high quality that accurately represent the shape and colour of flowers of different structures, sizes (2–8 cm) and symmetry (see Fig. 2 for a sample of flower models). The flower models presented in Fig. 2 were also deposited in SKETCHFAB (sketchfab.com/plantevolution), an online platform for hosting and visualising 3D models with textures, to allow a closer inspection of the models. In many cases, very minute details could be modelled, such as the delicate petal margins of *Rhytidophyllum vernicosum* (Fig. 2g) or the styles and stamens of several species (Fig. 2c–f). Overall, the flower shape and colour of the models were very accurate and were essentially identical to the real flowers.

Some structures are more difficult to reconstruct, particularly those that are slender, translucent or reflective. Pillose organs were also challenging, in part due to the difficulty of applying masks. Structures that are very close to each other such as overlapping petals (*Schlumbergera* sp.; Fig. 2b) or styles that are very close to petals (Fig. 2f,h,i) were also difficult to reconstruct independently from each other. Finally, very thin surfaces or parts that were partly concealed depending on the camera angles may require more source images to be further improved, such as below the basal petal surfaces on *Schlumbergera* sp., the petal margins inside the corolla on *Phaseolus coccineum*, and the dorsal midrib

common points between images during the alignment procedure to the flower itself (Fig. 1g). The picture alignment (Fig. 1h) generates a 3D cloud of matching tie points for each set of images (Fig. 1i). When different chunks of images are used separately, they need to be aligned together and then merged either automatically or using manually placed markers on distinctive features on the flowers on several images (e.g. tips of petals or sepals, anthers). If manual markers are used, a minimum of three markers spaced on the flower is required. The merging of images or groups of images results in a single tie point cloud (Fig. 1j).

Once all the images are aligned around a single tie point cloud, the model (mesh) can be generated, using depth maps generated for each photograph that represents the distance of the flower surface on the z axis for each camera positions (Fig. 1k). The mesh is composed of vertices, edges and faces, together forming polygons (Fig. 1l). During mesh reconstruction, the interpolated colour of the mesh polygons is calculated from images when using depth maps as source information (Fig. 1m). The resulting mesh may need minor touch-ups, such as removing unwanted portions of the inflorescence or the pin used to attach the flower.

We then scale the model by manually positioning landmarks on the scale bar in the original images and defining these landmarks as being spaced by the length of the scale, which resizes the

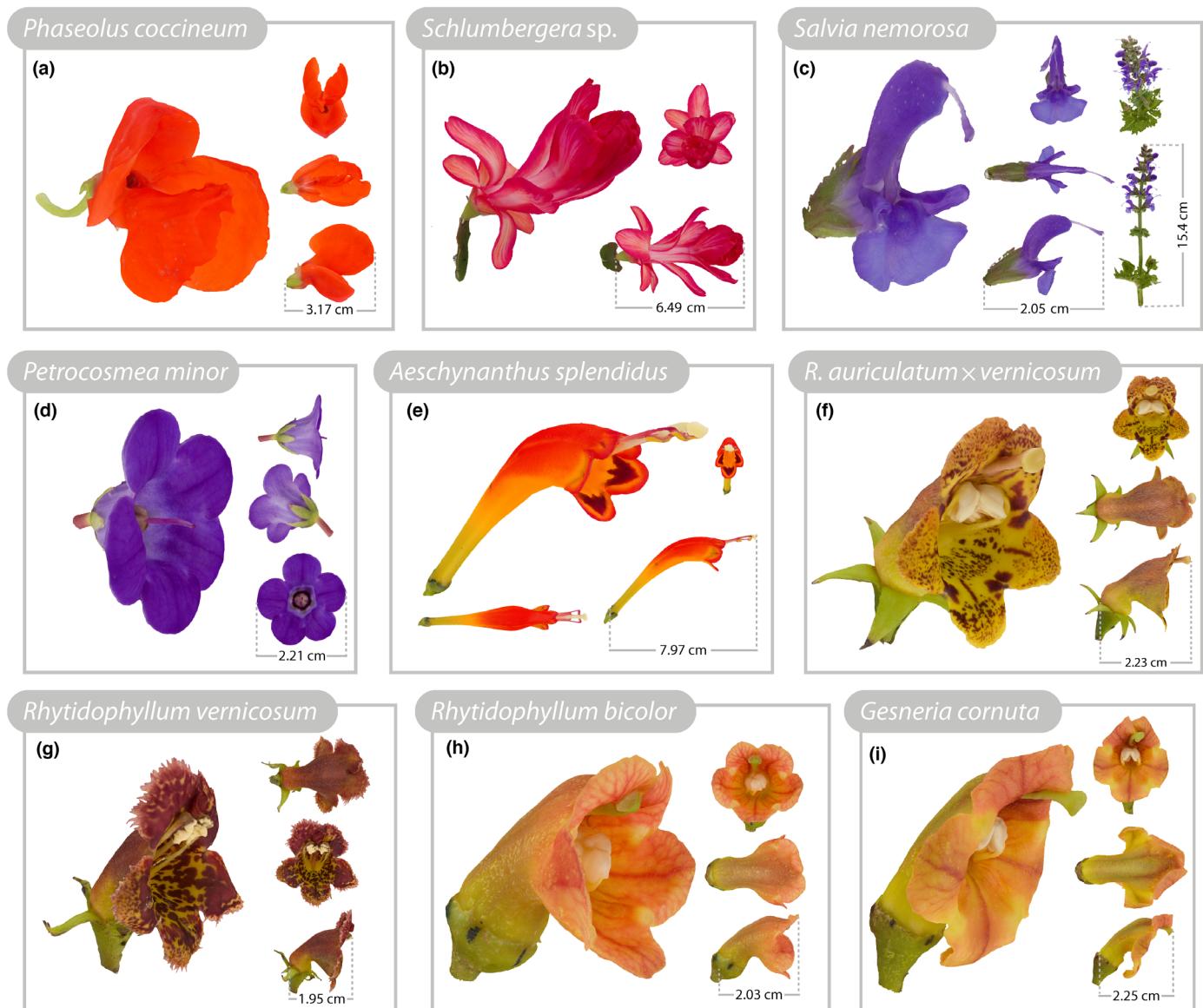


Fig. 2 Three-dimensional textured models derived from non-Gesnericeae (a–c) and Gesneriaceae flowers (d–i) using photogrammetry. *Phaseolus coccineum*, Fabaceae (a), *Schlumbergera* sp., Cactaceae (b), *Salvia nemorosa*, Lamiaceae (c), *Petrocosmea minor* (d), *Aeschynanthus splendidus* (e), the hybrid *Rhytidophyllum × vernicosum* (f), *Rhytidophyllum vernicosum* (g), *Rhytidophyllum bicolor* (h), *Gesneria cornuta* (i), and Gesneriaceae. The model of the inflorescence of *S. nemorosa* is illustrated under two different viewing angles (c).

of the sepal on *Salvia nemorosa*. Despite the occurrence of slight imperfections in some of the models, the global shape and colour of flowers reconstructed should allow most downstream applications.

Photogrammetry vs micro-CT comparison

To compare the models obtained by photogrammetry with models obtained by micro-CT scanning, the flowers of five species (*Gesneria acaulis* 1328-2021, *Kohleria* sp. 1828-2013, *Paliavana prasinata* 1432-2010, *Rhytidophyllum exsertum* 112-1991 and *Rhytidophyllum tomentosum* 1327-2021) were sent to the Integrated Quantitative Biology Initiative (IQBI) platform at McGill University for micro-CT scanning and model reconstruction.

Flowers were collected at the Montreal Botanical Garden and fixed in 4% paraformaldehyde (PFA) in 1× phosphate-buffered saline (PBS). The samples were gradually transferred to 70% ethanol from water and placed in 2% ethanolic phosphotungstic acid (EPTA) in 70% ethanol stain for 15 d. Flowers were scanned in 1% agarose in 15-ml tubes at resolution 22 µm at 60 V. The micro-CT models were reconstructed in DRAGONFLY (v.2020.2). The reconstruction of the models obtained from micro-CT took between 0.5 and 5 h.

To allow a visual comparison of the models obtained by photogrammetry and micro-CT, we presented the flower models of three species side-by-side in different figures (Figs 3, S1, S2). All micro-CT models were also analysed alongside the photogrammetry models in a geometric morphometric analysis (see the

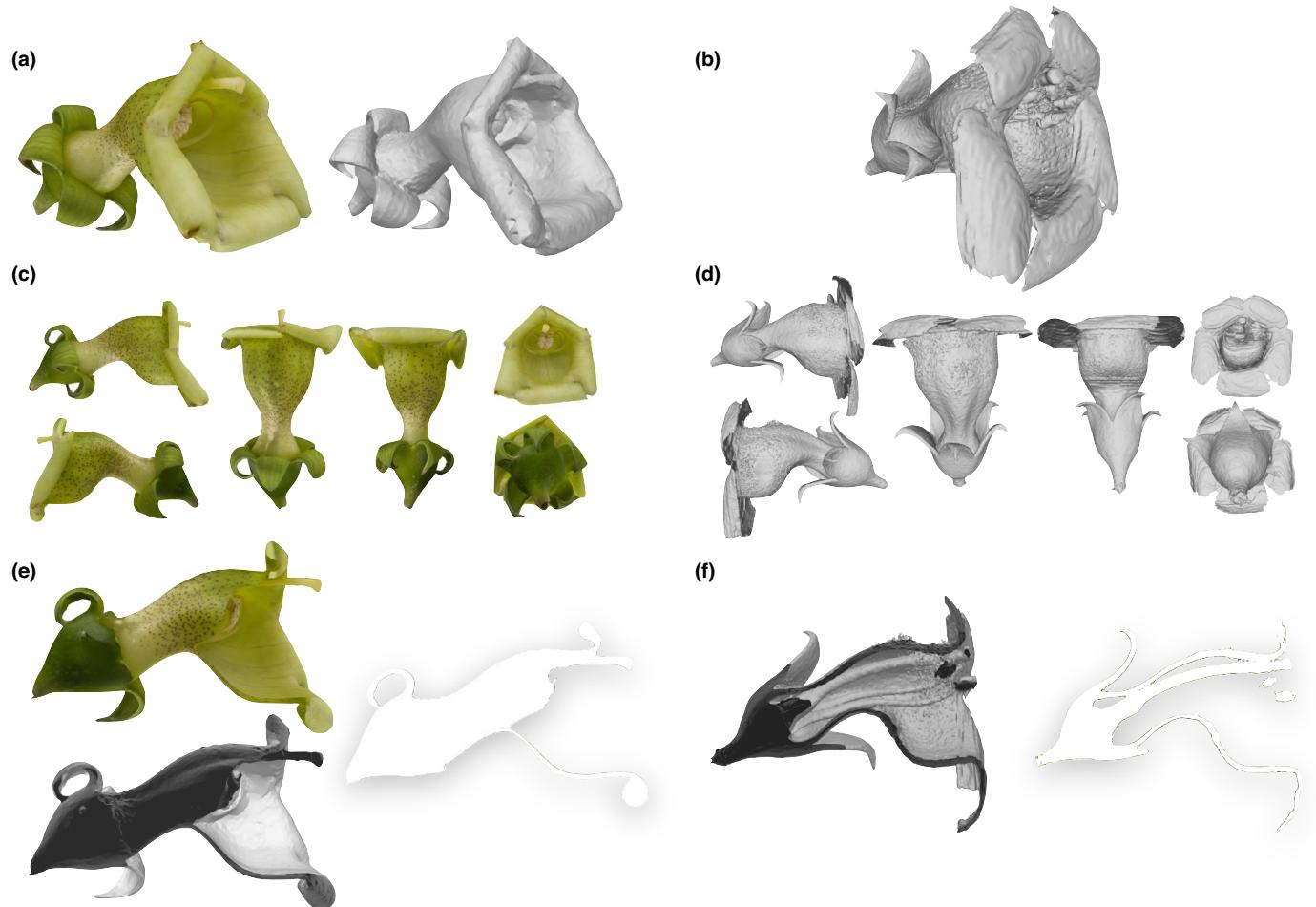


Fig. 3 Comparison of three-dimensional models of *Paliavana prasinata* derived from photogrammetry (left) and micro-computed tomography (micro-CT) scanning (right). The final solid models are represented for both photogrammetry (a, grey scale and colour) and micro-CT scan (b) under all viewing angles (c, d) (left and right lateral views, ventral, dorsal, anterior and posterior views). Sections of these models are represented by half models (grey scale and colour) and median plane sections of both models along the x-axis (e, f).

Application example section below). The micro-CT models were deposited in MORPHOSOURCE in the same project as the photogrammetry models.

The surface meshes of models reconstructed with the micro-CT scans included holes and bumps due to the detection of hairs during the scanning step, but overall represented the morphology of flowers similarly to photogrammetry (see details in the floral shape analysis application example). Due to the softening of tissues during the staining process, flower parts were occasionally distorted and flowers with naturally recurved petals and/or sepals during the anthesis were slightly unfolded on the CT-based model compared with the photogrammetry model and the real flower (see, for instance, *Paliavana prasinata* in Fig. 3a–d). Similar distortions of the corolla and sepals in micro-CT models are evident on the models of *G. rupinicola* and *R. exsertum* (Figs S1, S2). Internal and nonvisible organs were properly modelled using micro-CT scan, which could not be accurately reproduced using photogrammetry (Fig. 3e,f). However, anther and stigma position can be captured when visible from the flower opening (see Figs 2, 3).

Application example

Materials and methods

To demonstrate the potential use of flower models obtained from photogrammetry, we studied the shape and colour of 26 flowers from 18 species and one hybrid (*Rhytidophyllum auriculatum* × *vernicosum*) from the Gesneriaceae family that belong to three pollination syndromes: bird pollination, bat pollination and mixed-pollination (see details in Notes S1; Table S3). We provide a brief description of the methods used here, but detailed materials and methods are available as Supporting Information.

Floral shape analysis

The perianth of each flower was reconstructed in 3D using the described photogrammetry protocol above. We then used geometric morphometrics to compare the 3D flower shapes. Landmarks and semilandmarks for curves and surfaces were placed on the flowers (see Methods S1; Fig. S3; Table S4). After shape

alignment using a generalised Procrustes analysis (GPA), the coordinates of the landmarks and semilandmarks were projected onto the tangent space using principal component analysis (PCA) (Methods S1). The resulting morphospace places individuals from the same species very close to each other and allows the distinction of the pollination syndromes (Figs 4, S4). The estimated mean 3D shape of each syndrome also shows the main differences in shape between them (Fig. S5). The inclusion of the micro-CT flower models to the same morphospace shows that they fall very close to the photogrammetry models (Fig. S6).

Colour analysis

To illustrate the potential of the photogrammetric approach to study flower colour, the colour profiles of flowers were compared alongside the phylogeny of the 18 species of Gesneriaceae (see Methods S2 for phylogeny reconstruction). The colour of each flower surface texture was quantified in eight categories (bins) in terms of red, blue and green, which then allowed the computation of a colour distance between flowers (see Methods S3; Fig. S7). The resulting distance matrix was used to build a phenogram and compared with the phylogenetic relationships (Fig. 5). This example highlights that species tend to group by pollination syndromes when considering flower colour and that similar colour patterns show evolutionary convergence in the Gesneriaceae.

Discussion

Relevance of ultra-close-range photogrammetry for the study of flowers

Flower shape has attracted much interest in several subfields of plant sciences, but relatively few studies have used 3D flower models, despite the importance of precisely quantifying the size, shape and colour of flowers in 3D given that the vast majority of flowers have to interact in a 3D environment to be fertilised. The main aspects of the methods currently used that may limit the

number of 3D geometric morphometric studies on floral shape are the cost and lack of portability of micro-CT technologies. Although flowers can be fixed in the field in highly concentrated ethanol for later micro-CT scanning, doing so can damage or shrink flowers depending on their structure and thickness (Staedler *et al.*, 2013; Dellinger *et al.*, 2019). Moreover, contrasting reagents used to infiltrate the flower tissues before scanning may also produce additional leaching artefacts (Staedler *et al.*, 2013). We observed slight anatomical distortions in the flower models obtained with CT scans following fixations such as the unfolding of petals and sepals, distorting the shape and the relative positioning of the floral structures. Our micro-CT protocols could be improved to provide better and more accurate results; however, the use of standard protocols combined with an external service offers a fair comparison with our photogrammetry approach.

An array of flower sizes and colours for species belonging to the Gesneriaceae, Fabaceae, Lamiaceae and Cactaceae were successfully reconstructed using photogrammetry. Various sizes of flowers and inflorescences can be rendered accurately and in detail, and we were able to model all visible parts of the flowers, including the stigma and anthers in many species. The entire physical set-up (turntable, lightbox, tripod and colour chart) required to implement this method costs < 600 US\$, making this approach affordable for most laboratories that already own a suitable digital camera. Computer requirements are relatively reasonable for photogrammetry applications (16–32 GB of random access memory (RAM), and 4–8 central processing units (CPUs)). The academic professional edition of the Agisoft METASHAPE software we used to reconstruct the 3D models currently costs 549 US\$, although cheaper or open-source alternatives are available (see Medina *et al.*, 2020).

Ultra-close-range photogrammetry has several clear advantages over micro-CT – the gold standard in the field – for reconstructing 3D models of flowers. It is portable, affordable, time efficient both for image acquisition and model building, and can reconstruct the colour of the flower (see Table 1). Yet, photogrammetry is also subject to some limitations (Table 1). Most obviously, flower models can be reconstructed for only externally visible

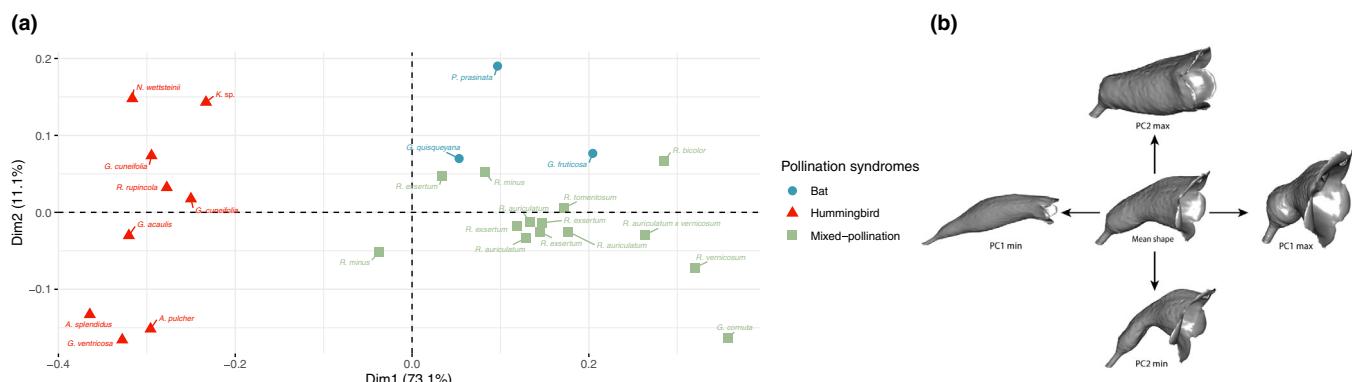


Fig. 4 Three-dimensional (3D) floral morphospace and corresponding shape variation along the axes for individuals belonging to the *Gesneria*, *Rhytidophyllum*, *Nematanthus*, *Aeschynanthus*, *Kohleria* and *Paliavana* genera. Specialists for bat (blue circles) and hummingbird pollination (red triangles) as well as mixed-pollination strategies (green squares) are represented along the first and second dimensions of the principal component analysis (PCA) (a). The mean 3D flower shape, as well as the maximum and minimum configurations of the 3D flower shape are shown for the first and second dimensions of the PCA (b).

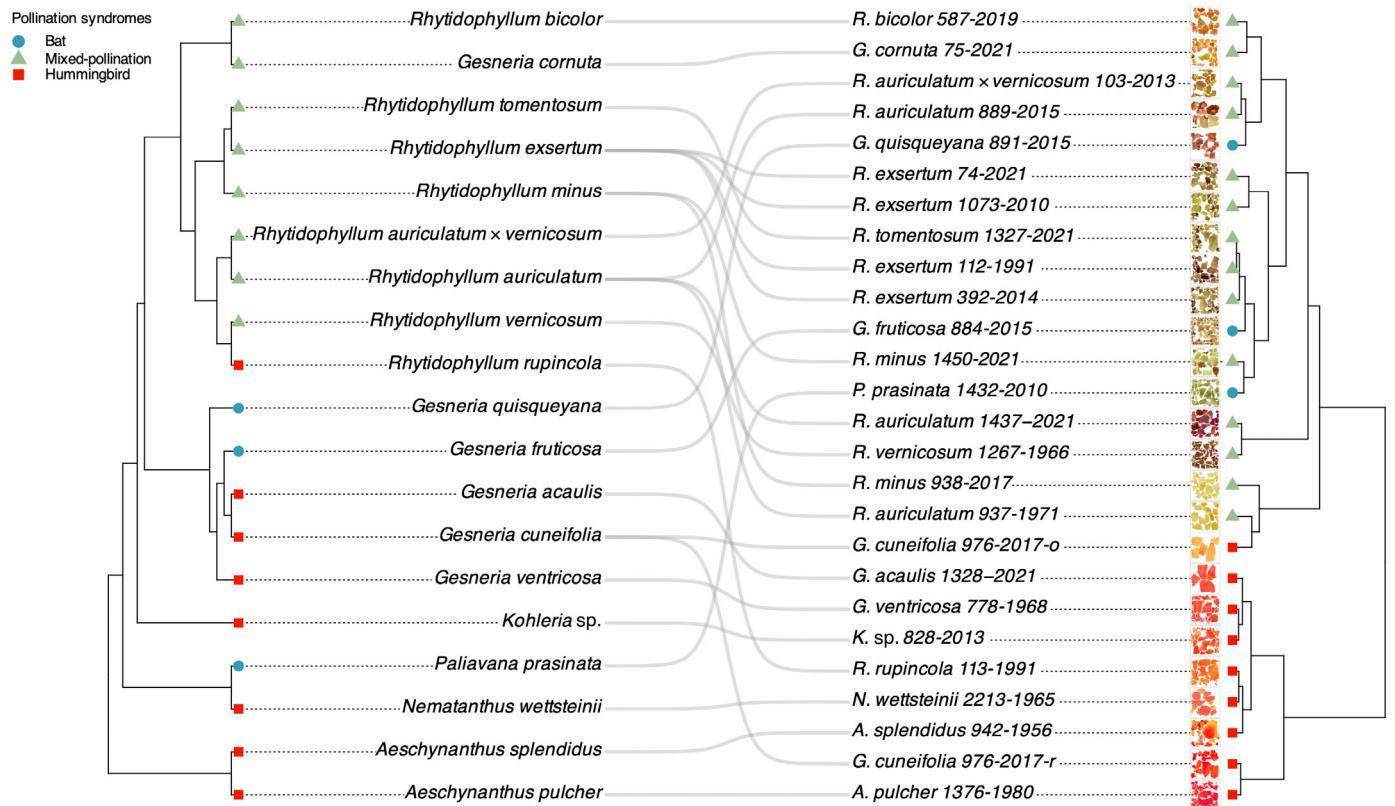


Fig. 5 Tanglegram linking the phylogenetic relations of Gesneriaceae species (left) with the colour distance dendrogram from flower specimen textures (large boxes at the tip of the dendrogram) using Ward's clustering method (right) according to their respective pollination syndromes.

parts of the flower. Hidden structures, such as reproductive organs in some species, may remain hidden in particularly closed corollas. Floral dissection might in some cases provide the means to characterise concealed structures in 3D using photogrammetry, for example removal of the corolla, or simply cutting the sepals to reveal the base of the corolla, as we did in the application example using Gesneriaceae. In addition, concealed organs can be photographed and thus be visible on the model texture, allowing them to be analysed. But if concealed organs are of main interest for a given study, micro-CT is clearly the best option.

Some flower characteristics also likely complicate the 3D model reconstruction with photogrammetry. One of these is the often very thin surfaces of flowers. Missing surfaces or edges in the final 3D model often result from a miscalculation of the position of sufficiently close outer and inner surfaces of an object to be modelled. Depth data from sets of images may intersect, thus creating holes in the 3D mesh. A contrasting background and accurate masking of flowers allow the recovery of thin structures at the extremities of the models such as fringed petals or stamen filament (e.g. *Rhytidophyllum vernicosum* and *Salvia nemorosa* in Fig. 2). Shiny or translucent surfaces also represented a challenge because the reflection of light or the features that are detected behind the translucent petals are not fixed features on the surface but are still captured in images. This issue can be solved using a softer light during the photography step. The presence of dense hairs on the flower also complicates model reconstruction because of the difficulty of adequately removing the background on

Table 1 Summary of the capabilities of both the micro-computed tomography (micro-CT) scan and photogrammetry approaches.

Characteristics	Approaches	
	Photogrammetry	micro-CT scan
3D reconstruction of occluded areas and internal structures	No	Yes
Model colour and texture	Yes	No
Portability	Yes	No, but flowers can be fixed on site
Adaptable to any type of flower	Yes, but very soft and hairy flowers and those with few clear visual landmarks are more difficult to reconstruct	Yes
Deformation of structures	No	Slight
Cost	From \$2000	\$200 000–\$1000 000
Time requirements per flower	Set-up and photography: 30 min Colour calibration: 10 min Reconstruction: 30 min–3 h	Staining: typically > 14 d Scanning: 1 h Segmentation: 30 min–5 h

Details on costs associated with photogrammetry are available in Supporting Information Table S1.

photographs and because the resulting model is often rugged, although it can generally be corrected by smoothing the 3D surface. Finally, radially symmetrical or spherical objects with no clear visual landmarks are typically difficult to reconstruct using photogrammetry (Ijiri *et al.*, 2018), such as some actinomorphic flowers. A straightforward solution is placing a few visually distinct markers on such flowers (i.e. coloured dots) to add artificial distinct features to help the software to align the photographs.

Overall, ultra-close-range photogrammetry is an useful alternative to micro-CT scanning and could be valuable for studies interested in the nonoccluded parts of a flower and its colour. One interesting avenue is to combine CT-based and image-based approaches to get the advantages of both approaches, as demonstrated by Ijiri *et al.* (2018).

Perspectives for floral morphology studies

Studying high-dimensional floral shape evolution One obvious application of 3D flower models obtained by photogrammetry is to study floral shape. Different methods exist for characterising biological shapes in 3D. The most popular type of method is geometric morphometrics that is based on the positioning of landmarks (homologous points) and semilandmarks (points that are positioned relative to others, along curves or surfaces). Landmark-free methods have also been developed and could be useful for smooth or featureless 3D surfaces for which landmark placement is not appropriate (Pomidor *et al.*, 2016). Our worked example on Gesneriaceae showed how photogrammetry can be used to study the 3D morphology of flowers. The accurate 3D reconstruction of flowers combined with landmark-based geometric morphometrics allowed better discrimination and understanding of the 3D structure of the distinct pollination syndromes (Fig. 4) compared with what can be obtained using 2D shape information from flowers in profile view (Joly *et al.*, 2018).

Studying flower colour in 3D One obvious advantage of photogrammetry over other approaches is to provide a very accurate reconstruction of flower colour in 3D. This opens many study opportunities as flower pigmentation is a major display signal for animal-pollinated plants. The complexity of floral colouration, including nectar guides, helps pollinators limit the time they spend locating rewards, thus improving their foraging efficiency (Leonard & Papaj, 2011). Moreover, both biotic factors, such as pollinator abundance and the colour of co-occurring plants in the community, and abiotic factors, such as solar radiation and low precipitation, can influence the colour perception and patterns of flowers (Dalrymple *et al.*, 2020).

We have shown that photogrammetry is a valuable tool to recover calibrated colour information from the entire 3D surface of flowers. Three-dimensional textures generated directly from calibrated high-quality photographs in colour analyses can account for the totality of variation in flower pigmentation, avoiding biases caused by overlooked concealed surfaces or the distorted importance given to certain regions of the flower (surfaces perpendicular to the camera compared with those that are more parallel) when using only a few 2D images in such analyses.

Moreover, the presence of colour on 3D models could facilitate the distinction of structures that vary in colour but not so much in shape. For example, some species of Merianieae have stamen appendages that are distinguished from anthers primarily by their colour. Retaining colour on 3D models could thus help the positioning of these structures to test alternative hypotheses of floral modularity (Dellinger *et al.*, 2019). The use of 3D models of flowers that retain colour information would greatly assist in distinguishing organs that differ in function and colour but are difficult to distinguish based on shape.

Photography is a convenient way to collect morphological and reflectance data (colour) from specimens, and to facilitate research in ecology and evolution. However, the lack of tools to make objective colour measurements and the fact that cameras generally used in scientific studies produce uncalibrated photographs make these images unreliable for quantitative colour measurements (Troscianko & Stevens, 2015). For this reason, a particular attention needs to be given to photo calibration and linearisation.

In addition, photography is not restricted to the visible spectrum (wavelength from 400 to 700 nm). Light can be detected by camera sensors in the UV range (320–400 nm) and IR (also called ‘heat radiation’) or near-infrared (NIR) range (over 700 nm to about 1 mm), making it possible to incorporate these components of the light spectrum into 3D models. This could be important to understand the patterns of reflectance evolution as most insect pollinators possess UV receptors (Chittka *et al.*, 2001; Schiestl & Johnson, 2013), and self-heating flowers or inflorescences (Thien *et al.*, 2000) as a reward or a means of enhancing the production and dissemination of floral scents (Seymour *et al.*, 2003). Furthermore, images could be converted to correspond to different animal visual system sensitivities (cone-catch values) (Troscianko & Stevens, 2015).

Experimental studies Advancements in 3D printing technology enable printing relatively small and intricate 3D models as well as remarkably detailed colour patterns, directly (using coloured filaments) or indirectly (by applying colour on models). In addition to colour, soft and flexible resins can be used thinly to resemble the soft tissues of flowers (see Fig. S8 for an example of a colourless soft 3D-printed artificial flower of a 3D model derived from photogrammetry). These methods should help expand the scope of experimental studies designed to test hypotheses about pollinator behaviour and flower shape, size and colour. As an example, printed artificial flowers and chimeric flowers made of artificial and natural flower parts were used to decouple and test the relative contributions of olfactory and visual signals to attract pollinators in a mimetic orchid *Dracula lafleurii* by Policha *et al.* (2016).

Natural history collections 3.0

Digitisation and archiving of information of material from natural history collections have revolutionised their current use. Three-dimensional modelling of natural history collections would further advance their value, accessibility and use. Such efforts are ongoing in entomological and ornithological

collections using photogrammetry (Ströbel *et al.*, 2018; Medina *et al.*, 2020). Unlike zoological specimens, which generally retain their 3D shapes in collections, plant specimens are usually kept pressed in herbaria, thus losing most of their natural shape. Although morphological data can be extracted from herbarium specimens (e.g. Bilbao *et al.*, 2021), morphological correlations between flower parts are generally lost. One strength of photogrammetry is that 2D data sets can be collected in the field to reconstruct 3D morphological features lost in herbarium specimens and can be subsequently linked to them in similar ways as other sources of information, such as genetic data or plant parts preserved separately from the specimens. Sharing such 3D models would significantly improve the quality of phenomic data obtainable from herbaria (Ströbel *et al.*, 2018; Medina *et al.*, 2020), or complement and extend the information on plant traits (morphological, anatomical, functional, biochemical, phenological and physiological) that is being centralised in global databases such as TRY (Kattge *et al.*, 2011, 2020) and PROTEUS (Sauquet, 2019). Photogrammetry could also be extended to living collections, such as botanical gardens, thus improving access to such collections by providing access to virtual plants all year round and from anywhere in the world, creating new opportunities for scientific studies and outreach (Maschner *et al.*, 2013). Open access databases dedicated to natural history, cultural heritage and scientific collections are already available for such applications (Boyer *et al.*, 2016).

Conclusions

Due to its simplicity and efficacy, photogrammetry has the potential to inspire new ways to quantify flower shape and colour and explore questions and collaborations in investigations of flowering plant evolution. Combined with genomic data, phenomic information obtained from 3D models using photogrammetry will open new areas of study of floral evolution. By combining practicality, reasonable costs, portability and user-friendly applications, photogrammetry has the potential to revolutionise studies of floral evolution and ecology.

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Competing interests

None declared.

Author contributions

DS, ML and SJ designed the research. ML, JB and SJ acquired the data. ML and SJ analysed the data. ML wrote the manuscript. ML, SJ, DS and JB edited and revised the manuscript. SJ and DS acquired funding.

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Data availability

The 3D model data are openly available in MORPHOSOURCE at <https://www.morphosource.org/>, reference ID: 000369440.

References

- Artuso S, Gamisch A, Staedler YM, Schönenberger J, Comes HP. 2021. Evidence for selectively constrained 3D flower shape evolution in a late miocene clade of malagasy bulbophyllum orchids. *New Phytologist* 232: 853–867.
- Artuso S, Gamisch A, Staedler YM, Schönenberger J, Comes HP. 2022. Evidence for an evo-devo-derived hypothesis on three-dimensional flower shape modularity in a tropical orchid clade. *Evolution*. doi: [10.1111/evo.14621](https://doi.org/10.1111/evo.14621).
- Bilbao G, Bruneau A, Joly S. 2021. Judge it by its shape: a pollinator-blind approach reveals convergence in petal shape and infers pollination modes in the genus *erythrina*. *American Journal of Botany* 108: 1716–1730.
- Boyer DM, Gunnell GF, Kaufman S, McGahey TM. 2016. MORPHOSOURCE: archiving and sharing 3-D digital specimen data. *The Paleontological Society Papers* 22: 157–181.
- Chittka L, Spaethe J, Schmidt A, Hickelsberger A. 2001. *Adaptation, constraint, and chance in the evolution of flower color and pollinator color vision*. Cambridge, UK: Cambridge University Press, 106–126.
- Christiansen F, Sironi M, Moore MJ, Di Martino M, Ricciardi M, Warick HA, Irschick DJ, Gutierrez R, Uhart MM. 2019. Estimating body mass of free-living whales using aerial photogrammetry and 3D volumetrics. *Methods in Ecology and Evolution* 10: 2034–2044.
- Cunliffe AM, Brazier RE, Anderson K. 2016. Ultra-fine grain landscape-scale quantification of dryland vegetation structure with drone-acquired structure-from-motion photogrammetry. *Remote Sensing of Environment* 183: 129–143.
- Dalrymple RL, Kemp DJ, Flores-Moreno H, Laffan SW, White TE, Hemmings FA, Moles AT. 2020. Macroecological patterns in flower colour are shaped by both biotic and abiotic factors. *New Phytologist* 228: 1972–1985.
- Davies TG, Rahman IA, Lautenschlager S, Cunningham JA, Asher RJ, Barrett PM, Bates KT, Bengtson S, Benson RB, Boyer DM *et al.* 2017. Open data and digital morphology. *Proceedings of the Royal Society B: Biological Sciences* 284: 20170194.
- Dellingler AS, Artuso S, Pamperl S, Michelangeli FA, Penneys DS, Fernández-Fernández DM, Alvear M, Almeda F, Scott Armbruster W, Staedler Y *et al.* 2019. Modularity increases rate of floral evolution and adaptive success for functionally specialized pollination systems. *Communications Biology* 2: 1–11.
- Evin A, Souter T, Hulme-Beaman A, Ameen C, Allen R, Viacava P, Larson G, Cucchi T, Dobney K. 2016. The use of close-range photogrammetry in

- zooarchaeology: creating accurate 3d models of wolf crania to study dog domestication. *Journal of Archaeological Science: Reports* 9: 87–93.
- Faegri K, Van Der Pijl L. 1979. *Principles of pollination ecology*. New York, NY, USA: Pergamon Press, 256.
- Fenster CB, Armbruster WS, Wilson P, Dudash MR, Thomson JD. 2004. Pollination syndromes and floral specialization. *Annual Reviews of Ecology Evolution and Systematic* 35: 375–403.
- Florey CL, Moore PA. 2019. Analysis and description of burrow structure in four species of freshwater crayfishes (Decapoda: Astacoidea: Cambaridae) using photogrammetry to recreate casts as 3D models. *The Journal of Crustacean Biology* 39: 711–719.
- Gamisch A, Staedler YM, Schönenberger J, Fischer GA, Comes HP. 2013. Histological and micro-CT evidence of stigmatic rostellum receptivity promoting auto-pollination in the Madagascan orchid *Bulbophyllum bicoloratum*. *PLoS ONE* 8: e72688.
- Giacomini G, Scaravelli D, Herrel A, Veneziano A, Russo D, Brown RP, Meloro C. 2019. 3D photogrammetry of bat skulls: perspectives for macro-evolutionary analyses. *Evolutionary Biology* 46: 249–259.
- Hsu H-C, Chou W-C, Kuo Y-F. 2020. 3D revelation of phenotypic variation, evolutionary allometry, and ancestral states of corolla shape: a case study of clade corytholoma (subtribe Ligeriinae, family Gesneriaceae). *GigaScience* 9: 1–16.
- Iglhaut J, Cabo C, Puliti S, Piermattei L, O'Connor J, Rosette J. 2019. Structure from motion photogrammetry in forestry: a review. *Current Forestry Reports* 5: 155–168.
- Ijiri T, Todo H, Hirabayashi A, Kohiyama K, Dobashi Y. 2018. Digitization of natural objects with micro ct and photographs. *PLoS ONE* 13: e0195852.
- Joly S, Lambert F, Alexandre H, Clavel J, Léveillé-Bourret E, Clark JL. 2018. Greater pollination generalization is not associated with reduced constraints on corolla shape in Antillean plants. *Evolution* 72: 244–260.
- Kattge J, Bönisch G, Díaz S, Lavorel S, Prentice IC, Leadley P, Tautenhahn S, Werner GD, Aakala T, Abedi M et al. 2020. TRY plant trait database – enhanced coverage and open access. *Global Change Biology* 26: 119–188.
- Kattge J, Diaz S, Lavorel S, Prentice IC, Leadley P, Bönisch G, Garnier E, Westoby M, Reich PB, Wright IJ et al. 2011. TRY – a global database of plant traits. *Global Change Biology* 17: 2905–2935.
- Katz D, Friess M. 2014. 3d from standard digital photography of human crania – a preliminary assessment. *American Journal of Physical Anthropology* 154: 152–158.
- Leonard AS, Papaj DR. 2011. ‘x’ marks the spot: the possible benefits of nectar guides to bees and plants. *Functional Ecology* 25: 1293–1301.
- Linder W. 2009. *Digital photogrammetry*, vol. 1. Berlin, Germany: Springer.
- Luhmann T, Robson S, Kyle S, Boehm J. 2013. *Close-range photogrammetry and 3D imaging*. Berlin, Germany: De Gruyter.
- Maschner HDG, Schou CD, Holmes J. 2013. Virtualization and the democratization of science: 3D technologies revolutionize museum research and access. In: *2013 Digital Heritage International Congress (DigitalHeritage)*. New York, NY, USA: IEEE, 265–271.
- Mathys A, Brecko J, Semal P. 2013. Comparing 3D digitizing technologies: what are the differences? In: *2013 Digital Heritage International Congress (DigitalHeritage)*. New York, NY, USA: IEEE, 201–204.
- Medina JJ, Maley JM, Sannapareddy S, Medina NN, Gilman CM, McCormack JE. 2020. A rapid and cost-effective pipeline for digitization of museum specimens with 3D photogrammetry. *PLoS ONE* 15: e0236417.
- van der Niet T, Zollkofer CP, de León MSP, Johnson SD, Linder HP. 2010. Three-dimensional geometric morphometrics for studying floral shape variation. *Trends in Plant Science* 15: 423–426.
- Policha T, Davis A, Barnadas M, Dentinger BTM, Raguso RA, Roy BA. 2016. Disentangling visual and olfactory signals in mushroom-mimicking *Dracula* orchids using realistic three-dimensional printed flowers. *New Phytologist* 210: 1058–1071.
- Pomidor BJ, Makedonska J, Slice DE. 2016. A landmark-free method for three-dimensional shape analysis. *PLoS ONE* 11: e0150368.
- Reich D, Berger A, von Balthazar M, Chartier M, Sherafati M, Schönenberger J, Manafzadeh S, Staedler YM. 2020. Modularity and evolution of flower shape: the role of function, development, and spandrels in erica. *New Phytologist* 226: 267–280.
- Rohlf FJ, Marcus LF. 1993. A revolution morphometrics. *Trends in Ecology & Evolution* 8: 129–132.
- Sauquet H. 2019. *PROTEUS: a database for recording morphological data and fossil calibrations*. v.1.27. [WWW document] URL <http://eflower.myspecies.info/proteus>.
- Schiestl FP, Johnson SD. 2013. Pollinator-mediated evolution of floral signals. *Trends in Ecology & Evolution* 28: 307–315.
- Seymour RS, White CR, Gibernau M. 2003. Heat reward for insect pollinators. *Nature* 426: 243–244.
- Staedler YM, Masson D, Schönenberger J. 2013. Plant tissues in 3D via X-ray tomography: simple contrasting methods allow high resolution imaging. *PLoS ONE* 8: e75295.
- Ströbel B, Schmelzle S, Blüthgen N, Heethoff M. 2018. An automated device for the digitization and 3d modelling of insects, combining extended-depth-of-field and all-side multi-view imaging. *ZooKeys* 759: 1–27.
- Stuppy WH, Maisano JA, Colbert MW, Rudall PJ, Rowe TB. 2003. Three-dimensional analysis of plant structure using high-resolution X-ray computed tomography. *Trends in Plant Science* 8: 2–6.
- Thien LB, Azuma H, Kawano S. 2000. New perspectives on the pollination biology of basal angiosperms. *International Journal of Plant Sciences* 161: S225–S235.
- Timerman D, Barrett SC. 2019. Comparative analysis of pollen release biomechanics in thalictrum: implications for evolutionary transitions between animal and wind pollination. *New Phytologist* 224: 1121–1132.
- Troscianko J, Stevens M. 2015. Image calibration and analysis toolbox – a free software suite for objectively measuring reflectance, colour and pattern. *Methods in Ecology and Evolution* 6: 1320–1331.
- Wang C-N, Hsu H-C, Wang C-C, Lee T-K, Kuo Y-F. 2015. Quantifying floral shape variation in 3d using microcomputed tomography: a case study of a hybrid line between actinomorphic and zygomorphic flowers. *Frontiers in Plant Science* 6: 724.
- Willmer P. 2011. *Pollination and floral ecology*. Princeton, NJ, USA: Princeton University Press.

Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Three-dimensional models of flowers of *Gesneria acaulis* (75-2021) obtained from photogrammetry and CT scanning.

Fig. S2 Three-dimensional models of flowers of *Rhytidophyllum exsertum* (112-1991) obtained from photogrammetry and CT scanning.

Fig. S3 Example of landmarks and semilandmarks placement on the corolla of *Gesneria cuneifolia* and *Gesneria cornuta*.

Fig. S4 Morphospace of individual three-dimensional floral shape variation along the second and third principal axes of the principal component analysis.

Fig. S5 Mean floral shapes for three pollination syndromes.

Fig. S6 Principal component analysis of floral shapes of Gesneriaceae according to pollination syndromes and the method used to reconstruct the three-dimensional floral shapes.

Fig. S7 Colour distance matrix heatmap and dendrogram using Ward’s distance.

Fig. S8 Example of a three-dimensional (3D)-printed flower of *Gesneria cornuta* in clear and soft resin.

Table S1 Summary of the materials used to scan and reconstruct three-dimensional flower models and their approximate price in 2022.

Table S2 Summary of the camera and turn table settings used to scan flowers.

Table S3 Species and collection numbers associated with the Botanical Garden of Montreal database of the specimens used to reconstruct three-dimensional models of flowers.

Table S4 Identification of the landmark and semilandmarks used for curves and surfaces.

Methods S1 Three-dimensional geometric morphometrics.

Methods S2 Phylogenetic analysis.

Methods S3 Flower colour variation analysis.

Notes S1 Taxonomic groups and flower material.

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