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CAPITULUM

THE INTERNATIONAL COMPOSITAE ALLIANCE NEWSLETTER



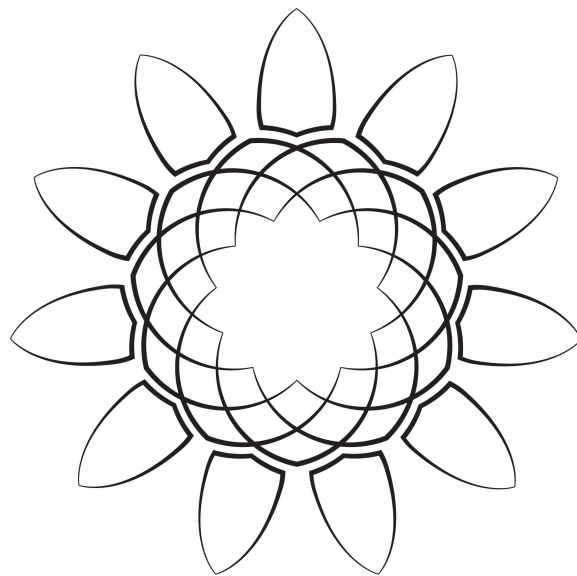
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THE INTERNATIONAL COMPOSITAE ALLIANCE

VOLUME 4(1) - NOVEMBER 2025

CAPITULUM

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For the community, by the community.



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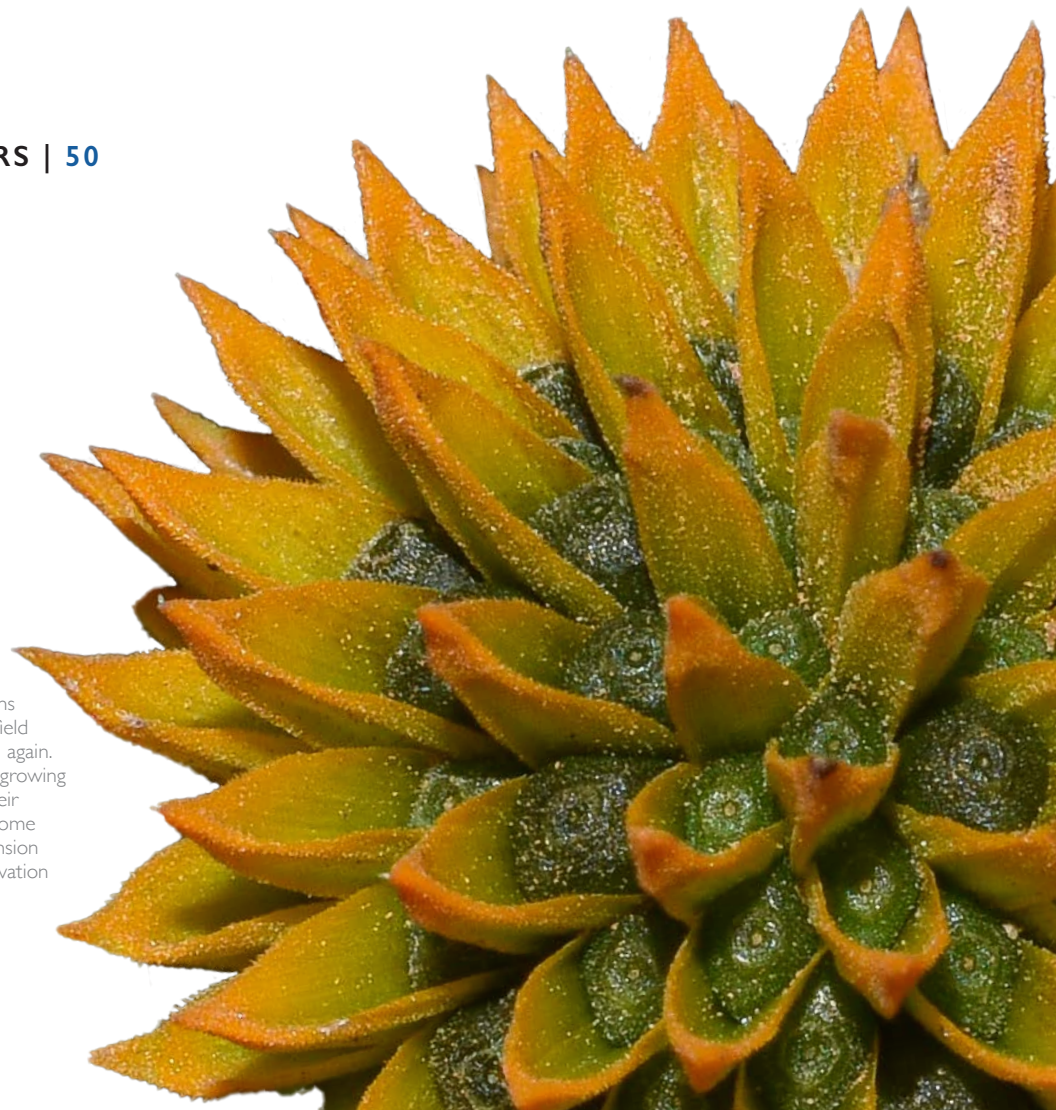
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Cover photo: One of only a handful of photographs taken of the woolly devil (*Ovicula biradiata*) in the field before the plants dried up and have not been seen again. In this issue Lichter Marck et al. report successfully growing *O. biradiata* from seeds to make observations of their reproductive biology, UV reflectance, and chromosome number. They also report a preliminary range extension for the woolly devil of over 100 km from an observation uploaded to inaturalist.

Photo by James Bailey, o



New insights into the natural history of the woolly devil *Ovicula biradiata* (Helenieae): chromosome number, UV reflectance, and a range extension

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ABSTRACT

The rare desert annual *Ovicula biradiata* was discovered in March of 2024 and recently described as a new genus and species. Few details about this enigmatic new composite are yet known, including its geographic distribution, reproductive biology, or chromosome number. Here we report a new record for the woolly devil and biological observations from four individual plants that were grown from seed. The cultivated plants of *O. biradiata*, from seedling to maturity, were not as densely white-woolly as plants in the field. The potted plants grew rapidly, producing solitary capitula at branch tips. The first flower parts to emerge from phyllaries were the strap-like limbs of 2–3 ray floret corollas, soon followed by the disc floret corollas already with yellow, pollen-laden anthers. A chromosome number of $2n=32$ was obtained for the species based on meiotic counts. We report preliminary evidence for reproductive self-incompatibility in the woolly devil, and the tendency for ray florets to fluoresce under ultraviolet light, suggesting they contrast with the overall cryptic appearance of the plants and aid in pollinator attraction. Finally, we report a new photographic observation recently uploaded to *iNaturalist* that, if substantiated, extends the range of the woolly devil eastward by over 130 km.

Keywords: ex-situ conservation, *iNaturalist*, pollination, self-incompatibility, Trans-Pecos Texas, UV reflectance.

INTRODUCTION

The woolly devil (*Ovicula biradiata* Manley) represents a charismatic new genus and species of Compositae in subtribe Tetraneurinae (Helenieae) that is remarkable for its small size, densely tomentose indumentum, and conspicuous 2–3 ray florets with elongated strap-like limbs, which are each white with four parallel maroon lines (Manley

et al., 2025). Everything known about this cryptic, minute, and annual Compositae has been gathered from photographs and specimens collected from two of three known localities in the backcountry of Big Bend National Park (BIBE) in the Chihuahuan Desert of Trans-Pecos Texas (Manley et al., 2025). Much remains to be learned about these plants, including their reproductive biology, chromosome number, population demographics, and ecology.



Figure 1. Senescing individuals of *Ovicula biradiata* in late spring. Photo by Dana Sloan in April 2024.

The first known individuals of the woolly devil were encountered and photographed on 2 Mar 2024 by BIBE personnel, Deb Manley and Kathy Hoyt, while hiking cross-country in search of rare plant populations (Manley et al., 2025). Taxonomists from nearby Sul Ross State University (SRSU) Michael Powell and Kelsey Wogan were invited by BIBE personnel to study the species, but by the time these investigators were granted permission to visit a population in the field on 25 Apr 2024, individual plants at all three localities had already begun to wither (Figure 1). It was not known if the delicate annual plants were fading due to drought and heat-wave conditions at the time or if this was part of a naturally accelerated annual life-history cycle. Dozens of partially desiccated individual plants were collected from two localities and the only known herbarium specimens of the woolly devil made so far were deposited at SRSU and CAS.

To discern evolutionary relationships and determine how to classify this new composite, DNA sequence data from the internal transcribed spacer (ITS) region was amplified using PCR and combined with the DNA sequence matrix for the epaleate tribes of the Heliantheae alliance originally published by Baldwin et al. (2002). Molecular phylogenetic evidence backed up by morphological observations suggested that the woolly devil is nested within

subtribe Tetraneurinae of the sneezeweed and blanket flower tribe Helenieae (Manley et al., 2025). Within Helenieae, the new species did not resolve within any of the widely-recognized helenioid genera and instead was supported as the sister lineage to the woolly paper-flowers (*Psilostrophe* DC.). The woolly devil was therefore described as a new genus and species, *Ovicula biradiata*.

Attempts to conduct further field studies in 2024 were thwarted by persistent drought conditions. Deb Manley and associates monitored the known localities and searched for any additional subpopulations of *O. biradiata* during the late winter and spring months of 2025 but did not find any individuals of the species growing (D.L. Manley, *pers. comm.*). The evidence available at the time suggested that the woolly devil is an ephemeral winter annual, initiating flowering in February or early March but only in good rain years, setting seed and withering before April.

To continue studying the woolly devil, we germinated and grew four plants from seed. The main purpose for growing plants of *O. biradiata* was to obtain a meiotic chromosome count, but this activity also provided an opportunity to learn more about the natural history of the species. Here, we report observations of the woolly devil from cotyledon to capitulum, including preliminary evidence for breeding system, mechanisms of pollinator attraction using ultraviolet light, and a significant range extension based on an observation uploaded to iNaturalist (www.inaturalist.org).

MATERIALS & METHODS

Fertile cypselae were obtained from Deb Manley's 25 Apr 2024 holotypic dried plant collection of *O. biradiata* from one of the three known BIBE localities of the species. Cypselae were placed in a paper envelope inside an unsealed plastic bag and stored at ordinary refrigerator temperatures for approximately four months. Attempts to germinate *O. biradiata* from seed were initiated in mid-May 2024. Approximately 10 cypselae were planted in a plastic 7 × 10 cm pot filled to ~1.5 cm from the top with fine commercial potting medium. The potting medium was soaked from below by placing the pot



Figure 2. **A.** Cultivated *Ovicula biradiata* individual with emerging capitula. **B.** Close up of capitula. **C.** Overview of individual with pendent sprawling stems overtopping the pot. **D.** Sparse woolly indumentum silhouetted by light. **E.** Detail of pendent stems.



Figure 3. **A.** Proximal view of *Ovicula biradiata* individual photographed at the type locality. **B.** Lateral view of dense indumentum on leaves and stems. Photos by Deb Manley (A) and James Bailey (B).

in a tray filled with water. Moisture at the medium surface was maintained by occasional spraying with a fine mist, and the pot was covered by a stiff, clear plastic sheet. These initial attempts to germinate seeds failed.

Following a second failed attempt to germinate woolly devil seeds using the same technique, in mid-June 2024, we decided to apply cold stratification to overcome dormancy (Baskin & Baskin, 2000). The third attempt to propagate *O. biradiata* from seed was initiated on 31 Aug 2024. Seeds were stratified over the course of three days in a home refrigerator at approximately 4° C. This time approximately 10 fertile (filled) cypselae were planted in non-compressed, fine potting medium. Moisture in the seed environment was maintained as in earlier trials. After 7–10 days, seven seeds had germinated.

Early-stage capitular buds picked for analyses of meiotic chromosome number counts were fixed in modified Carnoy's solution (4 parts chloroform; 3 parts absolute ethanol; 1 part glacial acetic acid). Standard meiotic squash techniques as outlined in Turner and Johnston (1961) were employed in preparing slides with acetocarmine stain (carmine

powder dissolved in 45% acetic acid) and in microsporocyte observations.

Once cultivated plants were flowering, we illuminated them with ultraviolet (UV) light using a 12W portable UV light. Plants were viewed under UV light for two nights, two days apart on 20 and 22 Feb 2025, three times each night, at ca. 7:00 pm, ca. 9:30 pm, and 11:00 pm. Herbarium specimens were viewed with UV light once during the day, in a dark room. Herbarium acronyms follow Thiers (2025).

RESULTS & DISCUSSION

Observations of development

Four seedlings survived and developed into mature plants. Upon germination, each individual plant presented two ovate cotyledons approximately 0.5 cm wide with only sparse silky trichomes. One of four seedlings, "Woolly Devil One" (hereafter WDI) grew vigorously [Figure 2A–E](#), surpassing the other plants. This dominant individual produced several prostrate stems that mingled with the other plants and overtopped the pot, partially shading other

The devil appears again

In February of 2025 an observation uploaded to iNaturalist by Heather Gully revealed the first known photographs of the woolly devil (*Ovicula biradiata*) taken in April 2019 in Sanderson, Texas, a range extension of over 130 km from the type collection.

Close up shot of *Ovicula biradiata* in Sanderson, Texas
Photo by Heather Gully



Figure 4. Small capitulum of *Ovicula biradiata* revealing itself with emergence of the first ray lamina. Photo by A.M. Powell.

plants and possibly hindering their development. Within a few days, leaves and stems of the seedlings produced a partial silky indumentum of woolly trichomes, but not the dense, whitish tomentum characteristic of plants observed in the field (Figure 3A–B). The pale greenish aspect of immature foliage and lack of dense trichomes persisted throughout the life span of the cultivated plants (Figures 2–3).

On 3 Dec 2024, a tiny capitulum approximately 3 mm across appeared at the tip of one stem of WDI. Additional heads were produced rapidly, all partially obscured among woolly trichomes and by tiny leaves, towards the distal branches of WDI. The small capitula were inconspicuous until the tiny strap-like distal portion of one ray floret corolla developed (Figure 4) and extended beyond the phyllaries. Second ray florets appeared and, in some cases, a third ray floret developed. After the ray florets, a single disc corolla extended from an opening head (Figure 5A–B) exhibiting

exserted style branches covered in yellow pollen (Figure 6A–E). Both ray and disc corollas were all white in some plants, while others were white with conspicuous, maroon, parallel veins (Figure 5, 6), as in plants observed in the field by Manley et al. (2025).

Woolly devil development from seed-to-flowering took three months. WDI was most vigorous in growth (Figure 2A–B) and prolific in producing approximately 200 heads. All the heads of WDI were triradiate (Figure 5), until later stages of growth, when a few biradiate heads (Figure 2E) appeared near branch tips (the latter noted on 3 Feb 2025). Two other individual plants that reached maturity also exhibited vitality in growth, and their numerous capitula were all biradiate. One individual plant, which was outcompeted by the other three plants, remained stunted and did not produce prostrate stems.

Chromosome counts support hypothesized phylogenetic relationships

On 11 Dec 2024, several heads in an early stage of development were picked and fixed in modified Carnoy's solution in preparation for a meiotic chromosome count. The first attempt, on 12 Dec, was unsuccessful because the bud material was in advanced stages of meiosis and pollen production. Nine additional attempts to obtain a chromosome number were made using a total of 42 buds. Capitular buds with microsporocytes in diakinesis and late anaphase I of meiosis were still very small and barely recognizable as heads.

We report a chromosome number of $2n = 32$ for *O. biradiata*, based on progeny derived from seeds of the type collection (Figure 7). The $x = 16$ base chromosome number for *Ovicula* Manley is consistent with molecular phylogenetic and morphological data that supports a closer relationship between *Ovicula* and *Psilostrophe* ($x = 16$; Rice et al., 2015; Strother, 2006) than to *Tetraneuris* Greene ($x = 15$; Rice et al., 2015) or other well recognized *Tetraneurinae* genera. We have also observed that the meiotic chromosomes of *Ovicula* and *Psilostrophe* are similar in size and show a tendency for deep staining with acetocarmine.

Reproductive biology of *O. biradiata*

The cultivation of *O. biradiata* was accomplished inside southeast-facing windows of a residence where no insects could be observed visiting the plants. On



Figure 5. *Ovicula biradiata* close up of capitulum. **A.** Lateral view. **B.** Frontal view. Photos by K. Wogan on December 2024.

8 Feb 2025, mature florets were examined under a dissecting microscope. No fertile cypselae were found. Again, in March and April 2025, numerous heads were harvested and examined for fertile cypselae, but none was found. Based on the lack of observed fertile cypselae, we preliminarily conclude that *O. biradiata* is most likely a self-incompatible annual.

To investigate the function of the densely white-woolly trichomes covering *O. biradiata*, UV light was directed at mature flowering plants. Plant tissues covered in trichomes appeared to absorb UV light, while the ray florets of both bi- and triradiate heads fluoresced (see full page insert on [page 8](#)). Some of the small, strap-like corollas, with limbs measuring $3\text{--}6 \times 0.6\text{--}1$ mm, were fluorescent from the proximal to the distal ends, while some only fluoresced in the distal half or less. Fluorescence in herbarium specimens of *O. biradiata* was essentially the same as in living plants, with trichomes appearing to absorb UV light while the ray florets fluoresced.

In April 2025, the vigorous, carefully nurtured cultivated plants of *O. biradiata* began to show signs of senescence.

Material was harvested from WDI, pressed, dried, and mounted as a chromosome-number voucher specimen. By mid-April senescence was evident in all four of the cultivated plants. At this time a considerable amount of the plant material was harvested and placed in RNAlater (Thermo Fisher Scientific) for use in whole-genome sequencing. We observed that the timing of senescence in our cultivated plants closely matched the life history of the species in the field, which we view as support for the conclusion that *O. biradiata* is an ephemeral winter annual.

A range extension for *O. biradiata*

An observation uploaded in February of 2025 to iNaturalist (Gully, 2025) includes photos of *O. biradiata* from April of 2019 growing with other common spring annual plants in the low Chihuahuan Desert, such as *Astragalus nuttallianus* DC., *Plantago ovata* Forssk., and *Scutellaria drummondii* Benth. The photographed plants were low annuals with numerous, spreading prostrate stems, resembling the same growth form as that observed in cultivated *O. biradiata*. The substrate on which the plants were growing appears to be calcareous gravel, the same substrate in which plants

Beyond visible, the woolly devil's spectral signature

Under UV light the dense woolly trichomes of this plant's leaves and stems absorb light while the ray florets reflect it, giving the capitula a conspicuous, spooky appearance.

Ovicula biradiata under UV light in the basement of the Sul Ross State University Herbarium
Photo by Kelsey Wogan



Figure 6. Developmental stages in *Ovicula biradiata* capitula. **A.** Prior to emergence of the style. **B.** Style emergence. **C.** Lateral view of secondary pollen presentation on unopened styles. **D.** Frontal view showing a mixture of receptive and pre-receptive disk florets with exposed stigmatic surfaces. Photos by A.M. Powell.

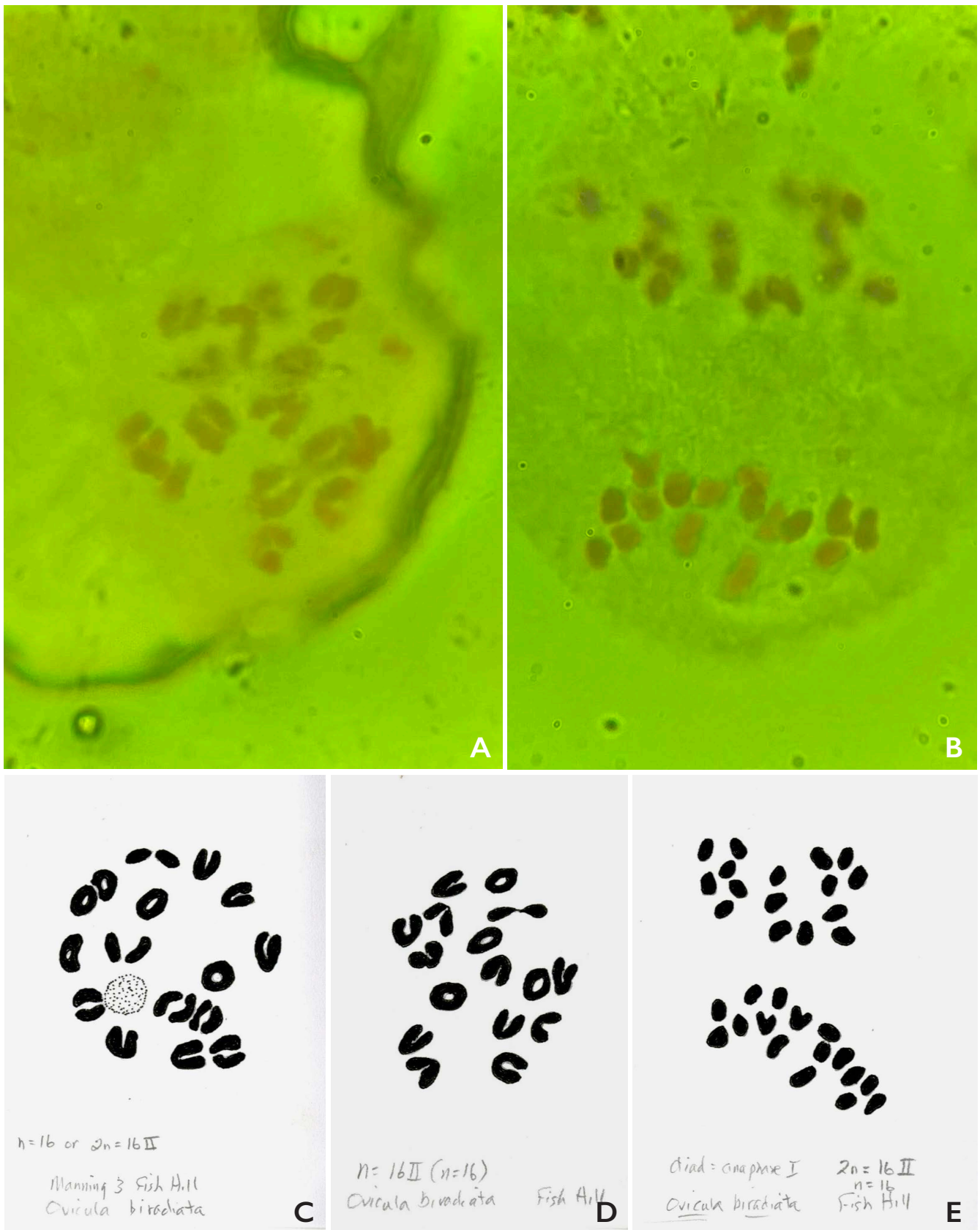


Figure 7. Meiotic chromosomes of *Ovicula biradiata*. **A.** Diakinesis. **B.** Anaphase. **C-E.** Free-hand drawings of chromosomal configurations. Photos and illustrations by A.M. Powell.

Ephemeral green

In a good year, the view from "the Window", a lookout in the Chisos Mountains of West Texas, stretches over the Chihuahuan Desert in a lush, inviting sweep of green. In a dry year, the same landscape turns harsh and unforgiving, the vegetation brown and brittle beneath the sun.

*Chisos Mountains, Texas
Photo by Isaac Lichter Marck*

had been observed growing at the type locality. In one photo a small fly (Diptera) is visible perched on the apex of one of the long ray florets. A fourth photo (Gully, 2025) was added in March 2025 to show an ant (Formicidae) crawling on top of the prostrate stems.

After speaking with Heather Gully by phone, it was confirmed that the observations were made on a private ranch near the town of Sanderson, in western Texas. By measuring the distance between the type locality and the town of Sanderson using Google Earth, it was determined that these new observations extend the range of the woolly devil by ca. 130 kilometers. Given extraordinary drought conditions that persisted through spring of 2025, it has so far been impossible to visit Sanderson to make herbarium specimens and study plants in detail at this new locality.

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LITERATURE CITED

- Baldwin, B. G., Wessa, B. L. & Panero, J. L.** 2002. Nuclear rDNA evidence for major lineages of helenioid Heliantheae (Compositae). *Syst. Bot.* 27: 161–198.
- Baskin, C. C. & Baskin, J. M.** 2000. Seeds: ecology, biogeography, and, evolution of dormancy and germination. Academic press.
- Gully, H. 2025. iNaturalist observation: <https://www.inaturalist.org/observations/263307985>. (accessed 1 Set 2025).
- Manley, D. L., Lichter Marck, I. H., Peralta, K., Castro, A. C., Wogan, K. A., Whiting, C. V. & Powell, A. M.** 2025. *Ovicula biradiata*, a new genus of Compositae from Big Bend National Park in Trans-Pecos Texas. *PhytoKeys* 252: 141–162.
- Rice A., Glick, L., Abadi, S., Einhorn, M., Kopelman, N. M., Salman-Minkov, A., Mayzel, J., Chay, O. & Mayrose, I.** 2015. The Chromosome Counts Database (CCDB)—a community resource of chromosome numbers. *New Phytol.* 206: 19–26.
- Strother, J. L.** 2006. *Psilostrophe*. Flora of North America north of Mexico. Vol. 21. Pp. 453–455. Oxford University Press, New York.
- Thiers, B. M.** 2025 (updated continuously). Index Herbariorum, New York Botanical Garden, New York U.S.A. Available from: <https://sweetgum.nybg.org/science/ih/> (accessed 5 May 2025)
- Turner, B. L. & Johnston, M. C.** 1961. Chromosome numbers in Compositae III. Certain Mexican species. *Brittonia* 13: 64–69.

Clear heads: simplified tissue preparation for studying plant anatomy and development in the Asteraceae

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ABSTRACT

The use of microscopy is instrumental for our understanding of plant anatomy and development. A major bottleneck is that conventional methods for plant tissue preparation can take weeks to complete and can involve hazardous materials that many labs are not equipped to handle. Furthermore, these methods traditionally involve embedding the fixed tissue in embedding substrates such as paraffin or plastic resins, which is both a laborious and time-intensive process. Here we describe a simplified and accessible method that accomplishes tissue fixation and clearing in just three days, minimizes the use of hazardous materials, and eliminates the need for embedding/sectioning. This method also utilizes structural autofluorescence of plant tissues and thus does not require any counterstaining to resolve structure. We demonstrate the utility of this preparation using both epifluorescence and confocal microscopy on examples from the Asteraceae family, making both techniques more approachable to biologists of all backgrounds and thus enabling a richer exploration of plant anatomy and development.

Keywords: anatomy, confocal, development, fluorescence microscopy, morphological diversity, tissue clearing.

INTRODUCTION

Light microscopy is an invaluable tool in studying plant anatomy and development. Specifically, the use of fluorescent microscopy has become an essential part of most plant cell, molecular and developmental biology laboratories' toolkits, complementing the vast number of molecular cloning tools that have come about in the last 30 years (i.e., fluorescent proteins). Most of these applications require transformation as well as live imaging of tissues to visualize fluorescent markers, limiting their utility largely to model systems.

This prevalence of fluorescent microscopy does mean, however, that some form of epifluorescence or confocal microscopy is increasingly available. To further facilitate the accessibility of fluorescence microscopy, here we describe a method of plant tissue preparation that is compatible with most sample types and approachable for researchers who are new to microscopy.

A key advantage of this protocol is chemical safety. While eco-friendly and low-risk methods of tissue clearing are growing in popularity, many still involve



Morphology begins with development

Flowering plants display remarkable morphological diversity, which is well-exemplified by Asteraceae capitula. Understanding diversity of form begins with understanding diversity of development.

*Lateral view of developed head of *Tagetes erecta* 'Antigua Orange'*
Photo by Reid Selby

harsh chemicals like chloral hydrate and xylenes. This protocol uses methyl salicylate, a common food flavoring agent that smells of mint or wintergreen, making it another useful alternative to more hazardous reagents. The tissue clearing properties of methyl salicylate were first described in an 1866 study comparing clearing agents with high refractive indices (Stieda, 1866). Because the refractive index of methyl salicylate (1.53) is close to glass (1.55), light passing through one medium into the other undergoes minimal distortion (limited diffraction). Methyl salicylate has since been well-described as an effective clearing agent in a variety of tissues (Hériché et al., 2022). In addition to using harsh chemicals, common methods used to prepare tissues are time consuming and can require specialized equipment (e.g., tissue embedding stations). Tissue preparation is still a major bottleneck for biologists working in diverse, non-model plant systems, especially when imaging larger structures at high resolution, which typically require experience and time to create quality sections. This technique is fast, approachable, and requires minimal equipment. To showcase the versatility of this method across sample types, we imaged a variety of tissues from multiple Asteraceae species. This method is particularly effective for imaging stages spanning capitulum development, demonstrating its utility in preparing large samples for detailed imaging. While methyl salicylate is not the only safe method of tissue clearing, it should be strongly considered by both experienced microscopists looking for a fast and easy alternative to other methods, and researchers new to microscopy looking for an approachable technique.

PROTOCOL

This protocol can be broadly categorized into four fundamental steps: dissection, fixation, clearing, and imaging (Figure 1).

Dissection:

While the rest of this protocol can be applied to most tissue types with limited modifications, dissection techniques will vary according to your tissue of interest. For many samples, only a single cut is necessary to both expose the structure for imaging and to allow for the proper penetration of fixing and clearing agents. A clean, flat surface

in the same plane as the region being imaged is necessary for the best quality image. A single stroke of a razor blade with minimal downward force is best, relying on slicing rather than chopping. It is worth noting that even the most careful cuts can result in some damage; however, sufficiently cleared tissue enables imaging on a focal plane a few cell layers in, bypassing damage from the cut site. For all images of developing capitula in this paper, we made a single longitudinal cut down the center of the bud with a fresh razor blade. If the structure being imaged is external or near external, such as leaf stomata or root tips, it is possible that limited or no dissection is required. For thicker samples, some additional sectioning may be necessary to allow the fixative and methyl salicylate to properly penetrate the tissue.

Fixation and dehydration:

Immediately after dissecting, place samples in ice cold FAA (2% (w/v) formaldehyde, 5% (v/v) acetic acid, 60% (v/v) ethanol) to fix at 4°C for ~24 hours while rocking—ideally with a nutating mixer, but any form of gentle agitation is suitable. While many fixatives can work with this protocol, we find that using FAA with 2% formaldehyde gently fixes the tissue and preserves cellular integrity well (Johansen, 1940; Jackson et al., 1994). Moreover, using a relatively low starting percentage of ethanol is sufficient for fixation, prevents excessive tissue hardening, and allows for a gentle transition to 70% ethanol in the next step. The day after fixation begins, decant the FAA, and add cold 70% ethanol for at least 30 minutes. Fixed tissue can then be stored in 70% ethanol for several months before continuing to the next step. Storing fixed samples at 4°C may further increase longevity. To prepare tissues for clearing, serially dehydrate them by replacing the 70% ethanol with 80%, then 90%, 95%, and finally 100% twice. Wait 30 minutes between each step of the dehydration. Make sure to use sufficient volumes of FAA and ethanol, such that the sample is never greater than 10% of the total volume. While fixation and dehydration are possible at room temperature, we find that using chilled FAA and ethanol slows dehydration as well as plasmolysis, preventing cellular distortion.

Clearing and imaging:

Once the tissue is fully dehydrated, decant the ethanol and replace it with 100% methyl salicylate. Allow the methyl salicylate to clear the tissue for

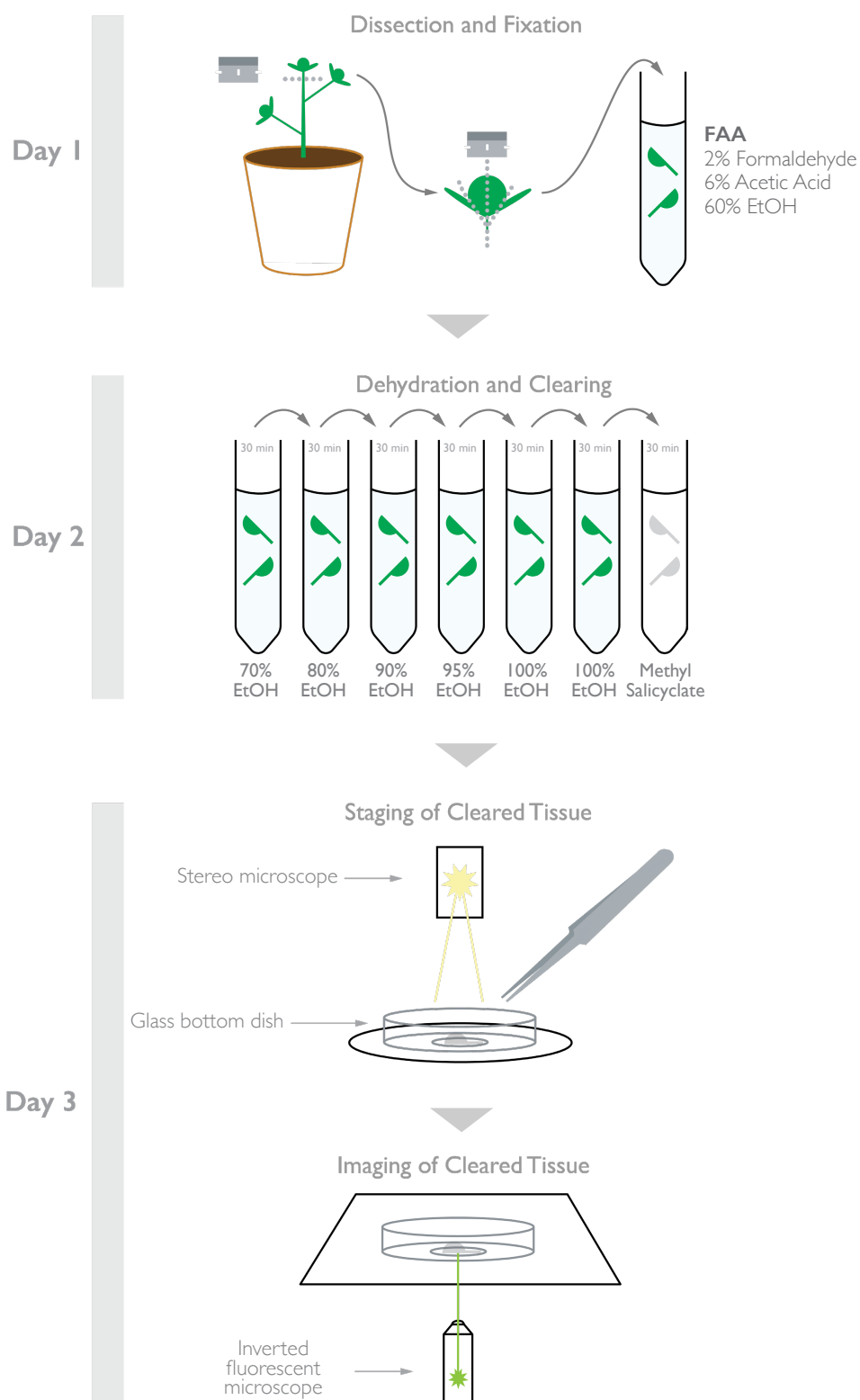


Figure 1. A workflow for imaging with methyl salicylate. This example of fixing and clearing tissue with methyl salicylate is spread across three days. Day 1, the tissue is dissected and fixed overnight in FAA (2% formaldehyde, 5% acetic acid, 60% ethanol). Day 2 the tissue is serially dehydrated by placing in 70% ethanol for 30 minutes, 80% ethanol for 30 minutes, 90% ethanol for 30 minutes, 95% ethanol for 30 minutes, and 100% ethanol twice for 30 minutes each. The tissue is then placed in methyl salicylate overnight. Day 3 involves staging the cleared tissue on a stereo microscope before imaging on an inverted fluorescent microscope.

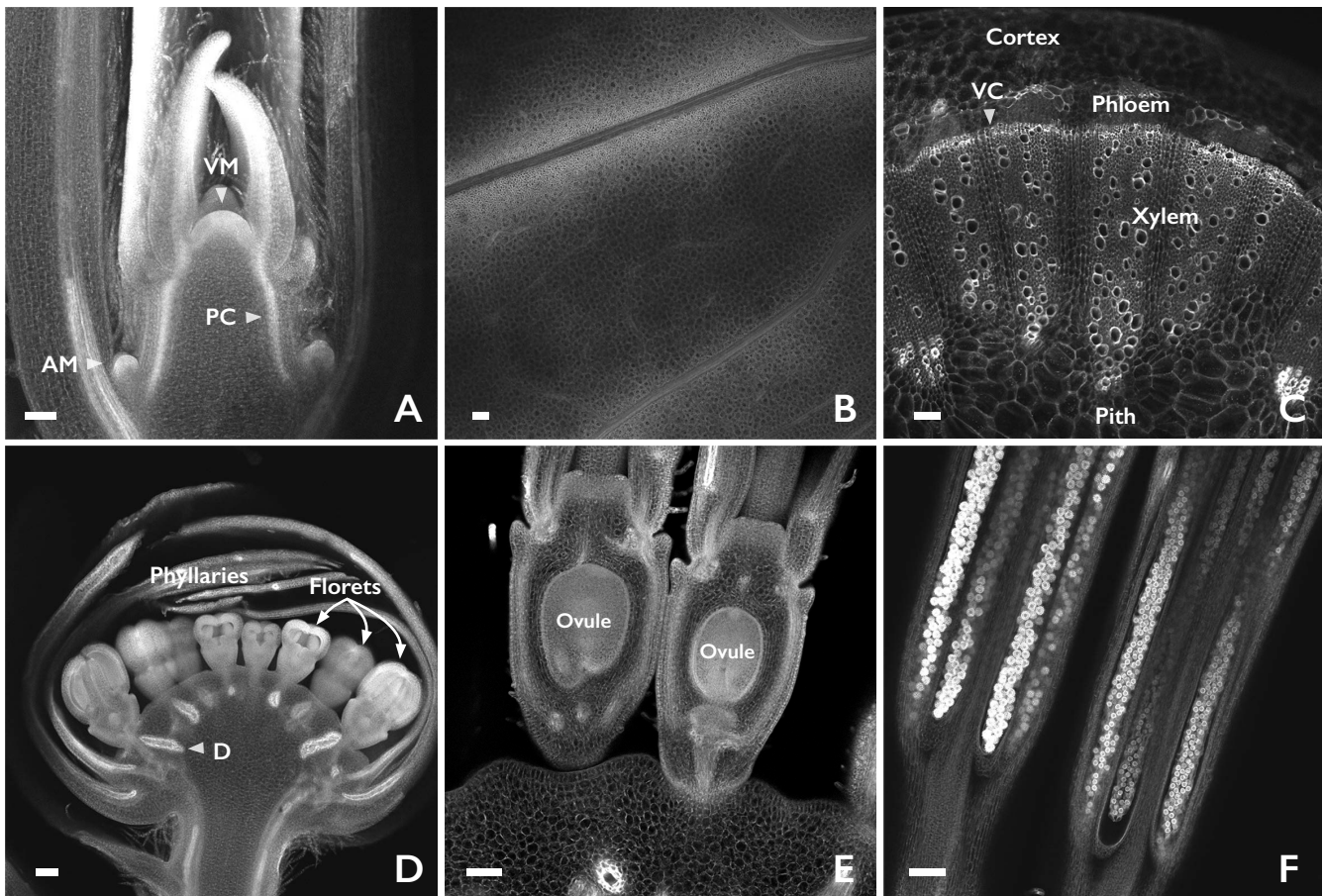


Figure 2. A showcase of tissues from *Jamesianthus alabamensis*. Images were captured on a Nikon A1 CLSM. **A.** A shoot apex with vegetative apical meristem (VM), axillary meristems (AM), and procambium (PC). **B.** An undissected leaf with mesophyll, a major vein, and minor veins. **C.** A cross-section of stem tissue with substantial secondary growth showing cortex, phloem, vascular cambium (VC), xylem, and pith. **D.** A developing capitulum with florets at various stages of floral organ formation, each with developing pappus, corolla, stamens, gynoecia, and ovules; phyllaries surround the developing florets and extracellular ducts (D) are present within the receptacle. **E.** A pair of developing florets with nearly mature ovules. **F.** Anthers containing pollen, just prior to anthesis. Scale bars all represent 100 μ m.

at least 24 hours. Samples can be stored in methyl salicylate indefinitely, and many tissues become clearer with time; however, samples stored in methyl salicylate are more fragile than those stored in 70% ethanol. Storing cleared samples at 4°C may increase longevity. When tissues are fully cleared, simply mount samples in methyl salicylate and image. A major advantage of this protocol is that samples can be prepared with minimal dissection, which typically means the only flat surface is often the cut face to be imaged. Therefore, we recommend using a glass bottom petri dish on an inverted microscope, with the cut surface facing downward. To help prevent air bubbles from distorting the image, place a small drop of methyl salicylate on the slide before mounting the sample. The images in this article

were captured on either a Nikon A1 confocal laser scanning microscope (CLSM) or an Echo Revolve, then processed in Fiji or ImageJ (Schindelin et al., 2012; Schneider et al., 2021) (see “Considerations in Imaging Method” for details).

METHYL SALICYLATE IS A BROADLY EFFECTIVE CLEARING AGENT

Diverse Tissues:

One of the largest benefits of methyl salicylate as a clearing agent is its ability to clear most tissues. Here we use *Jamesianthus alabamensis* S.F.Blake & Sherff, a rare

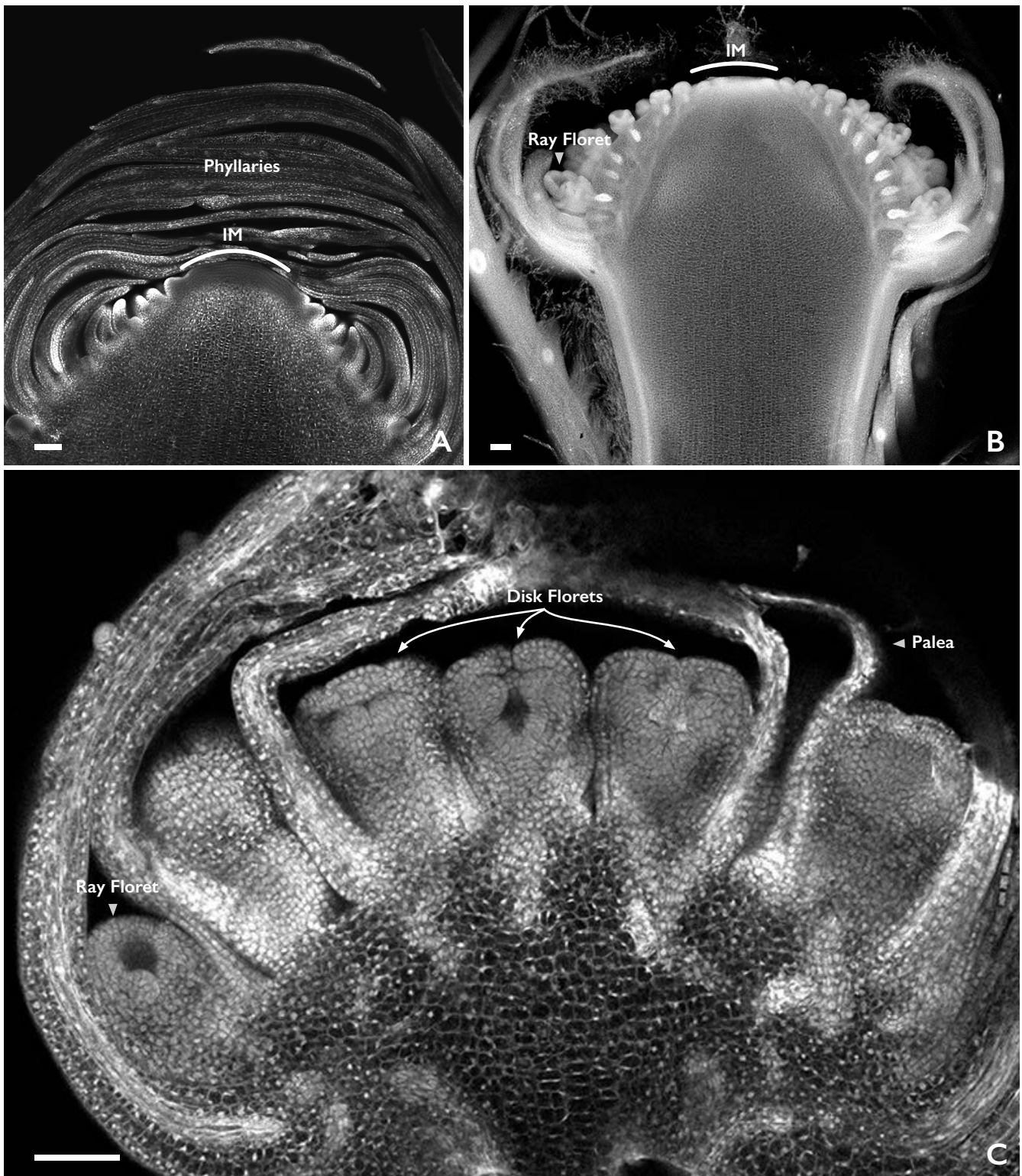
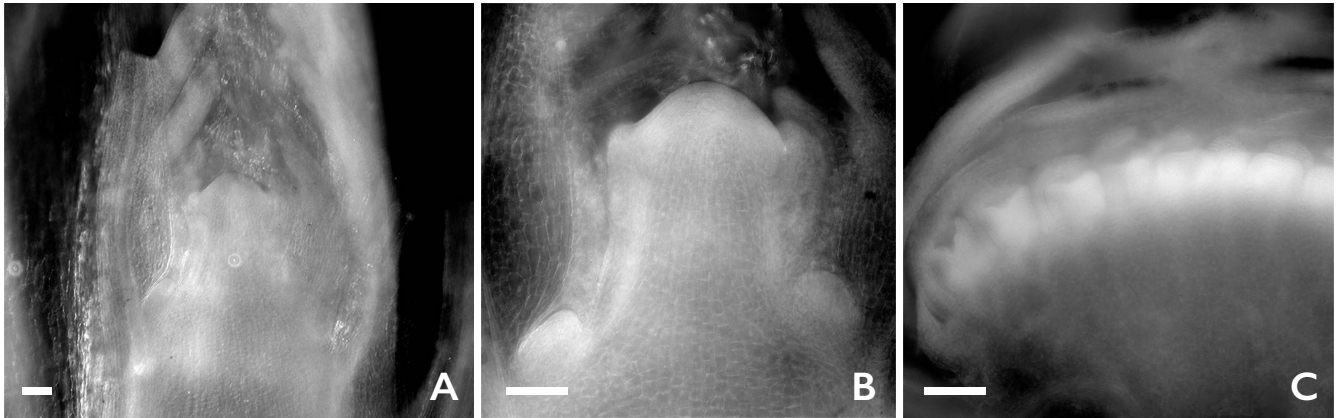


Figure 3. Developing capitula in a variety of species. Images were captured on a Nikon A1 CLSM. **A.** *Dahlia* 'Edna C' with conspicuous phyllaries and unconsumed inflorescence meristem (IM). **B.** *Tagetes erecta* with acropetally maturing florets ranging from visibly zygomorphic ray florets to unconsumed inflorescence meristem (IM). **C.** *Bidens forbesii* already showing distinct corolla morphologies between the zygomorphic ray florets and actinomorphic disk florets, as well as substantial paleae. Scale bars all represent 100 μm.

EPIFLUORESCENCE



CONFOCAL

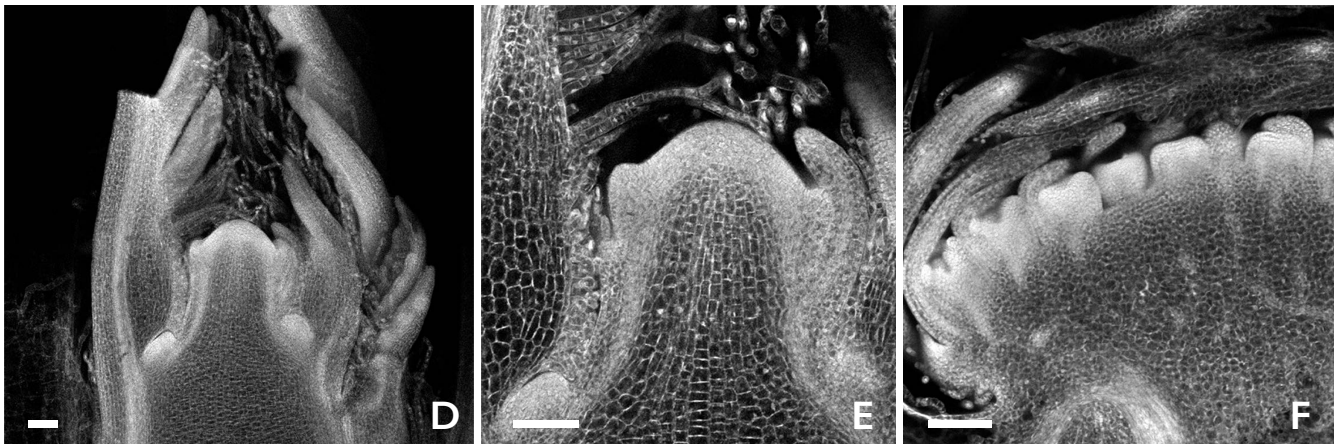


Figure 4. Confocal vs epifluorescence microscopy. Confocal images taken on a Nikon AI CLSM, and epifluorescence images taken on an Echo Revolve. **A-B.** *Tagetes erecta* vegetative meristem imaged on an epifluorescent microscope. **C.** A developing capitulum from *Bidens* 'Compact Yellow' imaged using epifluorescence **D-E.** *Tagetes erecta* 'Antigua Orange' vegetative meristem imaged on a confocal microscope. **F.** A developing capitulum from *Bidens* 'Compact Yellow' imaged using the confocal microscope. A and D were captured with a 4x objective. B, C, E, F captured with 10x objective. Scale bars all represent 100 μ m.

species endemic to freshwater streams and riverbanks in northern Alabama and Georgia, to demonstrate the variety of tissues that can be imaged. Although we focus on the population dynamics and conservation of *Jamesianthus alabamensis*, the simplicity of methyl salicylate clearing enables a thorough exploration of this rare plant's anatomy and development (Figure 2). The shoot apex with leaf primordia, vegetative shoot meristem, and developing procambial strands (Figure 2A), leaf anatomy (Figure 2B), secondary growth in the stem (Figure 2C), a developing capitulum soon after all floral whorls have been specified (Figure 2D), developing ovules (Figure 2E), and anthers before anthesis (Figure 2F).

Diverse Species:

Methyl salicylate is also effective across diverse species; methods have been described for *Oryza sativa* L., *Arabidopsis thaliana* (L.) Heynh., *Beta vulgaris* L., and more (Zeng et al., 2007; Rodriguez-Leal et al., 2019; Kwiatkowska et al., 2019; Jones et al., 2021; John et al., 2023). In addition to *Jamesianthus alabamensis*, we captured micrographs of developing capitula from multiple additional species (Figure 3). These include two ornamentals; *Dahlia variabilis* (Willd.) Desf. 'Edna C' (Figure 3A), which even while developing can reach almost 4 cm in diameter, and *Tagetes erecta* L. 'Antigua Orange' (Figure 3B), and a capitulum from

Development made clear

While morphological diversity is most obvious in mature structures and organs, variation in form begins at the earliest stages of development. A vegetative shoot apical meristem and developing leaves of *Bidens* 'Compact Yellow' illustrate this well. By coupling this approachable tissue-clearing technique with fluorescence microscopy, we can easily capture the details of meristem and organ primordia morphology.

Image of Bidens 'Compact Yellow' viewed in two channels (FITC in green and TRITC in red), highlighting the distinct fluorescent properties of naturally occurring compounds. Photo by Daniel S. Jones

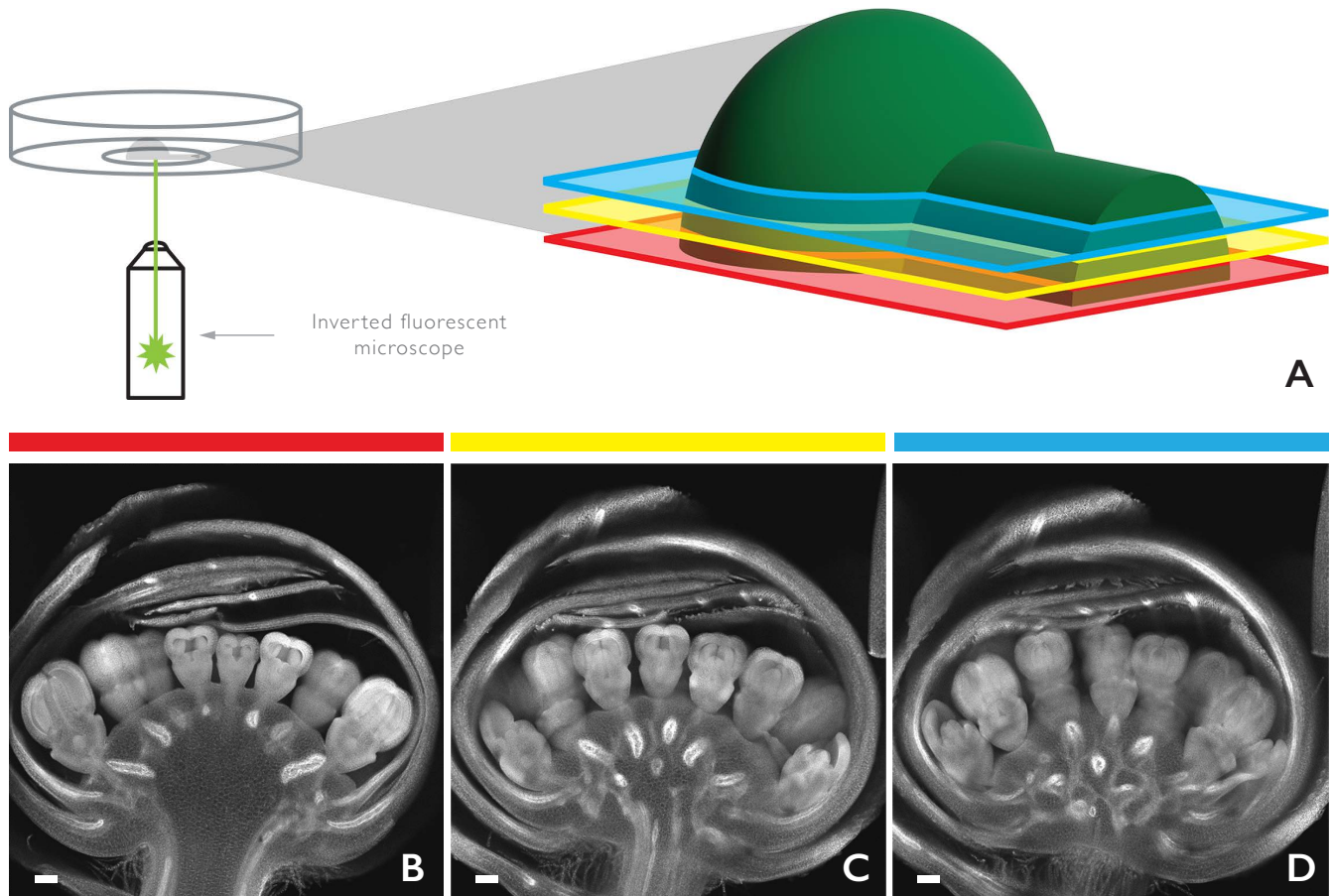


Figure 5. Optical sectioning of *Jamesianthus* ducts. Images were captured on a Nikon A1 CLSM. **A.** An illustration of the optical sections represented in images B-D; colors correspond to relative depth of focus. **B.** An image captured from a focal plane immediately adjacent to the initial physical section. **C-D.** Images captured from focal planes progressively deeper into the cleared capitulum. Scale bars all represent 100 μm .

Bidens forbesii Sherff (Figure 3C), a species endemic to the Hawaiian Islands. The difference in size across all three capitula may cause concerns for image acquisition and quality, especially for the large *Dahlia* Cav. samples. However, we show that even in *Dahlia* (Figure 3A), well-resolved images can be obtained with minimal dissection. Images of *Dahlia variabilis* 'Edna C' and *Tagetes erecta* 'Antigua Orange' (Figure 3A–B) illustrate the broad range of developmental stages that may coexist within a single capitulum. While the center may have unconsumed inflorescence meristem that has not formed any floral primordia yet, floral primordia at the periphery may be well into the development of all floral whorls. This enables a unique snapshot of the progression of floral ontogeny in a single image. In contrast,

Bidens forbesii (Figure 3C) exhibits a more synchronized developmental pattern, with florets progressing within a similar temporal window. Methyl salicylate clearing enables these rapid, high-contrast comparisons among species and across developmental time points.

CONSIDERATIONS IN IMAGING METHOD

Confocal vs Epifluorescence:

Due to point-scanning and a pinhole aperture blocking out of focus light from detectors (Elliot, 2020), confocal microscopy will always provide more resolution than standard epifluorescence microscopy.

However, epifluorescence is sufficient for many research questions and is typically more accessible and less expensive than confocal microscopy. All images prior to this section used a confocal microscope, but methyl salicylate clearing is suitable for both modes of imaging. To compare both methods, we imaged the same samples using epifluorescence (Echo Revolve) and confocal (Nikon AI CLSM) microscopes. The same vegetative meristem from *Tagetes patula* L. and the developing capitulum of *Bidens* 'Compact Yellow' (Figure 4A–F) are shown, illustrating not only the increased resolution and contrast achieved through confocal microscopy but also the level of analysis possible using epifluorescence—even with minimal sample preparation.

Optical Sectioning:

The ability to restrict incoming light to a small plane of focus via the pinhole aperture enables a technique in confocal microscopy known as optical sectioning. When dissecting samples, it is nearly impossible to make a cut that is precisely in the same plane you wish to image. Additionally, imaging the same tissue at various depths can provide valuable insight into its three-dimensional structure. Cutting the sample into multiple thin layers (sectioning) addresses both these points and is often achieved by methods such as paraffin embedding, enabling researchers to view multiple slices of the same sample. Thin sectioning can be resource- and time-intensive and reduces the three-dimensional context of structures by physically separating them. Clearing allows optical sections to be taken by adjusting the focal plane of the microscope. The cleared tissue minimizes light diffraction and allows for deeper light penetration. This, combined with the capability of confocal microscopes to omit signal from out-of-focus regions, allows for clear micrographs to be captured from a range of focal planes (Conchello & Lichtman, 2005). Therefore, imaging is not restricted to regions immediately adjacent to the initial cut site. Here we demonstrate this technique by imaging the ducts found within the receptacle of a *Jamesianthus alabamensis* capitulum (Figure 5). While ducts are visible in the plane of focus adjacent to the initial cut site (Figure 5B), the three-dimensional organization of these ducts becomes more apparent when viewed in multiple planes of focus progressing deeper into the tissue (Figure 5C–D).

Leveraging Autofluorescence:

This technique does not require any staining or labeling. Instead, it utilizes the abundance of naturally fluorescent compounds that plants produce. While this autofluorescence can create problems when imaging fluorescent stains or proteins like GFP, it can also be leveraged for stain-free structural analysis. Because FAA is a potent solvent, many autofluorescent molecules (i.e., pigments) will be washed out prior to clearing. However, there is still a considerable amount of autofluorescent material after clearing and fixing, especially in cell walls. There are many different sources of autofluorescence, and each molecule has different excitation and emission wavelengths (Donaldson, 2020). Therefore, it can be difficult to predict the most useful channel or filter to use when imaging with this method. While channels for fluorescein isothiocyanate (FITC) or green fluorescent protein (GFP) often give the best signal; however, this varies between samples. It is best to experiment with different channels or filters to find the optimum for a given sample. It is also possible to combine multiple channels. All the images in this article were captured using a combination of FITC (ex: 488 nm and em: 520 nm) and tetramethylrhodamine isothiocyanate (TRITC) fluorescent filters (ex: 550 nm and em: 580 nm). The levels of each channel were then adjusted and merged using Fiji (ImageJ) to produce a single balanced image. Although colored look-up tables (LUTs) can aid in visualizing multichannel images, grayscale presentation is preferable for structural analysis.

CONCLUSIONS

This simplified method offers an accessible, fast, and safe alternative to conventional tissue clearing techniques. While fluorescent counterstains remain necessary for some applications, leveraging autofluorescence from plant cell walls provides an in-depth look at developing structures with minimal treatment post-fixation. While this method is especially suitable for imaging capitula, methyl salicylate's ability to clear a variety of tissues makes this method broadly applicable. Additionally, the increasing availability of fluorescence microscopy necessitates accessible protocols. Our hope is that this method will lower the barrier to entry for fluorescence microscopy and open avenues of research previously unconsidered by those studying the Asteraceae and beyond.

ACKNOWLEDGEMENTS

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LITERATURE CITED

- Conchello, J.-A. & Lichtman, J. W.** 2005. Optical sectioning microscopy. *Nat. Methods* 2: 920–931.
- Donaldson, L.** 2020. Autofluorescence in plants. *Molecules* 25: 2393.
- Elliott, A. D.** 2020. Confocal Microscopy: Principles and Modern Practices. *Curr. Protoc. Cytom.* 92: e68.
- Hériché, M., Arnould, C., Wipf, D. & Courty, P.-E.** 2022. Imaging plant tissues: advances and promising clearing practices. *Trends in Plant Sci.* 27: 601–615.
- Jackson, D., Veit, B. & Hake, S.** 1994. Expression of maize KNOTTED1 related homeobox genes in the shoot apical meristem predicts patterns of morphogenesis in the vegetative shoot. *Development* 120: 405–413.
- Johansen, D. A.** 1940. Plant microtechnique. New York: McGraw-Hill.
- John, A., Smith, E. S., Jones, D. S., Soyars, C. L. & Nimchuk, Z. L.** 2023. A network of CLAVATA receptors buffers auxin-dependent meristem maintenance. *Nat. Plants* 9: 1306–1317.
- Jones, D. S., John, A., VanDerMolen, K. R. & Nimchuk, Z. L.** 2021. CLAVATA Signaling Ensures Reproductive Development in Plants across Thermal Environments. *Curr. Biol.* 31: 220–227.e5.
- Kwiatkowska, M., Kadłuczka, D., Wędzony, M., Dedicova, B. & Grzebelus, E.** 2019. Refinement of a clearing protocol to study crassinucellate ovules of the sugar beet (*Beta vulgaris* L., *Amaranthaceae*). *Plant Methods* 15: 71.
- Rodriguez-Leal, D., Xu, C., Kwon, C.-T., Soyars, C., Demesa-Arevalo, E., Man, J., Liu, L., Lemmon, Z. H., Jones, D. S., Van Eck, J., Jackson, D. P., Bartlett, M. E., Nimchuk, Z. L., & Lippman, Z. B.** 2019. Evolution of buffering in a genetic circuit controlling plant stem cell proliferation. *Nat. Genet.* 51: 786–792.
- Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, S., Rueden, C., Saalfeld, S., Schmid, B., Tinevez, J.-Y., White, D. J., Hartenstein, V., Eliceiri, K., Tomancak, P., & Cardona, A.** 2012. Fiji: an open-source platform for biological-image analysis. *Nat. Methods* 9: 676–682.
- Schneider, C. A., Rasband, W. S. & Eliceiri, K. W.** 2012. NIH Image to ImageJ: 25 years of image analysis. *Nat. Methods* 9: 671–675.
- Stieda, L.** 1866. Ueber die Anwendung des Kreosots bei Anfertigung mikroskopischer Präparate. *Arch. Mikrosk. Anat* 2: 430–435.
- Zeng, Y. X., Hu, C. Y., Lu, Y. G., Li, J. Q. & Liu, X. D.** 2007. Diversity of abnormal embryo sacs in indica/japonica hybrids in rice demonstrated by confocal microscopy of ovaries. *Plant Breed.* 126: 574–580.

Hecastocleis shockleyi (Hecastocleideae), the desert enigma: close encounters with one of Compositae's most solitary lineages

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ABSTRACT

The desert subshrub *Hecastocleis shockleyi* is the sole member of tribe Hecastocleideae and sub-family Hecastocleidoideae, a species that raises questions about the biogeographic history of the Compositae. It features a unique floral morphology, with single-flowered capitula aggregated together in a terminal inflorescence that is subtended by spiny, papery bracts. Additionally, its corolla and style branches resemble those of basal members of the family. Molecular phylogenetic data support *H. shockleyi* as an early diverging lineage sister to all other members of the large radiation of subfamilies Carduoideae and Asteroideae, yet it is found only in the southwest U.S., far from other early diverging lineages found in South America. In spite of this mysterious biogeography, which links the family's early radiations to its eventual global spread, little is known about the ecology and genetic diversity of the species. This report briefly summarizes three field trips in 2023, 2024, and 2025 that aimed to address this gap in our understanding of this charismatic and important Compositae.

Keywords: Asteraceae, biogeography, dispersal, Great Basin, Mojave, natural history, phenology, pollination.

INTRODUCTION

Few Compositae stand out to synantherologists as much as the desert subshrub *Hecastocleis shockleyi* A. Gray (Hecastocleideae), lone member of sub-family Hecastocleidoideae. Described in the late 1800s (Gray, 1881), *Hecastocleis* bears a name aptly derived from the Greek *hekastos* ("each") and *kleis* ("shut up"), pointing to the plant's unique floral structure (Williams, 1977). Each capitulum of *H. shockleyi* features just a single floret subtended by a multiseriate involucre, but 3–5 of these capitula are aggregated together at the end of stems and enveloped by 4–5 ovate to orbiculate bracts with spiny margins that turn from light green to white

during flowering. The specific epithet commemorates a prolific, early collector of Nevada's flora, William H. Shockley (1855–1925).

The morphology of *H. shockleyi* is even more striking in relation to its biogeography. It shares many characteristics with other early-divergent lineages of the family. For example, its actinomorphic corolla features five, deeply dissected lobes that reflex at ninety degrees, and its style features reduced branches with a continuous stigmatic surface, both common characters of the Barnadesieae. Yet, unlike those early-divergent lineages it is restricted to western North America, far from the family's South American origins. In the dated phylogeny of Mandel

Refuge on the ridge

Hecastocleis shockleyi establishes on exposed limestone ridges and gravel slopes of the Mojave Desert. The reason why *H. shockleyi* is only found on these xeric, nutrient poor substrates remains unknown.

Spring Mountains, Nevada
Photo by Colin Hoag

et al. (2019), *Hecastocleis* diverges from the rest of the family in the Eocene, approximately 50MYA, and occupies a position between the South American lineages and the remaining members of the family. In this way, *Hecastocleis* represents a crucial link between the family's early radiations and its eventual global spread. As Funk and Hind (2009) point out, this suggests one of three possibilities: 1) after arriving in North America, it left by long-distance dispersal to Africa, perhaps via Europe or Asia; 2) it was part of a larger radiation, of which it is the sole remaining member; or 3) the genetic evidence is incorrect. The latter seems unlikely, as molecular phylogenetic analyses to date consistently support the placement of *Hecastocleis* with high confidence (Mandel et al., 2019).

This story has captured the synantherological imagination for generations. Gray (1881) considered its discovery "a remarkable addition to the few known North American [Mutisieae], to stand near *Ainsliea*, but altogether *sui generis* and of peculiar habit." More recently, Funk and Hind (2009) also explain: "Very little is known about the biology of *Hecastocleis*; no pollinators were seen during visits to the Red Pass/Titus Canyon populations [in Death Valley National Park]. Since the florets and bracts are whitish, perhaps they attract night visitors. Likewise, there is no information on the ecology or ethnobotany." Indeed, 15 years since their writing, my literature search turned up only scattered mentions about its habitat preferences, ecology, and distribution (Williams, 1977; Ackerman, 2003; Funk and Hind, 2009; Ward & Bittman, 2024). I learned that it prefers rocky, gravelly soils on steep slopes between 1280-2,200masl, typically on a limestone or shale substrate. From digitized herbarium specimens, I learned that it occupies the Mojave and Great Basin Deserts in scrub and chaparral plant communities. Although long considered a species of conservation concern, recent field research has revealed that *H. shockleyi* is far more widespread than previously assumed (Ward & Bittman, 2024). Some of these populations feature thousands of individuals, and because it prefers such inaccessible habitats we can be confident that additional populations are yet to be found.

As an ecologist captivated by this historical biogeographic story sketched by researchers like Funk and Hind—and *magno amore in familiam*

Synantherearum captus—I needed to investigate further. What explains its patchy distribution? How is it pollinated and dispersed? How is it structured on the landscape? How are its populations, which occur often in discrete, disjunct, and difficult to access sites, related to one another? Where is its genetic diversity concentrated? And how might answers to those questions inform our interpretation of its longer-term geographical movements?

With the generous guidance of Mauricio Bonifacio and Jennifer Mandel, I set out in the late spring of 2023 to examine its genetic diversity, and collect leaf material from populations across the plant's range in Nevada and California. In the early summers of 2024 and 2025, I returned to focus entirely on one site in the Spring Mountains of Nevada, making natural history observations and mapping the structure of a single population. The following briefly describes my encounters with this famously enigmatic comp.

MAY 2023: A TOUR OF ITS RANGE

Hecastocleis shockleyi is often associated with Death Valley, but this is misleading. It occurs in Death Valley, and the association certainly gives a sense of its extreme habitat preferences, but in fact the plant lives quite widely across the Great Basin and Mojave Deserts. Thanks to digitized herbarium specimens deposited by researchers over decades, I was able to locate nine populations across its range in California and Nevada.

The first was just outside Las Vegas in the stunning Spring Mountains National Recreation Area, part of the Humboldt-Toiyabe National Forest. My visit was a rude awakening about the difficulties of *Hecastocleis* research—and one answer to my question about why so little is known about its ecology. The site was located seven miles off Highway 160, a full hour's drive down a gravelly 4x4 double-track that traversed Mojave Desert washes full of large rocks and flanked by sprawling creosote bushes (*Larrea tridentata* (DC.) Cov., Zygophyllaceae) and Joshua trees (*Yucca brevifolia* Engelm., Agavaceae).

From the pull-out where I parked my rented truck, the population was a short but arduous hike up



In Search of *Hecastocleis*

Across the Mojave and Great Basin, purple dots mark studied populations of one of *Compositae*'s most solitary lineages. These locations traced the narrow range of *Hecastocleis shockleyi* — from sun-baked bajadas to wind-cut ridges, a journey through extraordinary desert landscapes.



Where the land and the plants are one

Water is fundamental for plant life, yet on these steep, layered slopes of calcareous rocks where soil depth and moisture retention are minimal - a plant community as austere and singular as the landscape itself is able to thrive.



Spring Mountains, Nevada
Photo by Colin Hoag



Figure 1. *Hecastodeis shockleyi* in bloom, its bracts turning milky white as the florets open, set against the dramatic mountain landscape of the Mojave Desert.

a steep, rocky slope past cacti (e.g., *Ferocactus cylindraceus* (Engelm.) Orcutt, Cactaceae), buckwheats (e.g., *Eriogonum inflatum* Torr. & Frém., Polygonaceae), herbaceous Compositae (e.g., *Baileya multiradiata* Harv. & A. Gray, Helenieae), and subshrubs (e.g., *Ephedra viridis* Cov., Ephedraceae; *Coleogyne ramosissima* (Ridl.) M.W.Chase & Schuit., Rosaceae; *Ericameria* spp., Astereae). Clambering over rock ledges and periodically losing my footing on the gravelly slopes, I followed my handheld GPS to the point—all the while worried about the wisdom of embarking on this difficult project in the late-May heat.

Then I found one! And another! As so often happens in field botany, once I saw one, I saw them everywhere. When they first leaf out in spring, the

bracts surrounding the flower heads are light green in color and easy to recognize from afar. As spring turns to summer, they become white and even more conspicuous (Figure 1). Sadly, none had yet flowered, but it was clear that they were on the verge of doing so.

Without permits to collect leaf material in Death Valley National Park, I drove through to the Owens Valley. Whereas the Spring Mountains near Las Vegas represent the southern edge of the plant's distribution, populations in the Owens Valley on the western slopes of the Inyo Mountains appear to occupy its western edge. Surrounded by the most fantastical geological formations I have ever seen, including the snowcapped Sierra Nevada Mountains

An extravaganza of unique traits

Cluster of single flowered capitula of *Hecastocleis shockleyi*, enclosed by spiny, papery bracts. The florets are exposed, with styles extending beyond the syngenesious anther tube. The style is stout and glabrous, with slight thickenings below the apex and short style branches, features characteristic of early-divergent lineages within the Compositae.



Spring Mountains, Nevada
Photo by Colin Hoag



Figure 2. **A.** After flush, flowerheads emerge with bracts light green. **B.** Just before flowering, the bracts become whiter. **C.** In flower. **D.** After flowering, bracts become brittle and somewhat translucent before heads fall.

Desert opportunities

A bee visits the solitary floret of a *Hecastocleis shockleyi* capitulum. Each capitulum bears a single tubular flower subtended by papery, spine-tipped bracts — an unusual condition within Compositae. The floret's pale corolla and exposed anthers facilitate access for small insect visitors.

Spring Mountains, Nevada
Photo by Colin Hoag



Figure 3. **A.** Medium-sized individual growing among *Coleogyne ramosissima* on a rocky slope; note the conspicuous inflorescence bracts that turn milky white at flowering. **B.** A shoot from this year (right) alongside one from last year (bottom). New shoots produce spiny, sparsely toothed leaves that broaden at the base. In the subsequent year, these turn brown along with the stem, while soft, linear leaves emerge in brachyblasts from the axils of those spiny leaves. **C.** A rhizome. **D.** A close-up of brachyblasts in the axils of already senescent leaves.

looming to the west, I scrambled up rocky slopes in search of these small, prickly shrubs.

My next stop was near the plant's possible northern limit at Walker Lake, 170 km south of Reno. This area is decidedly not Mojave Desert, lying firmly in the Great Basin, which receives somewhat more annual rainfall and is dominated by sagebrush (*Artemisia tridentata* Nutt., Anthemideae) rather than creosote and Joshua trees. The two populations I found inhabited parallel valleys of the Wassuk Range running east-to-west. Unlike previous

populations that I'd found, which were on north- or west-facing slopes, these faced south, though the sites still featured steep, gravelly slopes and some plant species common to other sites, such as *Stanleya pinnata* (Pursh) Britton (Brassicaceae), *Xylorhiza tortifolia* var. *tortifolia* (Torr. & A. Gray) E. Greene (Asteraceae), and *Sphaeralcea ambigua* A. Gray (Malvaceae).

On my way back toward Las Vegas, I located two additional populations of *Hecastocleis*. The first was located in the geographic center of its range along

Holding ground

Hecastocleis shockleyi is capable of growing on loose gravel slopes where many botanists would lose their footing. Its shallow roots bind the coarse soil together and keep it from eroding away with the shifting landscape.

Wassuk Range, Mineral County, Nevada
Photo by Colin Hoag

the road near Coaldale in the Great Basin. It was the easiest population to access of them all—near to the highway and on a very moderate slope—but the individuals did not appear to be thriving. They were relatively small, with fewer flowerheads, and often displaying insect damage and signs of water stress including brittle, yellowing leaves. Their flowers seemed furthest from bloom compared to other populations.

The second population was near the possible eastern edge of *Hecastocleis shockleyi*'s range: back in the Mojave Desert near Alamo, Nevada. This population was much healthier, but mystifyingly it was situated on an east-facing slope! This variation in aspect would be unremarkable if it weren't so often the case that each population was narrowly circumscribed on the landscape by aspect. Populations may be abundant on one slope yet absent on the next, as though confined by subtle, unseen boundaries in the terrain.

JUNE 2024: ECOLOGY & NATURAL HISTORY I

Because of my ambitious goals during my first trip, I had little time for observation and site description. In the first week of June 2024, I decided to focus more narrowly on a single population in the Spring Mountains National Recreation Area near Las Vegas. This time I would arrive a week later to ensure that I could see the plant in flower. My goals were to make natural history observations about the site, to try to observe and collect pollinators, and to map the structure of the population on the landscape with a high-precision Global Navigation Satellite System device.

When I first arrived, the plants were not yet in flower, and I feared the worst. But within two days, it was as though someone had flipped a switch: dozens and then hundreds of plants were blooming. The flowers are quite small and somewhat inconspicuous from a few feet away, but the bracts by this time had turned a deep, milky-white color, making the plant even more conspicuous on the landscape than before ([Figure 2](#)). A single plant can feature many hundreds of florets.

My interest in mapping individuals across the landscape stemmed from my first impressions of the spatial structure of desert flora as someone unfamiliar with these arid landscapes. For example, during my first trip I was struck by just how rare it was to encounter juvenile plants, desert recruitment being extremely difficult. And, more so than in other landscapes I'd visited, plants seem to clump together, given that the micro-characteristics of a site (particularly soil moisture) act as a strong filter on plant presence or absence.

I was struck by just how expansive some individual plants can be, too. When *Hecastocleis* flushes in spring, new shoots emerge from buds in old stems, as well as from the base of the plant. These basal shoots push the circumference of the plant even wider, but some are apparently rhizomatous. I dug at the base between neighboring plants and encountered dead rootstock that had clearly once connected the two. From the gnarled, woody base of another plant, I found new shoots extending outward underground that were soft, white, and clearly not intended as aerial shoots ([Figure 3](#)).

Funk and Hind (2009) suggested sensibly that the white flower might attract night-time visitors such as moths. My camera trap and light trap failed to capture anything overnight, I did record a variety of insects visiting the plant ([Figure 4](#)). Notably, this included two species of bees that were active on pollen-covered florets. I managed to photograph and capture one of them, which appears to be *Lasioglossum* in the *Dialictus* group (Sam Droege, 2024, *pers. comm.*). Scanning electron microscope (SEM) images of the bee showed the distinctive *Hecastocleis* pollen described by Funk & Hind (2009).

I am still unclear about how *Hecastocleis* disperses. It is rare to find an individual with heads and bracts from the previous year, as they are completely shed during the brutally hot and dry summer, or during the rains and winds of winter. The pappus is a crown of scales (Panero & Funk, 2002), limiting its ability to disperse by wind or fur. The achene is glabrous and unlikely to stick to animals, though the entire inflorescence could do so, given the spines on the bracts (or even perhaps entire branches, given the spiny leaves on new flower-bearing shoots). Water dispersal from torrential rains is almost certainly a

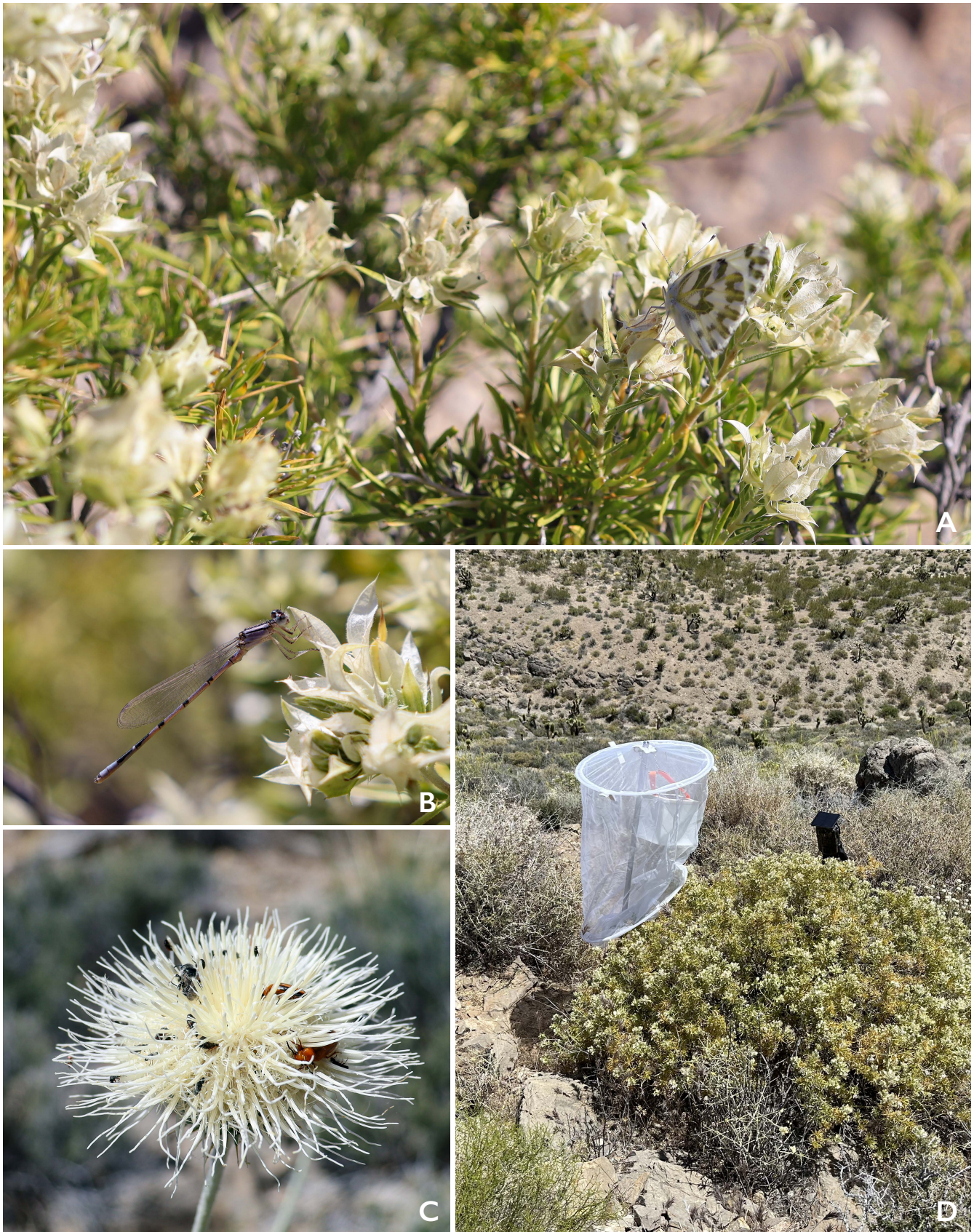


Figure 4. Observations and trapping efforts were conducted to document potential pollinators of *Hecastodeis* and associated desert Compositae. **A–B.** Lone rangers of the desert flora visiting *Hecastodeis*. **C.** *Cirsium neomexicanum* A. Gray (Cardueae) offers abundant florets that attract multiple visitors to a single head. **D.** Unsuccessful overnight moth trap.

The perennial ephemeral

Observed on the central Mojave margin, *Hecastocleis shockleyi* entering senescence as temperatures rise. Leaves, previously glaucous-green, now turn yellow and desiccate within as little as a week from anthesis. The species exhibits a brief vegetative and reproductive phase following seasonal moisture, retreating rapidly to dormancy under increasing aridity — a survival strategy finely tuned to desert pulse dynamics.

Spring Mountains, Nevada
Photo by Colin Hoag



Figure 5. Some other Compositae from Mojave desert. **A.** *Psathyrodes ramosissima* A. Gray (Helenieae). **B-C.** *Psilostrophe cooperi* (A. Gray) Greene (Helenieae). **D.** *Erigeron concinnus* Torr. & A. Gray (Astereae). **E.** *Ericameria linearifolia* (DC.) Urbatsch & Wussow (Astereae).

In transit

SEM of *Hecastocleis shockleyi* pollen grains adhering to the body of a bee. The grains are spheroidal to suboblate and tricolpate, with finely microechinate ornamentation characteristic of early-divergent Compositae.

Pollen grains found on body of bee depicted on page 21.
Photo by Eva Bruce

Extreme diversity

The Mojave is vast. Compositae are regular components of the bajadas stretching between distant ridges, as seen here, but finding them safely takes preparation. Even with these sweeping views, it's easy to lose sight of your truck among the arroyos, creosote, and Joshua trees.



Spring Mountains, Nevada
Photo by Colin Hoag

factor, but this only works downslope. Perhaps birds consume and disperse them.

Seeing desert Compositae just a week later than on my previous visit offered valuable insight into the ecological dynamics of the Mojave (Figure 5). I fell short of my goal to sample every plant in the valley because I ran out of time, but I did manage to sample an entire hillslope, including a total of over 3,000 points. Using a digital elevation model and my natural history site descriptions, I will analyze these data for clues about the structuring of the population on the landscape. With the leaf material I collected from those 9 populations in 2023, I hope to investigate their genetic diversity in relation to one another.

JUNE 2025: ECOLOGY & NATURAL HISTORY II

The following summer, I returned to this site—this time in the second week of June rather than the first. I came to capture additional points for my mapping project, which now includes 5,000 individual plants and counting. Additionally, I hoped to make observations of the plant further along in its annual life cycle. The temperatures were markedly hotter, and my workday therefore shorter. The plant community at large had begun to head toward dormancy, and *Hecastocleis* was no different. In place of the dark-green leaves with bright, white flowerheads, I found *Hecastocleis* almost entirely yellow. Most plants were no longer in flower, and many lacked flowerheads. Last year's brown stems had now turned grey, and this year's green stems had turned brown. The leaves were so brittle they fell off when touched. I noticed no visiting insects, though many capitula had been bored through by an insect seeking its fruit.

This visit taught me two important things. First, it appears that the plant's active period is quite narrow, in flower for just over a week, pointing to the opportunism required to survive and reproduce in the narrow window of a desert rainy season. Second, I learned that the plant likely disperses locally via the tumbleweed action of the aggregated capitula surrounded by the spiny bracts. As I brushed up against a plant, these structures would

often spring off the plant, rolling and blowing away on the ground. This might help explain why these plants sometimes aggregate at the base of other plants and, more broadly, why they have such a patchy dispersion across the landscape. How they manage to travel beyond that landscape is still an open question.

CONCLUSIONS

The geography of *Hecastocleis shockleyi* is mysterious, not only in its phylogeographic history but also in its current distribution. My three field research trips took me through some striking environmental changes, from the arid Mojave to the Owens Valley at the edge of the Sierra Nevada Mountains, to the sagebrush steppe of the Great Basin. Seeing a diversity of sites where *Hecastocleis* occurs only reinforced my sense for just how peculiar the spatial patterns of this plant are: narrowly restricted yet abundant within a given site, and occurring across a large geographic range on slopes facing every cardinal direction in two distinct desert environments.

What could be the driver of this strange pattern? Did the plant previously occur more widely before becoming restricted to higher elevation zones by climatic changes? After all, we know this process from elsewhere in the world (Sandel et al., 2011), and the Mojave and Great Basin were both cooler and wetter even just 10,000 years ago (Walker & Landau, 2018). Or, is this a story of stochastic dispersal events? What prevents *Hecastocleis* from colonizing new spaces that otherwise appear to suit them? My research will not answer these questions definitively, but hopefully it can shine some light on their edges.

I contacted Jerry Tiehm, the herbarium curator at University of Nevada, Reno—a long-time field botanist and an authority on the flora of Nevada—to ask him why *Hecastocleis* occurs where it does. He confirmed how difficult it was to characterize its preferences, explaining: “you don't so much go looking for the plant as you do stumble upon it.”

Dear reader, when you do stumble upon this incredible plant—and someday I hope you do—be careful. The slopes are steep, the desert is hot, and the plant is quite spiny.

ACKNOWLEDGEMENTS

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LITERATURE CITED

- Ackerman, T. L.** 2003. A Flora of the Desert National Wildlife Range, Nevada. *Mentzelia* 7: 1–90.
- Funk, V.A. & D. J. N. Hind.** 2009. Hecastocleideae (Hecastocleidoideae). Pp. 261–65, in: *Systematics, Evolution, and Biogeography of Compositae*. Eds, V. Funk, A. Susanna, T.F. Stuessy, R. J. Bayer. Vienna: IAPT.
- Gray, A.** 1881. Contributions to North American Botany. *Proc. Amer. Acad. Arts* 17: 163–230.
- Lessing, C. F.** 1829. “De synanthereis herbarii regii Berolinensis dissertatio prima.” *Linnaea* 4: 240–356, Tab. II.
- Mandel, J. R., Dikow, R. B., Siniscalchi, C. M., Thapa, R., Watson, L. E. & Funk, V. A.** 2019. A fully resolved backbone phylogeny reveals numerous dispersals and explosive diversifications throughout the history of Asteraceae. *Proc. Natl. Acad. Sci. U.S.A.* 116(28): 14083–88.
- Panero, J., and Vicki Ann Funk.** 2002. Toward a phylogenetic subfamilial classification for the Compositae (Asteraceae). *Proc. Biol. Soc. Washington* 115(4): 760–73.
- Sandel, B., Arge, L., Dalsgaard, B., Davies, R. G., Gaston, K. J., Sutherland, W. J. & Svenning, J. -C.** 2011. The influence of late quaternary climate-change velocity on species endemism. *Science* 334(6056): 660–64.
- Walker, L. R., & Landau, F. H.** 2018. *A Natural History of the Mojave Desert*. Tucson, AZ. University of Arizona Press.
- Ward, M. & Bittman, R.** 2024. Rare plant status review: *Hecastocleis shockleyi*. California Native Plant Society, Rare Plant Program. Accessed May 21, 2024. <https://rareplants.cnps.org/443/Plants/Details/2093>.
- Williams, M. J.** 1977. *Hecastocleis shockleyi* A. Gray. *Mentzelia* 3:18.

STYLE

Where art and science converge



THE LAND OF THE STRANGE

A lateral view of *Soliva stolonifera* (Brot.) Sweet (ANTHEMIDEAE) cypselid. Note the deep furrows on the fruit's surface as well as the two sharpened wing apices and the incurved style. The pappus is absent. Image taken through an Apexel 200x Mobile LED Microscope Lens.

Alexander N Schmidt-Lebuhn



READY FOR DISPERSAL

When the fruits of *Perityle brandegeana* Rose (PERYTILEAE) are mature, the phyllaries reflex and the pedicel turns outward, presenting the sticky epappose cypselae to a lucky passersby.

Isaac Lichter Mark



THE LONE RANGER

A thrips (Thysanoptera) feeding on the pollen of
Osteospermum ecklonis (DC) Norl. (CALENDULEAE)
as seen through an Apexel 200x Mobile LED Microscope Lens.

Alexander N Schmidt-Lebuhn



DANCING IN THE DARK

Two cypselae of *Hypochaeris albiflora* (Kuntze) Azevêdo-Gonç. & Matzenb. (CICHORIEAE) showing the distinctive plumose pappus bristles typical of subtribe Hypochaeridinae

Ary Mailhos

TICATIMES

selected Compositae news and updates from
THE INTERNATIONAL COMPOSITAE ALLIANCE

Edited by Jennifer R. Mandel

12-15 January 2026

DIGITAL FRONTIERS 2026: TRANSFORMING RESEARCH AND TRAINING IN SYNANTHEROLOGY

This exciting event will bring together TICA members to explore how digital tools are transforming research and training in synanthology. Highlights include strategic planning for the second edition of the Compositae Book, hands-on training for editing the Compositae Global database, and a TICA meeting featuring presentations from across the community. Don't miss this key opportunity to collaborate, learn, and shape the future of Compositae research!

Venue:

*The Botanical Research Institute of Texas (BRIT),
Fort Worth, Texas, USA*

There is still time to register for virtual attendees.

Deadline: 12 December 2025

[Register Here](#)

Questions? Contact local organizers:

Oyetola Oyebarji, ooyebarji@fwbg.org
Morgan Gostel, gostelm@upenn.edu

Overview Agenda

Jan 12th
Evening: Mixer and Welcome Event
Jan 13th
Global Compositae Database Workshop
Jan 14th
Group Breakouts for discussion of TICA business
including 2nd edition of the Compositae book
Jan 15th
All-members TICA Meeting including
presentations by attendees





Edited by Jennifer R. Mandel

TICA Talks is one of the highlights of our community. Monthly, we come together to hear from a range of experts on Compositae from morphology to development, systematics and genomics. If you missed one, you can catch up on our YouTube channel: <https://www.youtube.com/@theinternationalcompositae2637/featured>

TICA Talks 2025 kicked off in February with talks from Jennifer Mandel (University of Memphis, USA) and Mauricio Bonifacino (Universidad de la República, Uruguay) who introduced TICA, provided a brief history, and discussed its current and past progress.

In March, we heard from Reid Selby (Clemson University, USA), discussing work on developmental transitions in lettuce, and Yannick Woudstra (Stockholm University, Sweden) sharing about urban ecology in dandelions.

April brought us Carme Blanco Gavalda (Autonomous University of Barcelona, Spain) presented the HAP clade in tribe Gnaphalieae, and Paige Ellestad (University of Memphis, USA) discussed discordance and hybridization events at the base of the family. Isaac Lichter Marck (California Academy of the Sciences, USA) introduced a new species from Big Bend National Park in Texas (USA) that made national news in the US (see article this issue).

In May, Peter Innes (University of Colorado, Boulder, USA), presented work on the regulation of gene expression in Great Sand Dunes prairie sunflowers, and Anthony Melton (University of Montevallo, USA) updated us on *Artemisia tridentata* subsp. *tridentata* genomics.

June's talks were Lucía D. Moreyra (Botanical Institute of Barcelona, Spain), who presented their work on the origin and diversification of *Cirsium*, Brannan Cliver (Clemson University, USA) shared genome and

morphology work on Hawaiian *Bidens*, and Vinicius Bueno (Universidade Federal de Uberlândia, Brazil), presented on tribe Neurolaeneae.

In July, we host Jaydeep Sharma (The Maharaja Sayajirao University of Baroda, India), who presented their work on Gujarat composites, and Erika Moore-Pollard (University of Memphis, USA) who introduced a draft genome assembly for Barnadesieae.

August highlighted Island Biogeography, featuring two talks on evolutionary patterns and plant diversity in island systems. Our speakers were Lizzie Roebble (Naturalis Biodiversity Center & University of Groningen, Netherlands; University of Hawaii, USA), who presented on Hawaiian Compositae taxa, and Bruce Baldwin (University of California, Berkeley, USA) who presented on the phylogenomics of the silversword alliance.

In September, we followed the theme of Development, featuring two talks on floral head development in Compositae. Our speakers included Jakub Baczyński (University of Warsaw, Poland), who told us about secondary heads, and Paula Elomaa (University of Helsinki, Finland) who spoke on the evolution of capitula.

Finally, October featured three talks by Mariana Grossi (Museo de La Plata, Argentina) on reproductive traits in Eupatorieae (talk in Spanish), Benoit Loeuille (Royal Botanic Gardens, Kew, UK), on the Compositae in Flora Zambesiaca, and Jairo Patiño (Island Ecology and Evolution Research Group (GEEI), Institute of Natural Products and Agrobiology, Spanish National Research Council (IPNA-CSIC), Tenerife, Spain), on oceanic island Compositae evolution.

If you are interested in giving a TICA Talk, contact Dr. Colin Hoag: choag@smith.edu.



TICA TALKERS.

A. Jennifer Mandel & Mauricio Bonifacino. **B.** Reid Selby. **C.** Yannick Woudstra. **D.** Carme Blanco Gavaldá. **E.** Paige Ellestad. **F.** Isaac Lichter Mark. **G.** Peter Innes. **H.** Anthony Melton. **I.** Lucía Moreyra. **J.** Brannan Cliver. **K.** Vinicius Bueno. **L.** Jaydeep Sharma. **M.** Erika Moore-Pollard. **N.** Lizzie Roeble. **O.** Bruce Baldwin. **P.** Jakub Baczyński. **Q.** Paula Elomaa. **R.** Benoit Loeuille. **S.** Jairo Patiño.

GUIDELINES FOR AUTHORS

Types of articles and editorial process

CAPITULUM accepts contributions matching the following sections, as long as they are relevant to the plant family Compositae (sunflower, daisy or aster family, a.k.a. Asteraceae). HEAD TOPICS & BREVIA: Section to showcase current research on Compositae. PIPET & PRESS: Articles about methods and techniques. THE HANDLENS: A close up look to a given species or morphological structure. THE CABINET: A window to the stories behind ancient or recent herbarium samples and the botanists behind them. FROM THE FIELD: The place where the bold and the adventurous share their latest finds in their eternal pursuit of the more recalcitrant comps. STYLE: Where science and art converge, artistic photography, botanical illustration or creative infographics. All is welcome if it comes with style. Manuscripts or photos intended for publication in CAPITULUM are to be submitted at www.compositae.org. Contributions will be sent to two external reviewers. Suggestions of suitable, qualified reviewers without conflicts of interest are encouraged.

General formatting and structure

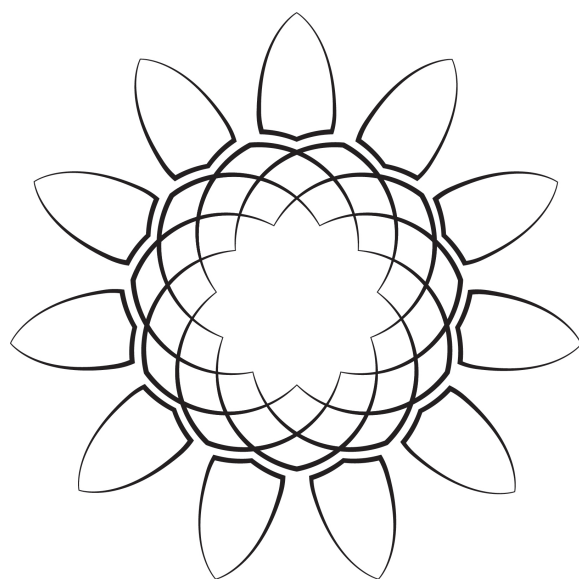
Consult a recent issue of CAPITULUM and follow these instructions. Language for submission is English. Use Times New Roman/Arial typography size 12. Provide a title and if needed, a subtitle. Include names of all authors, their ORCIDs, their professional affiliations and emails. Include a list of 5–6 keywords in alphabetical order. Provide an abstract of up to 250 words. There is no set structure on how to organize your text; however most cases will adapt well to the classic I-M&M-R-D plus Acknowledgements and Literature Cited; Tables; Appendices; and Figure Legends. Monographic works should include proper citation of names including types. Morphological descriptions, notes when needed, additional specimens examined (country, political division, political subdivision, locality, collector, collector #, date, herbarium code). Keys should be indented. Each couplet should be numbered 1a and 1b, 2a and 2b, etc. All scientific names at the rank of tribe or inferior should include authorship the first time they appear in the text, following Brummitt & Powell, *Authors of Plant Names* (Kew, 1992; info included in the International Plant Names Index [IPNI]). In text, a single space must follow a period, colon, semi-colon, or comma. Molecular phylogenetic and systematic studies should clearly state the sampling strategy including all details related to the sequencing and data analysis. Phylogenetic trees should have support values plotted.

Tables and figures

Tables must be prepared using MS Excel, please include different tables as different spreadsheets on the same file. Figures should be provided in either TIFF format (for photos) or as vector graphics such as AI or EPS for diagrams, illustrations, or phylogenetic trees. Maps should be provided as SHP files when possible or as vector graphics. You may send the photos and other graphics ordered and arranged following your preference, but you are advised that we could edit them to adjust them to CAPITULUM style. All figures must be uploaded as individual original files. Photos should be at least 300 dpi in resolution. Literature citation: please check that all your references are cited and vice versa. For the style of references check a recent issue of CAPITULUM.

Data availability requirements

CAPITULUM requires all data sets to be archived in a permanent, publicly accessible location. To enable readers to locate archived data, include a “Data Availability” section before the Literature Cited section. This should list the database, digital object identifiers (DOIs), stable URLs, and the respective accession numbers for all data from the manuscript, as appropriate. Note that accession numbers provided in a supplementary table (voucher table) accompanying the article do not need to be duplicated here in the data availability statement.



Magno amore in familiam Synanthearum captus
Lessing, 1829