



When Damage Becomes Diagnostic: Cracked Herbarium Specimens Reveal a New Taxonomic Character in *Heliampora* (Sarraceniaceae)

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Heliampora Bentham (1840: 432) (Sarraceniaceae) is a genus of carnivorous pitcher plants native to the “lost world” of South America: the tepuis of Venezuela, Guyana, and Brazil (Brewer-Carías *et al.*, 2024; McPherson *et al.*, 2011). Despite being the largest genus in the family, *Heliampora* often is the most difficult to identify to the specific level, especially with etiolated leaves or specimens of hybrid origin. We utilized microscopy on both cultivated and herbarium specimens to identify additional characters that can aid in the identification and delimitation of *Heliampora* species.

A new taxonomic character is described here: the morphology of the trichomes found in the most proximal portion of the interior of the pitcher (Fig. 1). This area is usually completely submerged by the pitcher fluid and is the location of prey death by drowning (Jaffe *et al.*, 1992). In this area, two trichome morphologies are typically observed. Here, for the first known time, we introduce and name these trichomes as either short and compact (SC) or long and thin (LT). SC trichomes are much shorter, subulate, and fine-pointed, and arise from a distinct pedestal, often larger or the same width as the trichome itself. LT trichomes are longer, thinner, and straight (Payne, 1978). These two morphotypes are shown on herbarium specimens and cultivated specimens in Figure 1. Although trichomes in other parts of the pitcher body may be similar morphologically, the morphological character states we define as SC or LT only relate to the trichomes at the base of the interior of the pitcher. Neither of these trichome morphologies have been observed to have the cuticular folds that were first reported in trichomes at the upper, more distal end of the pitcher, also called the bell (Bauer *et al.*, 2013; Lloyd, 1942). Scanning electron microscopy (SEM) was used to image SC and LT trichomes on cultivated *Heliampora* specimens (Fig. 1) as part of an ongoing doctoral dissertation study (Walston *et al.*, 2026). Microscopy was performed at the Newcomb Imaging Center (NIC), Department of Botany, University of Wisconsin–Madison, using a FEI Quanta 200 microscope, and at the Ray and Mary Wilcox SEM Lab and Electron Microbeam Laboratories, Department of Geoscience, University of Wisconsin–Madison, using a Hitachi S-3400N Type-II Variable Pressure microscope and Hitachi S-3700N microscope. Before microscopy, the samples were dissected from pitchers and dehydrated to 100% ethanol. Specimens were prepared at the NIC using a Leica EM CPD300 to dry the specimens with a speed setting of five. A Leica EM ACE60022 was used to sputter-coat the specimens with 8µm of platinum-palladium. Images were taken in secondary electron mode with an average high-voltage of 20kV and working distance of 10-15mm. Micrographs were not altered post-acquisition. All 24 species of *Heliampora*, as well as two undescribed and potential new species, were used in this study. A total of 54 specimens from living horticultural collections were imaged with SEM with 11 species having multiple accessions imaged. Two herbarium specimens were also imaged with SEM. More than 210 herbarium specimens from BH, BRIT, F, NCU, NY, and WIS were observed for this new taxonomic character.

The average length of SC trichomes is 0.32mm, and the average length of LT trichomes is 0.71mm. The differences in length between the SC and LT trichomes is statistically significant (7.28×10^{-37}), using an alpha value of 0.05. The densities of trichome distribution are also statistically significant between the two morphotypes (8.95×10^{-3}). FIJI was used to measure length and density of trichomes in the proximal interior of the pitcher of all 56 specimens (Schindelin *et al.*, 2012). For density, a 1x1mm square was placed in the center of the micrograph and used as the region of interest (ROI), and a trichome was only counted if more than 50% of its base was inside the ROI. All imaged specimens were used in these analyses unless trichome data was unquantifiable from the SEM images. The comparison of lengths and densities between SC and LT trichomes were accomplished using two-tailed Welch’s t-tests.

Most importantly, we predict these trichome morphotypes to be phylogenetically significant. Liu and Smith (2021) reported the most complete *Heliampora* molecular phylogenetic reconstruction to date. When observations of which species had SC or LT trichomes were compiled by us and then compared to the phylogenetic tree, the SC trichomes fit neatly into two of the five clades proposed (Liu & Smith, 2021). The species with SC trichomes are in the clades E2a and E3,

whereas the species with LT trichomes are in the clades W, E1, and E2b. The exception is *H. folliculata* Wistuba, Carow & Harbath (2001: 120), which has SC trichomes but is found in a clade with species that have LT trichomes. To confirm these phylogenetic observations, a more focused phylogenetic study would be required beyond the scope of this current paper.

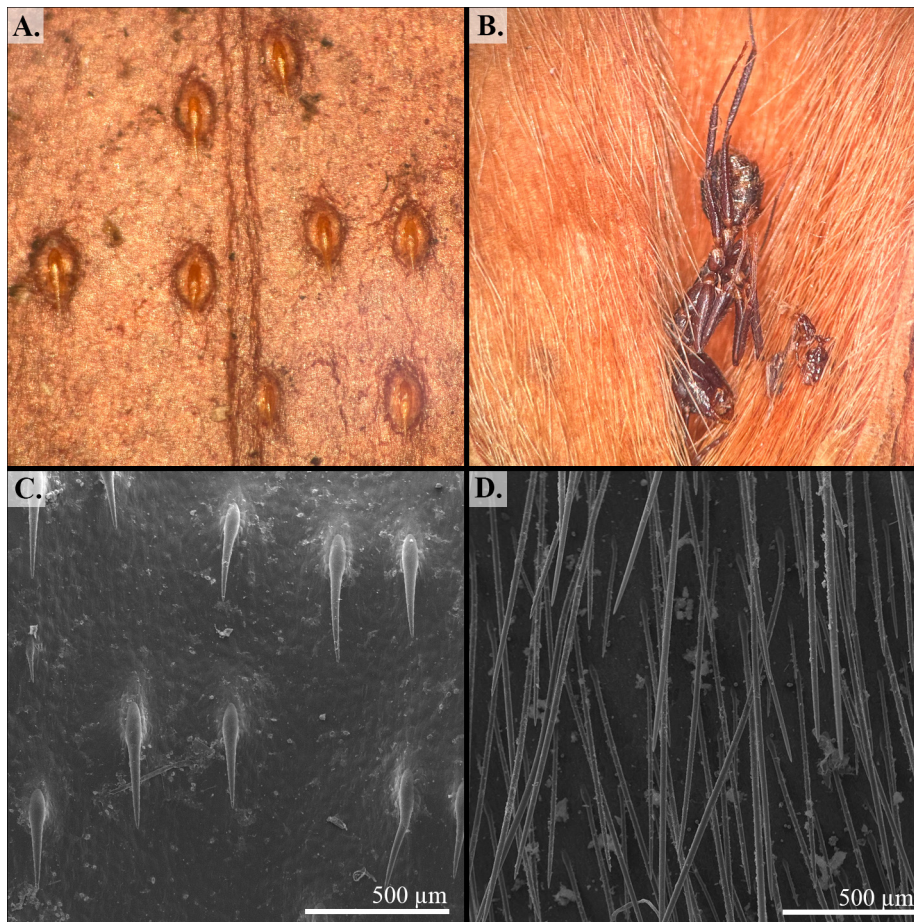


FIGURE 1. Comparison of the SC and LT character states on herbarium specimens and cultivated specimens. (A.) SC trichomes on a *Heliamphora* herbarium specimen at NY. (B.) LT trichomes on a *Heliamphora* herbarium specimen at NY. A and B are not to scale. (C.) SC trichomes imaged with SEM on a cultivated specimen of *Heliamphora ionasi* “Elegance.” (D.) LT trichomes imaged with SEM on a cultivated specimen of *Heliamphora minor* “Velvet, Luna Tepui.”

Liu and Smith’s phylogenetic reconstruction does not include the most recent species described in 2024, *H. electrum* Golos, Nerz, Mey & Wistuba (2024: 19), which had been known in the horticultural trade as “*H. sp.* Venamo” before publication (Golos *et al.*, 2024; Liu & Smith, 2021). We observed SC trichomes on *H. electrum*. Therefore, we hypothesize that, in an updated molecular phylogenetic tree, *H. electrum* will fall within the clades E2a or E3, along with other species with SC trichomes. The plant known in horticulture as *H.* “Black Cherry” has been hypothesized to be a cultivated variety or hybrid of *H. heterodoxa*, and because we observed SC trichomes on *H.* “Black Cherry,” this would support that theory of parentage. We speculate that the differing trichome morphologies may relate to differences in prey captured or microhabitat, with LT trichomes potentially being useful for pitchers that are more often flooded or catch larger prey. This is an untested hypothesis to be used for a future study. Lloyd (1942) hypothesized that the trichomes found in the proximal end of the pitcher were used for holding the captured prey in the pitcher until their death by drowning. He also observed cuticular folds on these trichomes, but we were unable to find them (Lloyd, 1942). Although the trichomes in this zone have been briefly mentioned in some protologues, this area of the leaf is almost always excluded from taxonomic treatments and illustrations (Bentham 1840). We now present it as a comparative character across the genus that provides insight into the evolution and geographic distribution of the genus (Liu & Smith, 2021).

Heliamphora is not easy to study in the field, and few people have access to substantial living material in cultivation. However, herbarium specimens, including types, offer a wealth of morphological characters, especially trichomes. Since their native habitat, the tepuis of South America, is so understudied and underexplored (McPherson *et al.*, 2011), herbarium specimens of this genus are rare and particularly valuable. One challenge in working with them is that curators are reluctant to allow destructive sampling and access to the pitcher interiors is often not possible. We have discovered a work around this

limitation. Most herbarium specimens are carefully pressed to reduce the amount of wrinkles, cracks, and other artifactual damage. *Heliamphora* leaves are notoriously rigid and do not bend without breaking. Therefore, many herbarium specimens of *Heliamphora* feature cracks and splits across the length of the pitcher body. This is in contrast to specimens of the related pitcher plants *Sarracenia* Tournefort ex Linnaeus (1753: 501) and *Darlingtonia* Torrey (1853: 4), which are usually better preserved with less structural damage (Fig. 2). These genera can still crack during the pressing process if they are older leaves that have already dried, have damage already, or were pressed poorly. However, we present here a case in which *Heliamphora* herbarium specimen damage during the pressing process actually yields specimens that are more useful taxonomically.



FIGURE 2. Comparison of herbarium specimens between *Sarracenia* (left) and *Heliamphora* (right) demonstrating the contrast in structural damage generally caused in the pressing process. Red boxes on the right illustrate areas where the new taxonomic character could be viewed through the cracks. *Heliamphora* image (right) is courtesy of the C. V. Starr Virtual Herbarium (<http://sweetgum.nybg.org/science/vh/>).

In many herbarium specimens of vascular plants, plant collectors try to press leaves showing both the abaxial and adaxial surfaces. Despite this, many *Heliamphora* specimens are not pressed to fully show the interior of the pitcher. This is likely due to a lack of understanding of the taxonomic importance of the interior of the pitcher until recently (McPherson *et al.*, 2011). This creates a unique challenge for systematists attempting to observe the interior of the pitcher in an herbarium specimen. However, the fractured nature of the rigid leaf works in favor of this, allowing small slits of visibility into the interior of the pitcher (Fig. 3). These cracks can occur across the pitcher body. Therefore, it is possible, and often likely, that the cracks will allow for observation of all or most areas of taxonomic importance of the interior of the pitcher. In historic herbarium specimens, particularly of types, these cracks are essential for the confirmation of trichome morphology and, most importantly, do not require any destructive sampling. As an example of the prevalence of these cracks, only 12 of the 142 herbarium specimens the authors examined at NY lacked fractures that allowed for observation of SC or LT trichomes.

For future collectors or mounters of *Heliamphora* specimens, whether cultivated or not, we suggest three options that allow for easier viewing of the pitcher interior, which contains taxonomically significant information. First, and preferred, more than one pitcher could be collected, allowing one pitcher to be pressed as usual and the other to be dissected down

the middle, with the interior of the pitcher visible. Second, one could press the pitcher as usual but cut holes periodically along its length to allow for interior observation. Third, two pitchers per plant could be collected. The first would be pressed as usual, allowing the cracks to form naturally during pressing, and the second pitcher could be filled with polyester fiber stuffing before pressing. This method is used in various museum preservation practices and helps preserve the form of the pitcher and often prevents excessive cracking, but the specimens can become quite thick and bulky (Baars, 2019). The polyester fiber stuffing, combined with the form preservation, makes these specimens less useful for observing the interior of the pitcher but ideal for studying pitcher form. Emphasis is often given to the preservation of the nectar spoon, or the highly modified appendage at the most distal end of the pitcher. The nectar spoon is very taxonomically important and should be pressed unobscured but not at the expense of the trichomes in the bell of the pitcher. Similarly, it is important to allow for observation of the exterior of the nectar spoon which can contain trichomes in some taxa.

The trichomes in the proximal end of the pitcher can also be observed from living specimens but require dissection of the leaf. To make sure that the portion of the leaf with the trichomes of interest is included, it is suggested to dissect the pitcher from as close to the rhizome as possible. It is imperative not to collect the youngest leaves from the plant as the immature leaves develop in the keel, or wing, of the most recently opened leaf. As *Heliampora* is heteroblastic and produces both distinct juvenile and adult leaves (McPherson *et al.*, 2011), it is not yet known whether juvenile leaves also contain these taxonomically significant trichomes; the authors intend to investigate this in a future study.

The rarity and value of the small herbarium record of *Heliampora* combined with most herbarium specimens of the genus only displaying the exterior of the pitchers makes observation of the taxonomically important interior of the pitcher difficult. Thus, the natural fracturing of the pitcher body provides taxonomists an opportunity to observe a newly reported taxonomic character, SC or LT trichomes, without destructive sampling.

The authors thank the New York Botanical Garden and the William and Lynda Steere Herbarium (NY) for the Visiting Scientist Travel Award that made this work possible. The authors acknowledge the Demeter Grant and Hugh H. Iltis Grant from the Department of Botany at the University of Wisconsin–Madison that funded the microscopy and herbarium travel. Jonathan Martin, Tyler Manzardo, Luke Larson, Sam Rickert, Hannah De Guzman, Leah Zuschlag, Jasmine Shaffar, and Samara Rouse are thanked for their microscopy and analysis, which helped with this research. Zachary Smith and Rose Andrews are thanked for their valuable edits. Nancy Marten and Billy Pinnix are thanked for tissue of the study species from their horticultural collections. The Wisconsin State Herbarium (WIS) is thanked for the use of the digitized specimen in Figure 2. The curators and directors of BH, BRIT, F, NCU, NY, and WIS are recognized and thanked for their assistance during the visitations, loans, and meticulous curation of their herbaria. This material is based upon work supported by the National Science Foundation Graduate Research Fellowship Program under Grant No. 2137424. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the National Science Foundation. Support was also provided by the Graduate School and the Office of the Vice Chancellor for Research at the University of Wisconsin–Madison with funding from the Wisconsin Alumni Research Foundation.

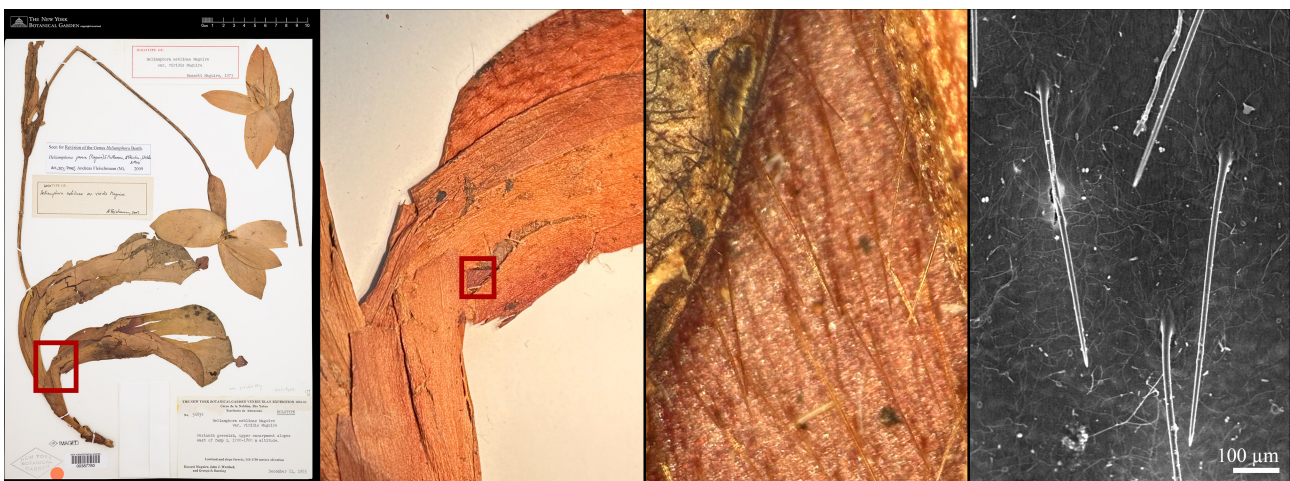


FIGURE 3. Demonstration of utilizing cracks in an herbarium specimen to view the trichomes in the proximal end of the interior of the pitcher in the holotype of *Heliampora neblinae* var. *viridis*. Left-most image courtesy of the C. V. Starr Virtual Herbarium (<http://sweetgum.nybg.org/science/vh/>). The right image is a SEM micrograph of the trichomes on a different cultivated *H. neblinae* specimen.

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Appendix

Herbarium voucher specimens: Taxon; voucher specimen (herbarium); catalog number; collection locality if known; horticultural name. **Herbarium voucher specimens:** Taxon; voucher specimen (herbarium); catalog number; collection locality if known; horticultural name. *Heliamphora Akopán* ined.; Walston & al 48 (WIS); v0435835WIS; Akopán tepui, cultivated; “sp. Akopán”. *Heliamphora Angasimá* ined.; Walston & al 80 (WIS); v0435814WIS; Angasimá tepui, cultivated; “sp. Angasimá”. *Heliamphora arenicola* Wistuba, A.Fleischm., Nerz & S.McPherson; Walston & al 74 (WIS); v0435793WIS; cultivated; “1988”. *Heliamphora “Black Cherry”*; Walston & al 97 (WIS); v0435845WIS; cultivated. *Heliamphora ceracea* Nerz, Wistuba, Grantsau, Rivadavia, A.Fleischm. & S.McPherson; Walston & al 74; (WIS); v0435829WIS; Cerro Neblina, Braz. Ven, cultivated; “AW Clone 1”. *Heliamphora chimantensis* Wistuba, Carow & Harbarth; Walston & al 42 (WIS); v0435799WIS; cultivated; “AW Old Clone”. *Heliamphora ciliata* Wistuba, Nerz, A.Fleischm.; Walston & al 43 (WIS); v0435805WIS; cultivated; “900 mes/meadow MS”. *Heliamphora collina* Wistuba, Nerz, S.McPherson & A.Fleischm.; Walston & al 56 (WIS); v0435842WIS; Testigos, cultivated; “Testigos AW.” *Heliamphora electrum* Golos, Nerz, Mey & Wistuba; Walston & al 87 (WIS); v0435856WIS; cultivated; “H. sp. Venamo Clone 01”. *Heliamphora elongata* Nerz; Walston & al 60 (WIS); v0435818WIS; Karaurin, cultivated; “Flat Lid Karaurin Tepui MS Clone 1”. *Heliamphora exappendiculata* (Maguire & Steyerem.) Nerz & Wistuba; Walston & al 91 (WIS); v0435809WIS; cultivated; “Ewok”. *Heliamphora exappendiculata* (Maguire & Steyerem.) Nerz & Wistuba; Walston & al 92 (WIS); v0435815WIS; cultivated; “Bubble Giant”. *Heliamphora folliculata* Wistuba, Harbarth & Carow; Walston & al 79 (WIS); v0435808WIS; Aparamán, cultivated. *Heliamphora glabra* (Maguire) Nerz, Wistuba & Hoogenstr.; Walston & al 63 (WIS); v0435800WIS; Ui-Wei Tepui, cultivated; “Ui-Wei Tepui M. Schach”. *Heliamphora heterodoxa* Steyerem.; Walston & al 67 (WIS); v0435807WIS; Sierra La Lema, Luepa, cultivated; “Sierra La Lema, Luepa / MS”. *Heliamphora heterodoxa* Steyerem.; Walston & al 93 (WIS); v0435821WIS; Venamo, cultivated; “Venamo Base”. *Heliamphora heterodoxa* Steyerem.; Walston & al 95 (WIS); v0435833WIS; Gran Sabana, cultivated; “Gran Sabana”. *Heliamphora heterodoxa* Steyerem.; Walston & al 78 (WIS); v0435802WIS; Kavanayen, cultivated; “Giant”. *Heliamphora huberi* A.Fleischm., Wistuba & Nerz; Walston & al 75 (WIS); v0435855WIS; Amuri, cultivated; “Amuri AW Clone 1”. *Heliamphora ionasi* Maguire; Walston & al 90 (WIS); v0435803WIS; cultivated; “Elegance”. *Heliamphora ionasi* Maguire; Walston & al 50 (WIS); v0435847WIS; cultivated; “Killer/Red Giant AW-H”. *Heliamphora macdonaldae* Gleason; Walston & al 66 (WIS); v0435801WIS; cultivated; “AW Clone 1”. *Heliamphora minor* Gleason var. *minor*; Walston & al 46 (WIS); v0435823WIS; Auyán tepui, cultivated. *Heliamphora minor* Gleason var. *minor*; Walston & al 61 (WIS); v0435812WIS; Luna tepui, cultivated; “Velvet Luna Tepui”. *Heliamphora minor* Gleason var. *minor*; Walston & al 73 (WIS); v0435843WIS; cultivated, Aonda shaft/sinkhole; “Aonda Shaft MS”. *Heliamphora minor* Gleason var. *pilosa*; Walston & al 53 (WIS); v0435860WIS; Auyán tepui, cultivated; “Auyán Clone 2”. *Heliamphora nutans* Benth.; Walston & al 88 (WIS); v0435862WIS; cultivated; “Giant mericlone”. *Heliamphora parva* (Maguire) S.McPherson, A.Fleischm., Wistuba & Nerz; Walston & al 71 (WIS); v0435831WIS; cultivated; “13/3 Red”. *Heliamphora purpurascens* Wistuba, A.Fleischm., Nerz & S.McPherson; Walston & al 52 (WIS); cultivated; “BCP Mixed Clones, T. Elliot”. *Heliamphora pulchella* Wistuba, Carow, Harbarth & Nerz; Walston & al 44 (WIS); v0435811WIS; Churí tepui, cultivated; “AW Clone 1”. *Heliamphora pulchella* Wistuba, Carow, Harbarth & Nerz; Walston & al 45 (WIS); v0435817WIS; Churí tepui, cultivated; “AW Clone 1”. *Heliamphora pulchella* Wistuba, Carow, Harbarth & Nerz; Walston & al 57 (WIS); v0435836WIS; Tirepón tepui, cultivated; “Tirepón AW”. *Heliamphora pulchella* Wistuba, Carow, Harbarth & Nerz; Walston & al 76 (WIS); v0435861WIS; Amuri tepui, cultivated; “Amuri AW Clone 1”. *Heliamphora pulchella* Wistuba, Carow, Harbarth & Nerz; Walston & al 82 (WIS); v0435826WIS; Akopán tepui, cultivated; “Akopán AW 01”. *Heliamphora pulchella* Wistuba, Carow, Harbarth & Nerz; Walston & al 83 (WIS); v0435832WIS; Churí tepui, cultivated; “Churí Fischer Clone 1”. *Heliamphora pulchella* Wistuba, Carow, Harbarth & Nerz; Walston & al 85 (WIS); v0435844WIS; Abacapá tepui, cultivated; “Apacapa GR”. *Heliamphora sarracenioides* Carow, Wistuba & Harbarth; Walston & al 55 (WIS); v0435848WIS; Ptari tepui, cultivated; “Ptari, Ven Clone 5/AW”. *Heliamphora tatei* Gleason; Walston & al 81 (WIS); v0435820WIS; cultivated. *Heliamphora uncinata* Nerz, Wistuba & A.Fleischm.; Walston & al 64 (WIS); v0435794WIS; cultivated; “E”. *Heliamphora uncinata* Nerz, Wistuba & A.Fleischm.; Walston & al 65 (WIS); v0435795WIS; cultivated; “Andy Smith Clone”. *Heliamphora uncinata* Nerz, Wistuba & A.Fleischm.; Walston & al 86 (WIS); v0435850WIS; cultivated; “Double Bubble MS Clone”.