*Phytotaxa* 669 (2): 075–086
https://www.mapress.com/pt/

Article

ISSN 1179-3155 (print edition)
PHYTOTAXA
ISSN 1179-3163 (online edition)

https://doi.org/10.11646/phytotaxa.669.2.1

# Pollen morphology and fruit anatomy of the enigmatic monotypic genus *Dicranocarpus* (Coreopsideae, Asteraceae)

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## Abstract

*Dicranocarpus* is a monotypic genus endemic to the Chihuahuan Desert with atypical attributes among species of tribe Coreopsideae. Phylogenetic position of *Dicranocarpus parviflorus* has been controversial. A molecular phylogeny showed that the taxon was closely related to *Dahlia*; however, further analyses with a wider sampling of *Dahlia*, also including *Hidalgoa*, identified the genus in a more distant clade. The objective of this study is to gather anatomical attributes from the cypselae as well as palynological characters along with macromorphology to determine whether *Dicranocarpus* shares characters with these genera in Coreopsideae. Our results show that although the pollen of *D. parviflorus* does not possess attributes that distinguish it from the rest of the tribe Coreopsideae, they were useful to confirm its membership in the tribe. The cypselae anatomy identified that *D. parviflorus* has characters of taxonomic value that differentiate this species from the rest of the Coreopsideae. The three most significant novel characters in *Dicranocarpus* were in the outer mesocarp, consisting of tracheoidal-like cells forming irregular rows up to 250 µm in length, an aerenchyma in the middle mesocarp, and the lack of phytomelanin and calcium oxalate crystals. Furthermore, the results of the phytomelanin patterns in the studied taxa highlight the need for further anatomical studies of the cypselae to fully understand the evolution of phytomelanin deposition in the tribe Coreopsideae and even in the Heliantheae Alliance. Macromorphological, palynological and anatomical attributes of the cypsela in *Dicranocarpus* were not shared with *Dahlia*. Moreover, the sexual condition of the disc and ray florets shared with *Hidalgoa* has evolved independently in lineages of Asteraceae.

**Key words:** cypsela anatomy, *Dahlia*, gypsicolous plant, *Hidalgoa*, phytomelanin, pollen

## Introduction

*Dicranocarpus* Gray (1854: 322) is a monotypic genus endemic to the Chihuahuan Desert in northern Mexico and the southern United States (Turner 2010; Fig. 1). A molecular phylogenetic analysis (Mort *et al.* 2008) showed that the taxon was closely related to *Dahlia* Cavanilles (1971: 56). However, further analyses that included *Dicranocarpus* with a wider sampling of *Dahlia*, also including species in *Hidalgoa* La Llave (1824: 15), identified the genus in a more distant clade (Sánchez-Chávez *et al.* 2019). *Dicranocarpus parviflorus* was described by Asa Gray in 1854 and placed in the subtribe Melampodiinae Lessing (1830) for presenting fertile pistillate ray florets and functionally male disc florets. However, this subtribe was regarded as a morphologically heterogeneous and polyphyletic group (Gray 1853, Turner & Johnston 1956, Turner & King 1962, Stuessy 1973, 1975). *Dicranocarpus* remained within this subtribe until Stuessy, in a review of Melampodiinae in 1973, suggested that the genus belonged to the subtribe Coreopsidinae, in tribe Coreopsideae, due to having pappus with two awns, and phyllaries and paleae with brown-orange longitudinal stria, characters that are diagnostic of the latter subtribe (Stuessy 1977). Later, Panero (2007) recognized a closer relationship of Melampodiinae to the tribe Millerieae and considered the members of the subtribe Melampodiinae to be part of Millerieae (Heliantheae alliance).

*Accepted by Alexander Sennikov: 10 Aug. 2024; published: 22 Oct. 2024*

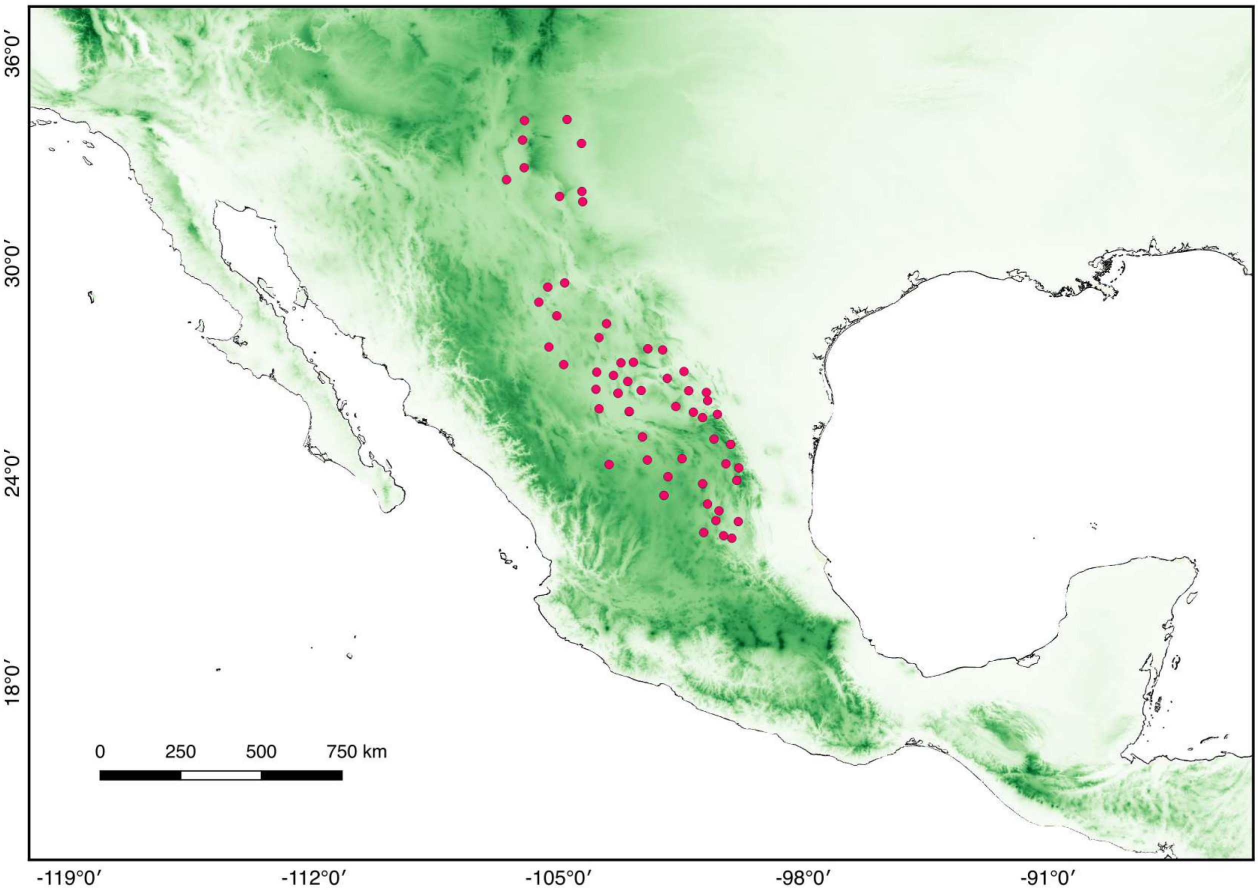

**FIGURE 1**. Distribution of *Dicranocarpus parviflorus,* a species endemic to the Chihuahuan Desert.

The morphology of *Dicranocarpus* is atypical among species in the tribe Coreopsideae (Fig. 2), although it shares characters with other members of the tribe such as *Hidalgoa*, *Moonia* Arnott (1836: 348), and *Oparanthus* Sherff (1937: 9), possessing fertile pistillate ray florets and functionally male disc florets (Robinson 1981, Ryding & Bremer 1992). However, it has been suggested that these features evolved independently in several lineages in the Asteraceae and should therefore be viewed with caution in the systematics of the group (Stuessy 1973, Turner 1978, Robinson 1981, Kimball & Crawford 2004, Mamut & Tan 2014).

Plants of *Dicranocarpus parviflorus* are succulent annual herbs with slender taproots, reduced capitula, and very reduced limbs of ray florets mostly covered by phyllaries (Fig. 3A). The ray florets are pistillate and fertile (Fig. 3B), while the disc florets are functionally male (Fig. 3C). The ray florets produce linear-elongated cypselae with horn-like rigid pappus awns at the apex (Fig. 3D). The plants are weedy, confined to desert or xeric habitats, growing exclusively over gypseous or alkaline soils, flowering in autumn. The distribution of *Dicranocarpus parviflorus* is exclusive to the Chihuahuan Desert; it has been recorded in New Mexico and Texas in the United States and Chihuahua, Coahuila, Durango, Nuevo León, San Luis Potosí, and Zacatecas in Mexico (Rzedowski 1975, Panero 2007, Crawford *et al.* 2009, Turner 2010, Flora of North America Editorial Committee 1993).

Ample morphological variation in the many genera of the tribe Coreopsideae has made it difficult to identify diagnostic characters for use in the delimitation of genera and species of this group because the vegetative and reproductive characters overlap (Kimball & Crawford 2004). Broad morphological variability has been reported in genera such as *Cosmos*, *Coreopsis*, *Bidens*, and *Dahlia* (Pandey & Singh 1982, Tadesse 1984, 1986, Ryding & Bremer 1992, Tadesse *et al.* 1995a, 1995b, Saar *et al.* 2003, Castro-Castro *et al.* 2014). In contrast, pollen attributes and cypsela anatomy have proved to be informative. For instance, a study of pollen in *Dahlia* and *Hidalgoa* identified that ora and colpus length and spine shape are diagnostic characters at the genus level (Sánchez-Chávez *et al.* 2022). In addition, comparative studies of the attributes of cypselae in several tribes in Asteraceae have identified traits of taxonomic importance that can help further our understanding of the evolutionary history of this family (Marques *et al.* 2022). Some examples of these characters include exocarp cells with pigmentation (Mukherjee & Sarkar 2001), variation in the number of layers of the mesocarp and cell layer arrangement of the sclerenchyma and parenchyma (Julio &

Oliveira 2009, Batista & De Souza 2017, Sánchez-Chávez *et al.* 2023), orientation of the exocarp cells (Jana & Mukherjee 2018), presence of phytomelanin with characteristic patterns and morphology (Pandey *et al.* 2014, Freitas *et al*. 2015, Sánchez-Chávez *et al.* 2023), and striations in the pericarp wall (Tadesse & Crawford 2014).

**FIGURE 2**. Capitulum of *Dicranocarpus parviflorus.* Photographs by Arturo Castro-Castro.

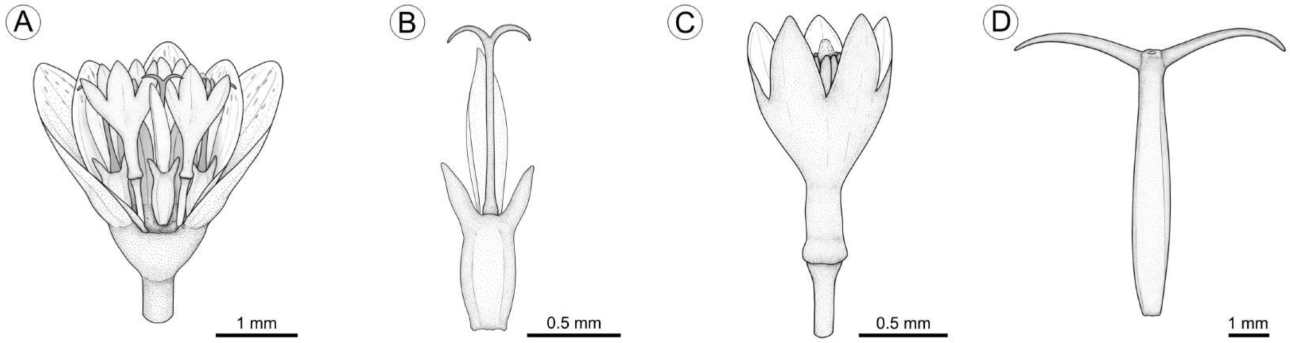

**FIGURE 3**. Capitulum of *Dicranocarpus parviflorus.* **A.** Capitulum morphology. **B.** Radial floret. **C.** Disc floret. **D.** Cypsela. Illustration by Teresa Jiménez.

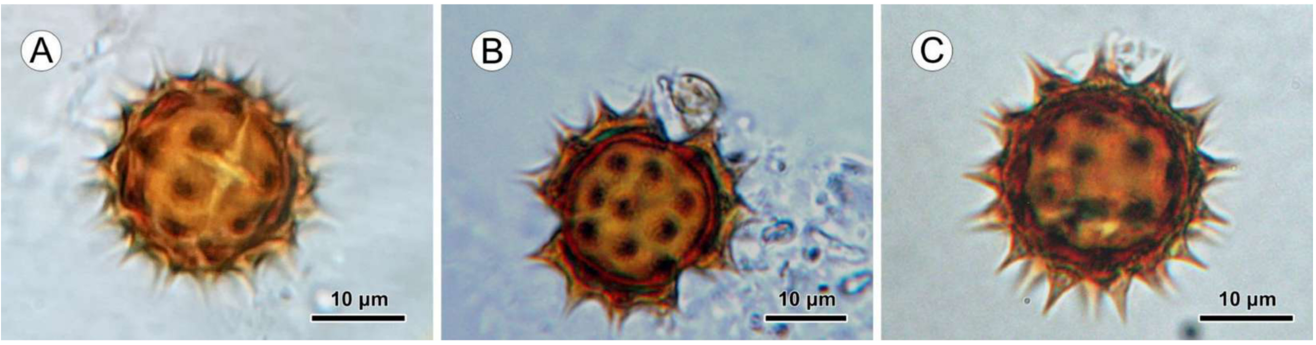

**FIGURE 4**. Pollen grains of *Dicranocarpus parviflorus* observed by light microscopy. **A.** Equatorial view, detail of ora and colpus. **B.** Polar view, detail of exine. **C.** Equatorial view, detail of spine.

The objective of this study is to document anatomical and palynological attributes of *Dicranocarpus parviflorus* not yet recorded and compare them along with macromorphological characters with those of other genera in Coreopsideae such as *Dahlia* and *Hidalgoa* to better understand how closely related these taxa are.

## Material and methods

Cypselae and pollen grains of *D. parviflorus* were obtained from the following specimens: *Villegas S. s/n* (CHAPA, XAL), *Villareal J.A. 5389* (ANSM, ASU, XAL), and *Hinton J. 18638* (TEX, XAL). To understand the distribution of *D. parviflorus*, taxonomic literature was consulted (e.g. Crawford *et al.* 2009, Turner 2010, Flora of North America Editorial Committee 1993), as well as the GBIF database (https://www.gbif.org/). To understand the macromorphology, the specimens of *D. parviflorus* in Table 1 were observed. Herbarium acronyms followed the Index Herbariorum (sweetgum.nybg.org/science/ih).

**TABLE 1.** List of *Dicranocarpus parviflorus* specimens examined. Herbaria acronyms according to Index Herbariorum.

| Locality | Voucher information |
|---|---|
| Chihuahua | *Gentry H.S. et al. 23090* (ARI, DES, MEXU), *Henrickson J. 6757* (ANSM), *Moore M.J. 1573* (MEXU, TEX), *Wendt L.T. 9821* (LL, MEXU). |
| Coahuila | *Avalos M.L. 23* (ANSM), *Chiang F. 9871* (MEXU), *Esperanza R.R 401* (ANSM), *García A. 3134* (ANSM), *Henrickson J. 13011, 6737b*, and *24108* (ANSM MEXU, TEX), *Hinton G.B. s/n, 2760* (ANSM), *Iltis H.H. 68* (MEXU), *Keil D. 7991* (OBI), *McGill L. 7991* (ASU), *Moore M.J. 1991, and 2559* (MEXU, TEX), *Noriega V. s/n* (ANSM), *Palacios S. 2992* (ANSM), *Pinkava D.J. 13664* (ASU), *Robert M.F. 65XX* (ANSM), *Villareal J.A. 3516, 5389, 6155,* and *9061* (ANSM, ASU, MEXU, TEX), *Wendt L.T. 1882* (ANSM, MEXU). |
| Durango | *García A. 2728* (ANSM), *Henrickson J. 7974,* and *17577* (MEXU). |
| Nuevo León | *Chiang F. 8045* (MEXU), *Conquist A. 9829* (MEXU, TEX), *Guerrero G. 811* (ANSM), *Hinton G.B. 2760* (ANSM), *Johnson I.M. 1270, 11198, and 11405* (MEXU), *Moore M.J. 1897* (MEXU, TEX), *Nesom G. 4332* (MEXU, TEX), *Patterson T.F. 7434* (MEXU), *Pearce R. 2206* (TTC), *Sundberg S. 1832* (MEXU, NY, TEX). |
| San Luis Potosí | *García P.J. 671* (ANSM, MEXU), *Henrickson J. 22785* (MEXU), *Lehto E. 22339* (ASU), *Nesom G. 6632* (OBI, UCR), *Pinkava D.J. 13535* (ASU), *Rzedowski J. 8141* (MEXU), *Templeton B.C. 8720* (RSA), *Villaseñor 191* (MEXU). |
| Zacatecas | *Estrada E. 10680* (ANSM), *Reveal J.L. 3362* (BRY, TEX), *Villareal J.A. 9367*, and *6155* (ANSM). |

*......continued on the next page*

**TABLE 1.** (Continued)

| Locality | Voucher information |
|---|---|
| New Mexico | *Kenneth D.H. 34755, 34769,* and *36409* (SJNM). |
| Texas | *Blassingame, J.L. 3263* (HPC)*, Burgess T.L. 2565* (TTC)*, Green III L.T. 135* (TTC)*, Hinckley L.C. 361 and 390* (BRIT), *Manning P. 4089* (JWC), *Powell A.M. 2205*, and *6751* (ANSM, LL, OKLA), *Warnock B.H. 211 and 10281* (BRIT, LL), *Waterfall U.T. 583, 5706* (OKLA)*, and 5793* (BRIT), *Worthington R.D. 12564* (BRIT, COLO, OBI, TEX, UTEP), *Wright C. 348* (GH). |

To observe their morphology, pollen grains were acetolyzed following the methodology of Erdtman (1960). The grains were immersed in glacial acetic acid for 24 hours before acetolysis and then transferred to the acetolysis mixture for 30 minutes and the temperature of the water bath raised to 96 ºC. For light microscopy (LM), the pollen grains were mounted in glycerol jelly, sealed, and then examined under a Carl Zeiss Fomi III Optical Microscope, equipped with a Cannon Power Shot G9 digital camera. Permanent slides were deposited in the Palynological Laboratory of the Instituto de Ecología, A.C. in Xalapa in Veracruz, Mexico. The following pollen measurements were obtained from 25 grains per sample: polar axis, equatorial diameter, colpus length, colpus width, ora width, ora length, spinae length, spine width at base, and number of apertures. The terminology of Halbritter *et al.* (2018) was followed.

For anatomical observations of the cypselae, dried ray cypselae were initially rehydrated in a solution of 20% NaOH for 24 h and then rinsed with tap water, dehydrated in an acetone series (following the protocol of Márquez-Guzmán *et al.* 2016), and embedded in JB-4 resin, following the manufacturer's protocol. The samples were sectioned at a thickness of 10–15 µm using a rotary microtome. The resulting material was stained with 0.05% toluidine blue in acetate buffer at pH 4.7 (modified from O'Brien *et al.* 1964) and mounted on slides using a synthetic resin. Images were obtained on a Carl Zeiss Fomi III photomicroscope, equipped with a Cannon Power Shot G9 digital camera. The images were edited using PhotoScapeX image-editing software (MOOII Tech, Korea). General anatomical terminology followed Roth (1977), while that of the pericarp anatomy and cypsela shape followed Beentje (2016) and Julio & Oliveira (2009) respectively.

## Results

### Pollen morphology

The analyzed pollen grains are tricolporate and oblate spheroidal (P/E = 0.96–1.01) and radially symmetric (Fig. 4). Pollen size is P = 21.5 (22.5) 23.1 µm, E = 21.9 (23.2) 24.3 µm, and corresponds to a small grain (Erdtman 1969). The lalongate ora length is 1.5 (2.0) 2.6 µm, and width is 11.4 (12.4) 12.8 µm (Fig. 4A), with acute apices. The colpus is elliptical, of length 8.4 (8.8) 9.5 µm, and width 1.7 (2.1) 2.3 µm (Fig. 4A), apex acute. The exine is thinner, 0.7 (0.9) 1.2 µm, excluding the spines (Fig. 4B). The ornamentation is echinate; spine length ranges from 4.7(5.1) to 5.7 µm and width at base 4.6 to 5.2 µm. The spine shape is more deltate than conical (Fig. 4C), with apex acute.

### Cypselae anatomy

In cross-section, the ray cypselae are compressed to rounded, with indentations (Fig. 5A). The exocarp is formed by uniseriate thin-walled, rectangular transversely cells (Fig. 5B). The mesocarp is divided into three regions (Fig. 5B). The outer mesocarp comprises one to two layers of tracheoidal-like cells, these lengthen towards the interior in a radial direction forming irregular rows of up to 250 µm length, the thickenings in the walls are ca. 120 µm (Fig. 5C–D). The median mesocarp possesses a multiseriate layer of sclereids. Aerenchyma-like intercellular spaces are evident in the outer and median mesocarp (Fig. 5B). The inner region also has parenchymatic cells that are generally consumed when seeds develop. Vascular bundles were observed in the inner mesocarp (Fig. 5A). Phytomelanin layers or crystals are absent (Fig. 5B and E). The endocarp is consumed during the seed development (Fig. 5B).

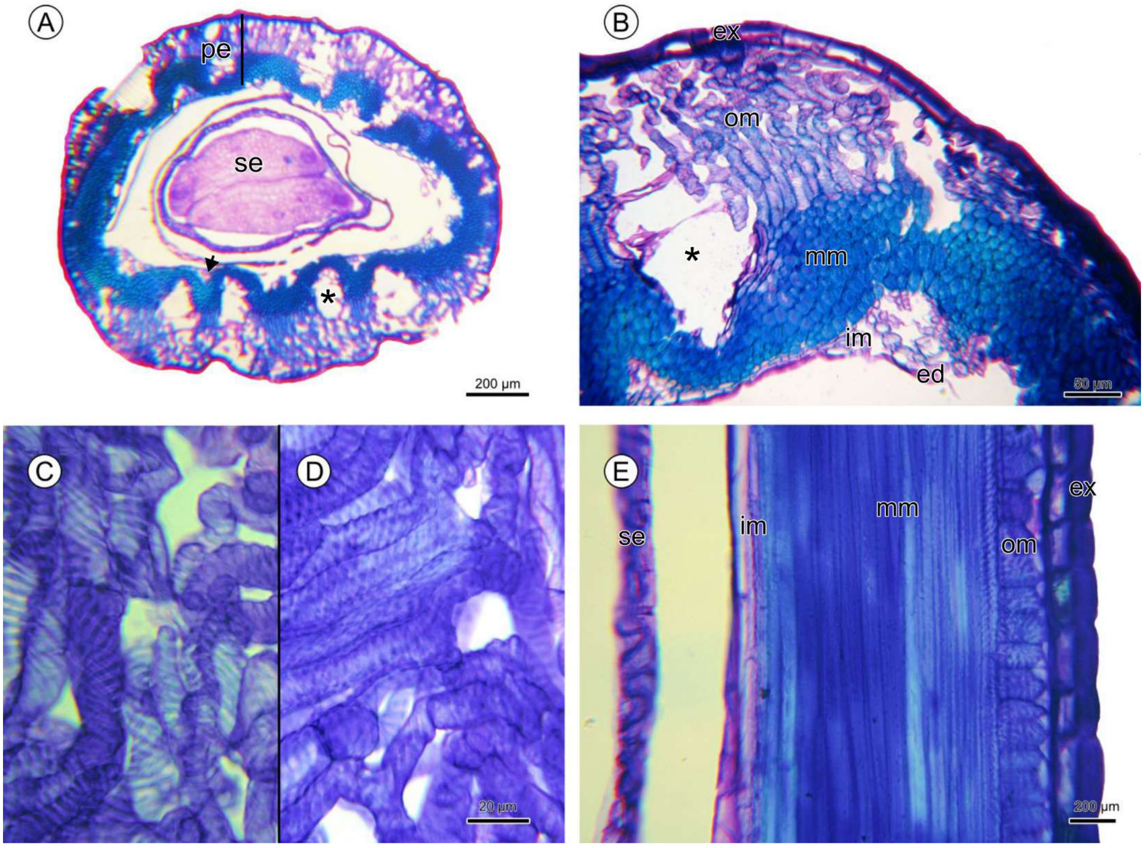

**FIGURE 5**. Cypsela of *Dicranocarpus parviflorus* observed by light microscopy. **A.** transversal cross-section. **B.** Detail of the wall. **C–D.** Detail of outer mesocarp. **E.** Longitudinal view. ex: exocarp; om: outer mesocarp; mm: median mesocarp; im: inner mesocarp; ed: endocarp; pe: pericarp; se: seed; asterisk: aerenchima-like; black arrow: vascular bundle.

## Discussion

The *Dicranocarpus* pollen shows similarity in attributes described for taxa of the tribe Coreopsideae (Tadesse *et al.* 1995b, Blackmore *et al.* 2009, Crawford *et al.* 2009, Tellería 2017). Pollen grains are echinate, spheroidal, round in both views, tricolporate, and with lalongate ora such as in species of Coreopsideae (Blackmore *et al.* 2009). Based on the classification proposed by Erdtman (1969), the pollen for Coreopsideae has a medium (25–50 µm) to small (10–25 µm) size (Tellería 2017). In addition, in the tribe, most species have tricolporate grains (Fig. 6A); however, some members in *Bidens* Linnaeus (1753) and *Dahlia* vary in terms of number of colpi (Wodehouse 1930, Tadesse *et al.* 1995b, Sánchez-Chávez *et al.* 2022). *Dahlia* differs by having tricolporate, hexacolporate, and much larger, pollen grains (Fig. 6B) (Wodehouse 1930, Sánchez-Chávez *et al.* 2022). Nevertheless, the pollen grains of *Dicranocarpus* are tricolporate and small (ranging from 21 to 24 µm). The ora and colpus in *Dicranocarpus* are similar to those observed in *Bidens, Coreopsis*, and *Hidalgoa* (Tadesse *et al.* 1995b, Sánchez-Chávez *et al.* 2022), the ora is lalongate, with width ranging from 11 to ca.13 µm, and the largest colpus is approximately 9 µm, with apices always acute in both apertures (Fig. 6C). The spines resemble those described in the tribe Coreopsideae (Tadesse *et al.* 1995b, Blackmore *et al.* 2009, Tellería 2017), deltate to conical, and smaller (4.7 to 5.7 µm). These similar characteristics between the pollen grain of *Dicranocarpus* and those of other genera in the tribe Coreopsideae support its membership in the group.

The anatomy of the cypselae in taxa in Coreopsideae has demonstrated uniform morphology (Pandey & Singh 1982, Julio & Oliveira 2009, Batista & De Souza 2017, Sánchez-Chávez *et al.* 2023), with some exceptions in *Fitchia* Hook.f. (Pandey *et al.* 2014), *Hidalgoa* (Sánchez-Chávez *et al.* 2023), and now in *Dicranocarpus*. The pericarp in species of the tribe consists of uniseriate compressed exocarp and a mesocarp with three regions: outer parenchymatic mesocarp, middle sclerenchymatic mesocarp, and inner parenchymatic mesocarp, with a phytomelanin layer deposited in a schizogenous space between the outer and middle mesocarps (Pandey & Singh 1982, Tadesse *et al.* 1995a, Julio & Oliveira 2009, Jana & Mukherjee 2014, Pandey *et al.* 2014, Tadesse & Crawford 2014, Batista & De Souza 2017, Souza-Filho *et al.* 2019, Mathur & Pandey 2020, Sánchez-Chávez *et al.* 2023). However, the cypsela of *D. parviflorus* differs in the outer mesocarp, which comprises one or two layers of tracheoidal-like cells, forming irregular rows reaching a length of 250 µm. Following a review of the specialized literature, we were unable to find any examples in Asteraceae that present this pattern in the outer mesocarp; however, it should be noted that many taxa in Coreopsideae remain to be studied. Our hypothesis is that these tracheoidal-like cells might function as protective tissue in the absence of phytomelanin. This attribute is probably an adaptation in response to dry environments, the recorded habitat of these plants. We suggest that further histochemical tests will determine composition of these cells; we were not able to conduct these tests because our samples were embedded in resin.

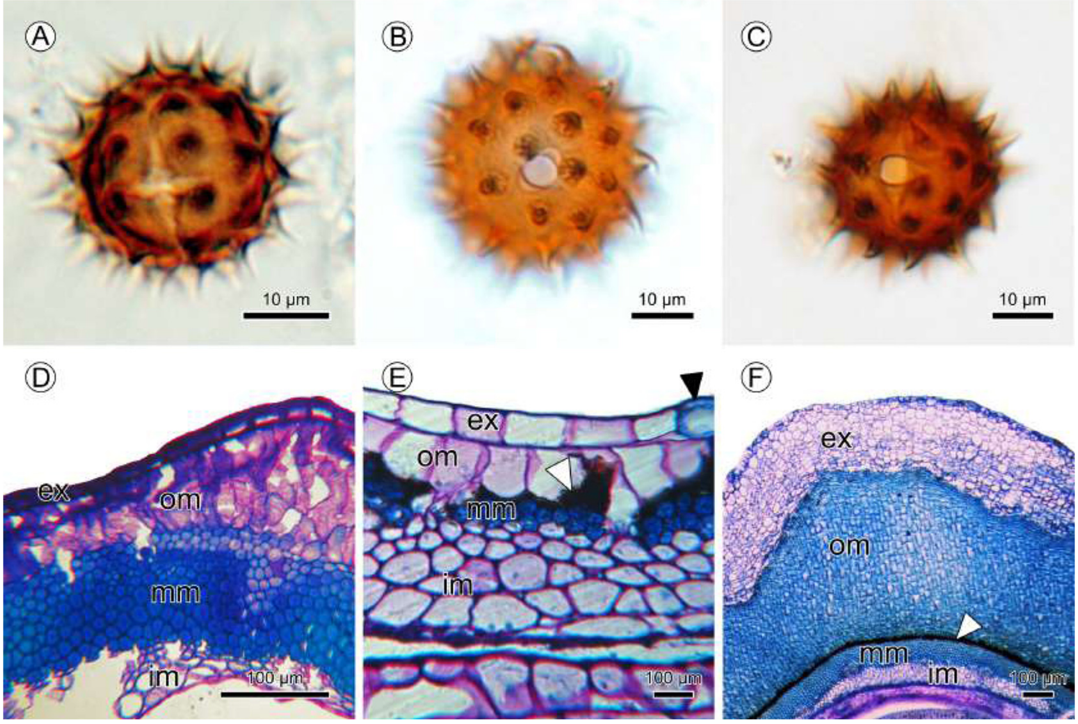

**FIGURE 6.** Pollen and cypselae comparison among *Dicranocarpus*, *Dahlia*, and *Hidalgoa.* **A–C.** Pollen grain in equatorial view. **A.** *Dicranocarpus parviflorus*. **B.** *Dahlia cuspidata*. **C.** *Hidalgoa ternata*. **D–F.** Detail of the wall of the cypsela, **D.** *Dicranocarpus parviflorus*. **E.** *Dahlia linearis.* **F.** *Hidalgoa pentamera.* ex: exocarp; om: outer mesocarp; mm: median mesocarp; im: inner mesocarp; white arrowhead: phytomelanin.

Despite the close phylogenetic relationship of *Dahlia*, *Dicranocarpus*, and *Hidalgoa* (Mort *et al.* 2008, Sánchez-Chávez *et al.* 2019), the pericarp structure differs among them. The exocarp in *Dahlia* is uniseriate, thin-walled, with isodiametric cells, longitudinally or transversely elongated, and several species possess some cells along the exocarp with a well-developed secondary wall (Fig 6E) (Sánchez-Chávez *et al.* 2023). The exocarp in *Hidalgoa* is formed by multiseriate, walled isodiametric cells (Fig. 6F) (Sánchez-Chávez *et al.* 2023), while the exocarp in *Dicranocarpus* is formed by uniseriate thin-walled, rectangular transversely cells (Fig 6D). The outer mesocarp is also distinctive among them. In *Dahlia* the outer mesocarp is uniseriate with thin-walled palisade parenchyma (Fig 6E) (Sánchez-Chávez *et al.* 2023). In *Hidalgoa* it is multiseriate, comprising several layers of rectangular cells of parenchyma, thickening to sclerification in mature cypsela (Fig 6F) (Sánchez-Chávez *et al.* 2023), while in *Dicranocarpus*, as mentioned previously, it comprises one or two layers of tracheoidal-like cells, forming irregular rows reaching a length of 250 µm (Fig 6D).

The presence of phytomelanin in the cypsela is a common attribute of most genera of the Heliantheae alliance (Anderberg *et al.* 2007). Previously, several studies determined phytomelanin as a synapomorphy to the Heliantheae alliance (Panero 2007, Robinson 2009); however, recent anatomical studies (Pandey *et al.* 2014, Freitas *et al*. 2015, Lusa *et al*. 2018, Mathur & Pandey 2020, Marques *et al*. 2021) have shown that the phytomelanin in Asteraceae is more widely distributed. Currently, a variety of shapes have been described in the walls of the cypselae in the Heliantheae alliance, and in other tribes of the family. In *Fitchia* (tribe Coreopsideae), which lacks phytomelanin, the presence of calcium oxalate crystals was described (Pandey *et al.* 2014). Another example in the Heliantheae alliance is Helenieae, the tribe that does not have phytomelanin but possesses crystals (Baldwin 2009). Tadesse & Crawford (2014) described six types of surface ornamentation in *Bidens* and *Coreopsis*, two of them characterized by the absence of phytomelanin but with deposition of dark brown secretions. Marques *et al.* (2021) demonstrated that in *Lychnophora salicifolia* (Vernonieae), it is possible for both substances, phytomelanin and calcium oxalate crystals to coexist in pericarp. Cypselae with phytomelanin occur in *Dahlia* and *Hidalgoa*. However, the phytomelanin deposition differ among them. In *Dahlia*, the phytomelanin forms a discontinuous layer, deposited between the outer and median mesocarp regions in a spine-like pattern (Fig. 6E) (Sánchez-Chávez *et al.* 2023). In *Hidalgoa*, the phytomelanin is also deposited between the outer and median mesocarp regions, forming a continuous layer (Fig. 6F) (Sánchez-Chávez *et al.* 2023). In *Dicranocarpus*, the lack of phytomelanin on the pericarp distinguishes it not only from these genera but also from other members of Coreopsideae; moreover, this is the first report of the lack of phytomelanin and crystals in the pericarp of Coreopsideae (Fig. 6D). Finally, it is interesting to highlight the presence of intercellular spaces, aerenchyma-like, in *Dicranocarpus* while in *Dahlia* and *Hidalgoa*, as well as in the rest of Coreopsideae secretory ducts are present.

Regarding macromorphological characters, *Dicranocarpus* shares attributes with *Hidalgoa* in terms of the sexual condition of the disc and ray florets (Table 2), although it has been suggested that the sexuality of the disc and even ray florets have evolved independently in lineages of Asteraceae (Stuessy 1973, Turner 1978, Robinson 1981, Kimball & Crawford 2004, Mamut & Tan 2014). Leaf attributes and habits differ among *Dicranocarpus, Hidalgoa*, and *Dahlia* (Table 2). In fact, *Dicranocarpus* was placed in tribe Coreopsideae based on characters such as pappus with two awns, and phyllaries and paleae with brown-orange longitudinal stria (Stuessy 1973). Hence, further molecular and morphological studies could therefore clarify the relationships among these tree genera and provide diagnostic characters that reflect the evolutionary history among these tree genera.

**TABLE 2.** Comparison of attributes of *Dicranocarpus*, *Dahlia*, and *Hidalgoa*.

| Features | *Dicranocarpus* | *Dahlia* | *Hidalgoa* |
|---|---|---|---|
| Duration | annual | perennial | perennial |
| Habit | herbaceous | herbaceous, shrub, liana | herbaceous |
| Leaf blade | segmented | segmented, simple | simple, segmented |
| Sexual condition of ray florets | pistillate, fertile | generally sterile, rarely pistillate | pistillate, fertile |
| Sexual condition of disc florets | functionally male | fertile | functionally male |
| Ray floret length | 0.5–2.5 mm | greater than 1 cm | greater than 2 cm |
| Ray floret color | yellow | white, pink, yellow, lavender to purple, red-orange, blackish- scarlet | yellow, orange |
| Disk florets | 3–8 | numerous | 25–55 |
| Polar axis of pollen (µm) | 21.5 (22.5) 23.1 | 25.3 (31) 39.4 | 25.7 (29.1) 34 |
| Number of apertures | tricolporate | tricolporate, hexacolporate | tricolporate |
| Ora length (µm) | 1.5 (2.0) 2.6 | 1.0 (2.5) 5.4 | 1.7 (2.4) to 4.8 |
| Ora width (µm) | 11.4 (12.4) 12.8 | 1.2 (3.6) 8.6 | 6.3 (9.3) to 13.0 |
| Colpus length (µm) | 8.4 (8.8) 9.5 | 2.2 (4.8) 9.2 | 11.2 (14.7) 16.8 |
| Colpus width (µm) | 1.7 (2.1) 2.3 | 1.2 (2.8) 5.8 | 2.6 (3.3) 4.2 |
| Os lalongate | lalongate | non-lalongate to rarely lalongate | lalongate |
| Spinae length (µm) | 4.7(5.1) 5.7 | 4.2 (7.7) 12.2 | 4 (5.6) 6.7 |
| Length of cypselae (mm) | 5 to 10 | 8 to 18 | 14 to 18 |
| Awn length, cypselae (mm) | 2 to 5 | inconspicuous, with minute teeth to 3 to 5 | 3 to 5 |
| Outer mesocarp in cypselae | uniseriate to biseriate | uniseriate | multiseriate |
| Phytomelanin | absent | present, irregularly in spine | present, irregular and smooth |
| Aerenchyma | present | absent | absent |
| Ploidy level | 10 | 16, 17, 18, 32 | 15, 16, 17 |

The results obtained in this study confirm the importance of pollen attributes for the recognition of *Dicranocarpus* as a member of the tribe Coreopsideae and that some of them are shared with *Hidalgoa* (Mort *et al*. 2008, Sánchez-Chávez *et al.* 2019). Moreover, a more extensive study of the anatomy of the cypselae in Asteraceae is recommended to help understand the evolution and the importance of characters such as pattern and presence of phytomelanin in the diverse lineages of this family.

## Conclusions

Although the pollen of *Dicranocarpus parviflorus* does not possess attributes that distinguish it from the rest of the Coreopsideae tribe, except *Dahlia*, it was useful to confirm the membership of the species in the tribe. The anatomy of the cypselae demonstrated that *D. parviflorus* has characters with taxonomic value that distinguish it from the rest of the Coreopsideae, as well as others that are shared with other species in the tribe. The three most significant novelties in *Dicranocarpus* were observed in the outer mesocarp consisting of tracheoidal-like cells forming irregular rows of up to 250 µm length, the aerenchyma-like cells in the median mesocarp, and the lack of phytomelanin and calcium oxalate crystals. The results regarding the patterns of phytomelanin in the studied taxa highlight the need for further anatomical studies of the cypselae of additional species to help understand the evolution of phytomelanin deposition in the Heliantheae Alliance.

## Acknowledgments

We thank Keith MacMillan for editing the English version of the paper. We thank Dr. José Angel Villarreal, curator of the ANSM herbarium, for digitalizing important specimens for this study. Thanks also go to Sonia Galicia for providing invaluable help in the anatomy lab and assistance with optical microscopy. We thank Teresa Jimenez for the illustrations. E. Sánchez-Chávez was supported by a Doctorate in Science scholarship from CONAHCyT, Mexico (753335).

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