

## Micrographic standarization of *Baccharis* L. species (Asteraceae)

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### Summary

Controversies still exist regarding the differentiation of some *Baccharis* sect. Caulopterae species known as “carqueja”. In the present work we studied anatomically five of these species: *Baccharis articulata* (Ba), *Baccharis crispa* (Bc), *Baccharis gaudichaudiana* (Bg), *Baccharis microcephala* (Bm) and *Baccharis trimera* (Bt). Ba and Bc are considered official in Argentinean Pharmacopeia and Bt in the Brazilian Pharmacopeia. Bg and Bm are considering possible substitutes. The qualitative and quantitative variables for each one were determined, emphasizing the importance of anatomic characters as taxonomically useful. Analytical micrographs of the studied species were elaborated as reference standards for quality control of the raw drug.

## Estandarización micrográfica de especies de *Baccharis* L. (Asteraceae)

### Resumen

Aún existen controversias con respecto a la correcta diferenciación de las especies de *Baccharis* de la sección Caulopterae conocidas como “carqueja”. En este trabajo se estudió anatómicamente a cinco de estas especies: *Baccharis articulata* (Ba), *Baccharis crispa* (Bc), *Baccharis gaudichaudiana* (Bg), *Baccharis microcephala* (Bm) y *Baccharis trimera* (Bt). Las especies Ba y Bc son consideradas oficiales en la Farmacopea Argentina y Bt en la Farmacopea Brasileña. Bg y Bm son consideradas posibles sustituyentes de las anteriores. Se determinaron las variables cualitativas y cuantitativas para cada una, resaltando la importancia de la anatomía en la obtención de caracteres taxonómicos para poder diferenciarlas. Con los datos obtenidos se elaboraron las micrografías analíticas de las especies estudiadas, para que puedan ser utilizadas como patrones de referencia para el control de calidad de la droga cruda.

### Introduction

*Baccharis* L. is the most numerous genus in the Astereae tribe, with a geographical distribution restricted to America, from Canada to Southern Argentina and Chile (Fielding, 2001).

Several authors have contributed to the infrageneric classification of *Baccharis* (Lessing, 1831; De Candolle, 1836; Weddell, 1855, 1856; Baker, 1882, 1884; Heering, 1904; Cuatrecasas, 1967; Ariza Espinar, 1973; Barroso, 1976). It was De Candolle (1836) who first subdivided the genus

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**Key words:** Anatomy - Asteraceae - *Baccharis* - carqueja - micrography - sect. Caulopterae.

**Palabras clave:** Anatomía - Asteraceae - *Baccharis* - carqueja - micrografía - sección Caulopterae.

into sections (sect.), based mainly on leaf morphology. More recently, Giuliano (2001) subdivided the 96 Argentine *Baccharis* species into 15 sect. Among them, sect. Caulopterae DC is characterized by the presence of species with winged stems. Species of this sect. are known popularly as “carqueja” and two of them, i.e., *Baccharis articulata* (Lam.) Pers. and *Baccharis crispa* Spreng. are included in the Farmacopea Nacional Argentina Ed. VI (1978) and a third one, *Baccharis trimera* (Less.) DC. in the Farmacopéia Brasileira Ed. IV (2002).

They are used as hepatic, stimulating to bile secretion, diuretic drugs, for ulcer healing and as external antiseptics, in infusions or decoctions. (Hieronymus, 1882; Sorarú and Bandoni, 1978; Toursarkissian, 1980; Martínez Crovetto, 1981; Correa, 1985).

Although there is abundant information about the genus in the literature, there are many controversies regarding the correct nomenclature as many synonyms exist especially in sect. Caulopterae DC. (Ariza Espinar, 1973; Lonni *et al.*, 2005; Simões-Pires *et al.*, 2005; Müller, 2006).

The species in sect. Caulopterae that have been most studied anatomically are *B. articulata*, *B. crispa* and *B. trimera* (Metcalf and Chalk, 1972; Ariza Espinar, 1973; Cortadi *et al.*, 1999; Barboza *et al.*, 2001; Müller, 2006; Freire *et al.*, 2007).

Müller (2006) differentiated *Baccharis genistelloides* subsp. *crispa* (here: *B. trimera*), that contains tufts of 3-7-celled clavate uniseriate hairs, from *B. articulata*, that contains tufts of 4-6-celled flagellate hairs. In contrast, Freire *et al.* (2007) reported that the trichomes of four species of the Caulopterae sect., *B. articulata*, *B. gaudichaudiana* DC., *B. microcephala* (Less.) DC. and *B. trimera*, are bulbiferous flagellate with triangular apical cells; however *B. trimera* differed from the other three species due to its anisocytic stomata.

Several authors also reported this type of stomata in *B. articulata* (Ariza Espinar, 1973; Cortadi *et al.*, 1999; Barboza *et al.*, 2001). In addition to these diverse studies, it is important to take in account that Baker (1882; 1884) considered that *B. gaudichaudiana* was the same species as *B. articulata*. Ariza Espinar (1973) studied the anatomy of the two official Argentine species (*B. articulata* and *B. crispa*) and Cortadi *et al.*, (1999) reported on the anatomy of the winged

stems of three species of the Caulopterae sect., *B. articulata*, *B. crispa* and *B. trimera*, finding some differences between them.

Another study aimed to discriminate two or three *Baccharis* species of the Caulopterae sect. by using micrographic characters or chemical composition of vouchers, only involved the presence or absence of isolated metabolites (Gianello *et al.*, 2000, Rodríguez *et al.*, 2008).

However, the information from these studies is incomplete and therefore they are inconclusive about the proper differentiation of some species of the Caulopterae sect. and so, there is a general consensus that a complete morphoanatomical examination of the some winged stem species to obtain both the qualitative and quantitative variables is needed.

Therefore the aim of this work is to perform a morphoanatomical exhaustive study of three official “carqueja” species: *B. articulata*, *B. crispa* and *B. trimera* and two possible substituents: *B. gaudichaudiana* and *B. microcephala* to achieve micrographic standardization of them.

## Materials

### *Plant material*

Specimens from the following herbaria: UNR, SI, CTES, BAF and LP (abbreviations according Holmgren *et al.*, 1990), or fresh material collected by the authors during five collecting campaigns in 2004, 2005, 2006, 2007 and 2008, were examined; all materials were collected with flowers and/or fruits to enable identification, checked by the authors and stored in the UNR herbarium (Table 1).

## Methods

### *Morphoanatomy*

Fresh material was fixed in F.A.A. (70° ethanol, glacial acetic acid, formaldehyde and water 50:5:30:15). The herbarium material was hydrated in boiling water with added drops of detergent.

Zeiss MC 80 Axiolab light microscope equipped with a photographic camera and Nikon Alphaphot YS light microscope with polarized light and a Nikon Type 104 stereoscopic drawing tube were used for the microscopic examination.

Table 1.- Data of analysed samples of *Baccharis* species

	Site	Date	Voucher
<i>Baccharis accharis</i>	<i>Prov. of Misiones</i>		
	Dpto. Cainguas, Loc. Campo Grande, Ruta Nac. 8	1/VIII/1987	Vanni <i>et al.</i> 976 (CTES)
	Dpto. Apóstoles	X/1977	Cabrera 28469 (SI)
	<i>Prov. of Entre Ríos</i>		
	Dpto. Federación, Loc. Santa Ana	24/IX/1961	Burkart 22823 (SI)
	Dpto. Colón, Loc. Colón, Parque Nacional El Palmar	11/II/2006	Gattuso S. 770 (UNR)
	Dpto. Uruguay, Loc. Pronunciamiento	VIII/ 2005	Gattuso S. 807 (UNR)
	Dpto. Uruguay, Loc. Pronunciamiento	11/II/2006	Gattuso S.769 (UNR)
	<i>Prov. of Chaco</i>		
	Dpto. Sgto. Cabral, Parque Nacional Chaco	23/XI/1991	Fortunato 2593 (SI)
	<i>Prov. of Santa Fe</i>		
	Dpto. Capital, Loc. Santa Fe	VIII/ 2005	Gattuso M. 189 (UNR)
	Dpto. Capital, Loc. Santa Fe	13/XII/2005	Gattuso M. 190 (UNR)
	Dpto. Belgrano, Loc. Las Rosas	15/IX/2005	Gattuso M. 186 (UNR)
	Dpto. Rosario, Loc. Rosario	18/I/2007	Gattuso S. 854 (UNR)
	Dpto. Rosario, Loc. Rosario	18/I/2007	Gattuso S. 855 (UNR)
	Dpto. Rosario, Loc. Arroyo seco	VIII/ 2007	Mc Cargo J. 29 (UNR)
	<i>Prov. of Buenos Aires</i>		
	Pergamino, cruce de Rutas 188 y 18 camino a Bavio	2/V/2006	Gattuso M. 222 (UNR)
		30/III/1975	Zardini 592 (SI)
	<i>Prov. of Tucumán</i>		
	Villa Nougues	12/VII/1907	Lizer 55 (SI)
	Villa Nougues	X/1966	Esk 02513 (SI)
	<i>Prov. of Córdoba</i>		
	Dpto. Punilla, Loc. Ongamira, Paraje: "Las dos Lunas"	25/II/2006	Gattuso S. 789 (UNR)
	Dpto. Punilla, Loc. Asconchinga, "Potrero de los peones"	25/II/2006	Gattuso S. 790 (UNR)
	Dpto. Punilla, Loc. La Cumbre	I/2007	Gattuso S. 856 (UNR)
	Dpto. Calamuchita, camino a Atos Pampa, a 1 km de la Ruta Villa Gral. Belgrano y La Cumbrecita	19/IV/2005	Gattuso M. 183 (UNR)
	Dpto. Calamuchita, Loc. Yacanto de Calamuchita	11/I/2006	Gattuso M. 191 (UNR)
	Dpto. Calamuchita, Loc. El Durazno	4/III/2006	Gattuso S. 799(UNR)
	Dpto. Calamuchita, Loc. El Durazno	4/III/2006	Gattuso S. 800 (UNR)
	Dpto. Calamuchita, Loc. El Durazno	4/III/2006	Gattuso S. 801 (UNR)
	Dpto. Calamuchita, Loc. El Durazno	4/III/2006	Gattuso S. 802 (UNR)
	Dpto. Calamuchita, Loc. El Durazno	4/III/2006	Gattuso S. 803 (UNR)
	Dpto. Calamuchita, Loc. El Durazno	4/III/2006	Gattuso S. 804 (UNR)
	<i>Prov. of Buenos Aires</i>		
	Tandil, en el cerro Albión	3/III/1946	Krapovickas 2989 (SI)
	<i>Prov. of Córdoba</i>		
	Dpto. Punilla, Loc. Ascochinga, "Potrero de los peones"	25/II/2006	Gattuso S. 791 (UNR)
	Dpto. Punilla, 5 km al O de Capilla del Monte	19/III/2005	Gattuso M.156 (UNR)
	Dpto. Punilla, 5 km al O de Capilla del Monte	19/III/2005	Gattuso M.157 (UNR)
	Dpto. Calamuchita, Loc. Los Reartes, puente San Ignacio de Loyola	11/III/2006	Gattuso M. 208 (UNR)
Dpto. Calamuchita, Loc. Yacanto de Calamuchita	18/IV/2005	Gattuso M.153 (UNR)	
Dpto. Calamuchita, Loc. Mina Clavero	III/1940	Valencia 2376 (SI)	
<i>Prov. of San Luis</i>			
Dpto. Cnel. Pringles, camino a la Mina Carolina	28/IV/1986	Volponi 939 (SI)	
Dpto. La Capital, Loc. El Volcán	1/IV/1983	Agiglia 6486 (SI)	
<i>Prov. of Mendoza</i>			
Dpto. Las Heras, Loc. Potrerillos, Salto	15/I/2006	Gattuso S. 778 (UNR)	
Dpto. Luján de Cuyo, Loc. Luján, "Las Chacritas"	20/III/1944	Covas 2038 (SI)	
Dpto. Tupungato, a 5 km al O de la estancia "La Selva"	17/IV/1986	Leuenberger & Arroyo 3571 (SI)	
Dpto. Tunuyán, entre Tunuyán y El Manzano	10/III/1945	Covas (SI)	

**Table 1.-** Data of analysed samples of *Baccharis* species (cont.)

	Site	Date	Voucher
<i>Baccharis gaudichaudiana</i>	<i>Prov. of Misiones</i>		
	Dpto. San Ignacio, Loc. Jardín América	20/II/2006	Gattuso, M. 209 (UNR)
	Dpto. San Ignacio, Loc. San Ignacio	12/X/2005	Gattuso M. 177 (UNR)
	Dpto. San Ignacio, Loc. San Ignacio	12/X/2005	Gattuso M.179 (UNR)
	Dpto. San Ignacio, Loc. San Ignacio	11/III/2005	Gattuso M. 181 (UNR)
	Dpto. San Ignacio, Loc. San Ignacio	20/II/2006	Gattuso, M. 210 (UNR)
	Dpto. San Ignacio, Loc. San Ignacio, camino de Loreto a San Ignacio	11/X/1975	Zardini <i>et al.</i> 658 (SI)
	Dpto. Candelaria, Loc. Santa Ana	3/IX/1912	Rodríguez 539 (SI)
	Dpto. Leandro N. Alem, Loc. Cerro Azul	11/III/2005	Gattuso, M. 182 (UNR)
	<i>Prov. of Jujuy</i>		
Dpto. Santa Bárbara, Loc. Abra de los Morteros	IX/1976	Cabrera 27975 (SI)	
Dpto. Capital, Loc. Capilla	22/IX/1981	Ahumada 4324 (SI)	
<i>Baccharis microcephala</i>	<i>Prov. of Misiones</i>		
	Dpto. Candelaria, Loc. Loreto	II/1945	Montes s. n. (SI)
	Dpto. Apóstoles, Ruta 14, al NE del cruce a San José	20/II/2006	Gattuso M. 212 (UNR)
	<i>Prov. of Corrientes</i>		
	Dpto. Ituzaingó, Ruta Nac. 12 a 10 km de Ituzaingó	25/I/1976	Romancksuk <i>et al.</i> 367 (SI)
	Dpto. Mercedes, Bañado del Ayuí	20/X/2005	Gattuso M. 185 (UNR)
	Dpto. Mercedes, Cuenca del Ayuí	20/X/2005	Gattuso M. 184 (UNR)
	Dpto. Monte Caseros, km 173, Campo Gral. Ávalos	21/2/1979	Schinini <i>et al.</i> 17491 (CTES)
	<i>Prov. of Entre Ríos</i>		
	Dpto. Uruguay, Loc. Concepción del Uruguay	6/X/1950	Hunziker 4384 (SI)
	Dpto. Paraná, Paracao, barrancas frente a cuarteles	31/X/1962	Boelcke & Correa 9137 (BAA)
	Delta del Paraná, Arroyo Martínez	13/X/1944	Boelcke 942 (SI)
	<i>Prov. of Formosa</i>		
Dpto. Laishi, Reserva "El Bagual"	17/II/2006	Gattuso M. 211 (UNR)	
<i>Prov. of Santa Fe</i>			
Dpto. Rosario, Loc. Rosario	27/X/2005	Gattuso S. 768 (UNR)	

The wings were dehydrated with increasing concentrations of alcohol and coated with gold-palladium. Observations were made using a JEOL scanning electron microscope, model 35-CI.

#### *Surface view of epidermis*

The wings of the stems were diaphanised according to Strittmatter's technique (1973) when KOH 10% was used to remove the resin layer.

#### *Cross-sections of winged stems*

The material was dehydrated in increasing ethanol concentrations, then with ethanol/xylene and xylene and lastly embedded in paraffin (Gattuso and Gattuso, 2002). Cuts were performed manually with a Minot microtome, obtaining 12 µm thick sections. Diluted Safranin and Safranin-Fast green were used for staining (Strittmatter, 1979). The material was also dehydrated in increasing acetone concentrations,

acetone/propylene oxide and propylene oxide and embedded in Spurr's epoxy resin (Union Carbide International Co.). The stem segments were cut into 1 µm sections obtained with an ultramicrotome, equipped with a diamond knife. Toluidine Blue 1% and Acid Fuchsin 1% were used for staining (D'Ambrogio, 1986).

Crystals were observed using weak diluted acid and polarized light analysis (Johansen, 1940).

## Results and discussion

### *Alate stems anatomy – Qualitative and Quantitative variables*

Table 2 summarizes the differential anatomical qualitative variables of five species of *Baccharis* with winged stems.

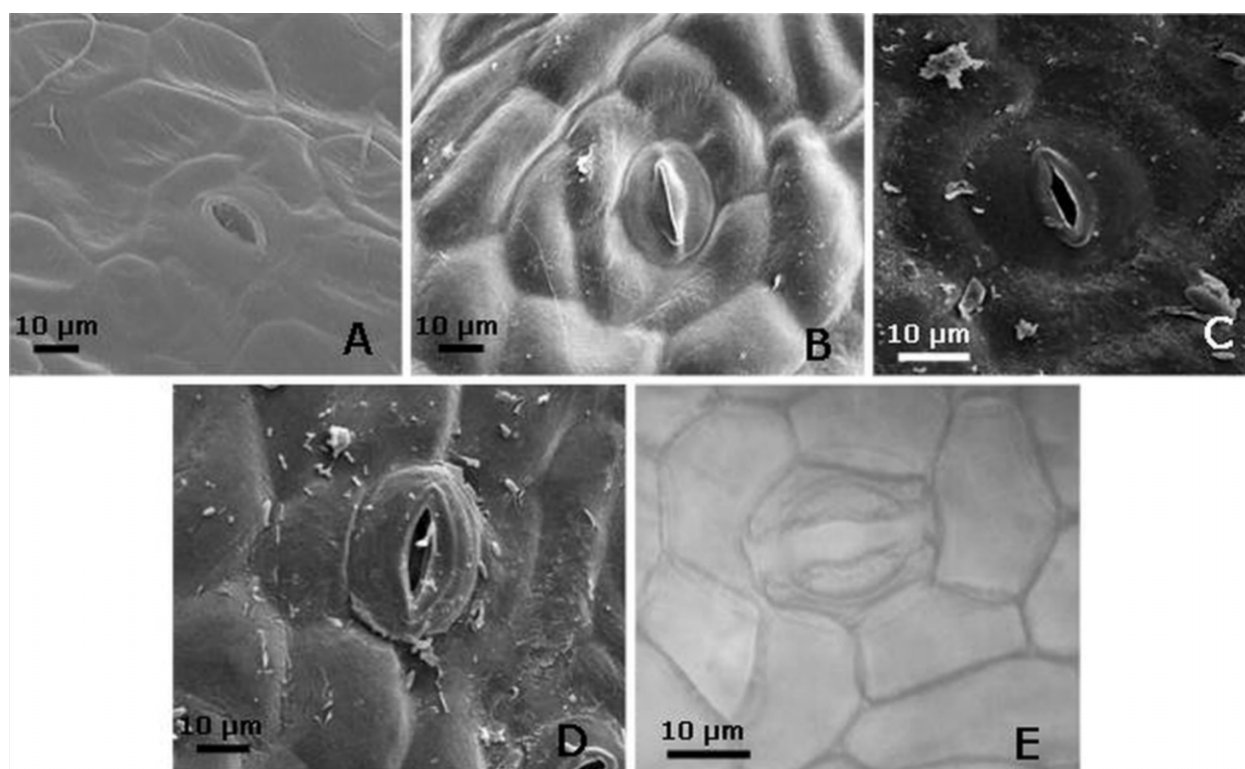
**Table 1.-** Data of analysed samples of *Baccharis* species (cont.)

	Site	Date	Voucher
<i>Baccharis trimera</i>	<i>Prov. of Misiones</i>		
	Dpto. Guaraní, Ruta Prov. 21, camino de Paraíso a Moconá, a 23 km de la Ruta Nac. 14	28/IV/1997	Morrone <i>et al.</i> 2177 (SI)
	<i>Prov. of Corrientes</i>		
	Dpto. Mercedes, Loc. Mercedes	8/VII/2005	Gattuso M. 187 (UNR)
	Dpto. Mercedes, Loc. Mercedes	2/II/2006	Gattuso M. 213 (UNR)
	Dpto. Paso de Los Libres, Estancia "El Recreo", costa del Río Uruguay	19/II/1979	Schinini 17333 (SI)
	Loc. Bonpland	13/III/2005	Gattuso M. 152 (UNR)
	Loc. Bonpland	13/III/2005	Gattuso M. 166 (UNR)
	Dpto. Alvear, ruta 14 entre Santo Tomé y Alvear	22/II/2006	Gattuso M. 244 (UNR)
	<i>Prov. of Entre Ríos</i>		
	Dpto. Federación, Loc. Colonia La Argentina	3/II/1983	Guaglianone <i>et al.</i> 1238(SI)
	Loc. Isthilart, camino a Calchaquí	18/I/1977	Troncoso 1562 (SI)
	Dpto. Colón, Parque Nacional El Palmar	14/III/2005	Gattuso M. 148 (UNR)
	Dpto. Colón, Parque Nacional El Palmar	14/III/2005	Gattuso M. 150 (UNR)
	Dpto. Colón, Parque Nacional El Palmar	11/II/2006	Gattuso S 772 (UNR)
	Dpto. Colón, Parque Nacional El Palmar	11/II/2006	Gattuso S. 773 (UNR)
	Dpto. Uruguay, Loc. Pronunciamiento	11/XII/2004	Gattuso M. 154 (UNR)
	Dpto. Uruguay, Loc. Pronunciamiento	11/II/2006	Gattuso S. 771 (UNR)
	Dpto. Uruguay, Loc. Pronunciamiento	11/II/2006	Gattuso S. 774 (UNR)
	Dpto. Uruguay, Loc. Pronunciamiento	11/II/2006	Gattuso S. 780 (UNR)
	Dpto. Uruguay, Loc. Pronunciamiento	22/III/2006	Gattuso S. 808 (UNR)
	Dpto. Uruguay, Loc. Pronunciamiento	22/III/2006	Gattuso S. 809 (UNR)
	Dpto. Uruguay, Colonia Dolores	11/XII/2004	Gattuso M. 155 (UNR)
	Dpto. Uruguay, Loc. Concepción del Uruguay, Colonia Elías	22/III/2006	Gattuso S. 816 (UNR)
	<i>Prov. of Buenos Aires</i>		
	Pergamino, cruce de Rutas 188 y 18	2/V/2006	Gattuso M. 223 (UNR)
	Elizalde, Praderas	14/III/1940	Cabrera 6337 (SI)
	Berisso, Ruta 11, km 11	8/IV/2006	Gattuso M. 220 (UNR)
Punta Lara	3/III/2002	Lara (BAF)	
Batolomé Bavio	IV/2003	Rivas (BAF)	
Tronquist, Sierra de la Ventana	3/III/1984	Hunziker & Wulff 12055 (SI)	
<i>Prov. of Santa Fe</i>			
Dpto. Rosario, Loc. Rosario	27/X/2005	Gattuso S. 767 (UNR)	
Dpto. Rosario, Loc. Rosario	IX/1997	Reyna (LP)	
San Marcos	16/II/1941	Nicora 17735 (SI)	
<i>Prov. of Salta</i>			
Dpto. Santa Victoria, Loc. Los Toldos, Río Toldos, frente a la Quebrada del Astillero	30/X/1987	Novara 7129 (SI)	
<i>Prov. of Catamarca</i>			
Dpto. Andalgalá, Loc. El Condado	4/V/1916	Jorgensen 1418 (SI)	

Table 3 summarizes the anatomical quantitative variables of the same species and table 4 shows the quantitative variables that differ between these five species.

Anomocytic stomata are present in the five studied species and this coincides with the reports of Metcalfe and Chalk (1972) for the Asteraceae; Cortadi *et al.* (1999) for *B. articulata*, *B. crispa*

and *B. trimera* and Freire *et al.* (2007) for *B. microcephala*. Cyclocytic stomata in *B. articulata* and anisocytic stomata in *B. trimera* were previously reported by Pertusi (1987). Other authors also reported anisocytic stomata in *B. trimera* (Cortadi *et al.*, 1999; Freire *et al.*, 2007) and *B. crispa* (Ariza Espinar, 1973; Cortadi *et al.*, 1999; Barboza *et al.*, 2001; Freire *et al.*, 2007).

**Figure 1.-** Stomata type present in the five studied species

**A-D:** Scanning electron micrograph; **E:** light micrograph. **A-B:** ciclocytic stomata of *B. gaudichaudiana* and *B. articulata*, respectively; **C:** anisocytic stomata of *B. trimera*; **D-E:** anomocytic stomata of *B. crispa* and *B. microcephala*, respectively.

**Table 2.-** Differential anatomical qualitative variables of five species of sect. Caulopterae with winged stems

	Wings				
	<i>Ba</i>	<i>Bc</i>	<i>Bg</i>	<i>Bm</i>	<i>Bt</i>
<b>ST</b>	Anomocytic Ciclocytic	Anomocytic Anisocytic	Anomocytic Ciclocytic	Anomocytic	Anomocytic Anisocytic
<b>UTT</b>	3-4 basal cells, acutely curved, terminal cell not very long, with the subterminal cell larger than other cells and terminal cell narrower than remaining cells of the trichome (flagellate trichomes)	2-3 basal cells wider than long. Terminal cell is acute at apex and presents thick cell wall, which gives a smooth appearance to its surface (armed trichomes)	3-4 basal cells, acutely curved, terminal cell not very long, with the subterminal cell larger than other cells and terminal cell narrower than remaining cells of the trichome (flagellate trichomes)	3-4 basal cells, acutely curved terminal cell not very long, with the subterminal cell larger than other cells and the terminal cell narrower than remaining cells of the trichome (flagellate trichomes)	3-4 basal cells, curve triangular terminal cell, not very long, shaped "nails", with thin cell walls, giving it a rough appearance on the surface, with subterminal cell as wide as the terminal cell and not longer than the others. (clavate trichomes).
<b>MB</b>	Presence of laminar collenchyma	Absence of laminar collenchyma	Presence of laminar collenchyma	Presence of laminar collenchyma (only 1 or 2 rows)	Absence of laminar collenchyma

**MB:** marginal bundles; **ST:** stomata type; **UTT:** uniseriate trichome type.

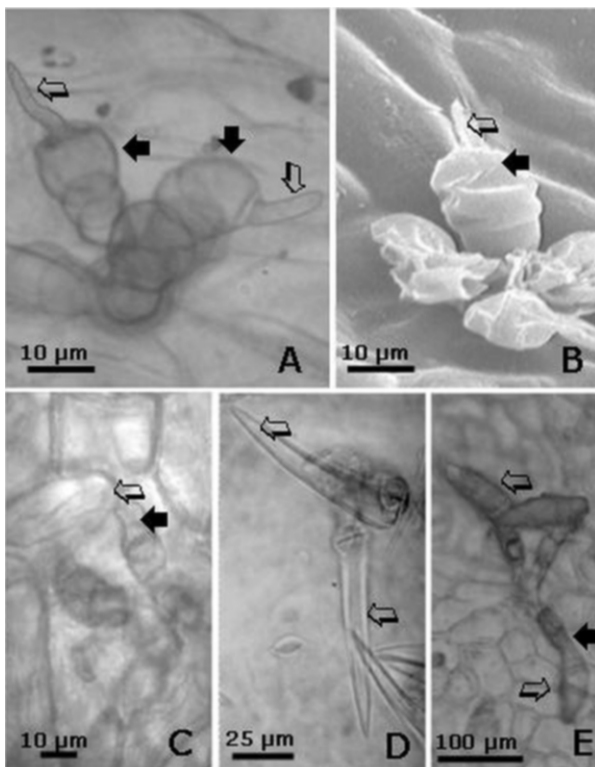
Anisocytic stomata were also reported in *B. articulata* by Ariza Espinar (1973), Cortadi *et al.* (1999) and Barboza *et al.* (2001), but in our study no anisocytic stomata were observed for this species. Freire *et al.* (2007) observed cyclocytic stomata in *B. articulata* and *B. gaudichaudiana*, in agreement with our results (Figure 1).

With regard to the uniseriate type of trichome, we distinguished at least three different types of uniseriate trichomes in the five studied species (Figure 2). Müller (2006) reports tufts of 3-7-celled clavate uniseriate hairs in *Baccharis genistelloides* subsp. *crispa*. In our investigation we observed this type of trichome in *B. trimera*, but not in *B. crispa*, which presented the trichome type described as a whip by Ariza Espinar (1973), or 1-armed trichome by Freire *et al.* (2007), and Metcalfe and Chalk (1972). Freire *et al.* (2007) described the bulbiferous flagellate tri-

chome type for *B. articulata*, *B. gaudichaudiana*, *B. microcephala* and *B. trimera*. In our study, we observed this trichome type in the first three species mentioned but not in *B. trimera*. Ariza Espinar (1973) reports the whip trichome type in most species of *Baccharis* with different lengths of the terminal cell according to the species and uniseriate glandular and whip trichomes in tufts of *B. articulata*.

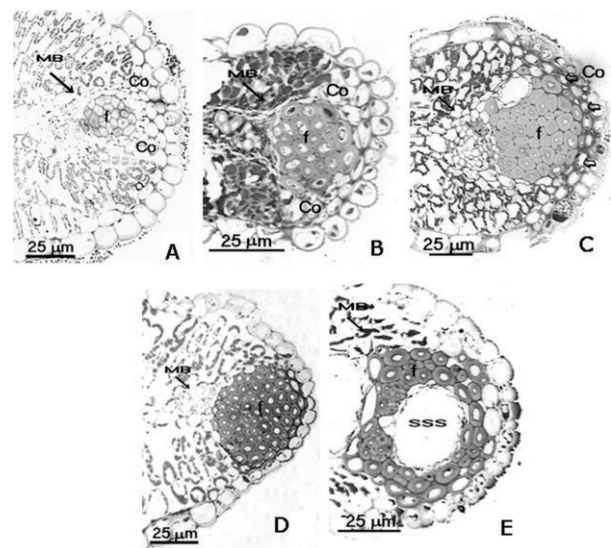
There is collenchyma in the wing margins of *B. articulata* and *B. gaudichaudiana*, which make these species different from *B. crispa* and *B. trimera*, that have a conspicuous cap of sclerenchyma fibres replacing the collenchyma in this position. *B. microcephala* presents only 1-2 rows of collenchyma in the wing margins. We propose that the presence of subepidermal collenchyma in the wing margin is a differential character between some of the species (Figure 3).

**Figure 2.-** Uniseriate type of trichome



**A, C-E:** Light micrograph and **B:** scanning electron micrograph. **A-C:** flagellate trichomes of *B. articulata*, *B. gaudichaudiana* and *B. microcephala*, respectively; **D:** armed trichomes of *B. crispa*; **E:** clavate trichomes of *B. trimera*. Arrows indicate terminal cells (white) and sub-terminal cells (black).

**Figure 3.-** Light micrograph of a wing transverse section



**A-B:** Presence of collenchyma in the wing margin of *B. articulata* and *B. gaudichaudiana*, respectively; **C:** Presence of 1-2 rows of collenchyma in the wing margin of *B. microcephala*; **D-E:** Absence of collenchyma in the wing margin of *B. crispa* and *B. trimera*, respectively (**Co:** collenchyma; **f:** fibres; **MB:** marginal bundle; **SSS:** secreting schizogenous structures).

**Table 3.-** Quantitative variable's mean and standard error of *B. articulata*

		<i>Ba</i>	<i>Bc</i>	<i>Bg</i>	<i>Bm</i>	<i>Bt</i>
<b>Stem (cross-section)</b>	Perimeter (mm)	2.14 ± 0.46	3.00 ± 0.62	3.17 ± 0.66	3.55 ± 0.31	3.10 ± 0.84
	Number of SSS	3.00 ± 1.00	1.00 ± 1.00	8.00 ± 2.00	1.00 ± 1.00	8.00 ± 3.00
	Number of SSS per Stem (mm)	1.23 ± 0.38	0.35 ± 0.30	2.51 ± 0.59	0.28 ± 0.18	2.77 ± 1.44
	SSS length (µm)	17.00 ± 5.33	24.80 ± 8.67	51.67 ± 13.29	25.50 ± 10.75	58.83 ± 14.08
	SSS width (µm)	28.33 ± 7.20	14.80 ± 5.76	34.00 ± 14.42	12.50 ± 3.00	29.33 ± 10.86
<b>Wings (cross-section)</b>	Wings width	0.8 ± 0.23	1.74 ± 0.87	2.94 ± 0.62	1.14 ± 0.47	2.60 ± 1.12
	Number of SSS	3.00 ± 1.00	2.00 ± 2.00	11.00 ± 3.00	1.00 ± 1.00	9.00 ± 4.00
	Number of SSS per Wing (mm)	3.73 ± 1.86	1.31 ± 1.12	3.60 ± 1.13	0.53 ± 0.51	3.50 ± 0.86
	SSS length (µm)	40.67 ± 20.42	22.80 ± 4.38	83.71 ± 26.26	46.43 ± 17.27	106.67 ± 30.55
	SSS width (µm)	33.00 ± 10.49	17.00 ± 5.83	48.00 ± 17.17	23.43 ± 3.09	54.50 ± 20.43
<b>Wings (diaphanised)</b>	Stomata density	47.00 ± 7.00	119.00 ± 29.00	48.00 ± 10.00	82.00 ± 5.00	138.00 ± 29.00
	Stomata index	7.03 ± 0.97	13.00 ± 1.42	5.00 ± 0.51	6.54 ± 1.00	13.83 ± 1.86
	Stomata length (µm)	53.27 ± 3.68	29.70 ± 0.74	58.54 ± 3.95	40.12 ± 5.63	31.93 ± 2.27
	Stomata width (µm)	41.03 ± 3.21	25.83 ± 1.18	49.00 ± 6.37	31.76 ± 3.72	28.97 ± 2.89
	Tuft of trichomes density	22.00 ± 5.00	28.00 ± 4.00	34.00 ± 18.00	20.00 ± 10.00	16.00 ± 7.00

**Ba:** *B. articulata*; **Bc:** *B. crispa*; **Bg:** *B. gaudichaudiana*; **Bm:** *B. microcephala*; **Bt:** *B. trimera*; SSS: schizogenous secreting structure.



**Table 4.-** Summary of quantitative variables that differ between sect. Caulopterae species with winged stems in Argentina

	<i>Ba</i>	<i>Bc</i>	<i>Bg</i>	<i>Bm</i>	<i>Bt</i>
<i>Ba</i>	Density, index, length and width of stomata, Stem number of SSS, Stem length SSS		Width wing, Wing number of SSS	Density, length and width of stomata, Number of SSS per wing	Density, index, length and width of stomata, Stem number of SSS, Stem length SSS, Width wing, Wing number of SSS, Wing length SSS
<i>Bc</i>		Density, index, length and width of stomata, Stem number of SSS, Number of SSS per Stem, Wing number SSS, Wing length SSS		Stomata index	Stem number of SSS, Number of SSS per Stem, Stem length SSS, Wing number SSS, Number of SSS per wing, Wing length SSS, Wing width SSS
<i>Bg</i>				Density, length and width of stomata, Stem number of SSS, Number of SSS per Stem, Wing number of SSS, Number of SSS per Wing	Density, index, length and width of stomata
<i>Bm</i>					Density, index and length of stomata, Stem number of SSS, Number of SSS per Stem, Stem length SSS, Wing number SSS, Number of SSS per wing, Wing length SSS
<i>Bt</i>					

Quantitative variables show statistically significant differences between the species ( $p < 0.05$ ).

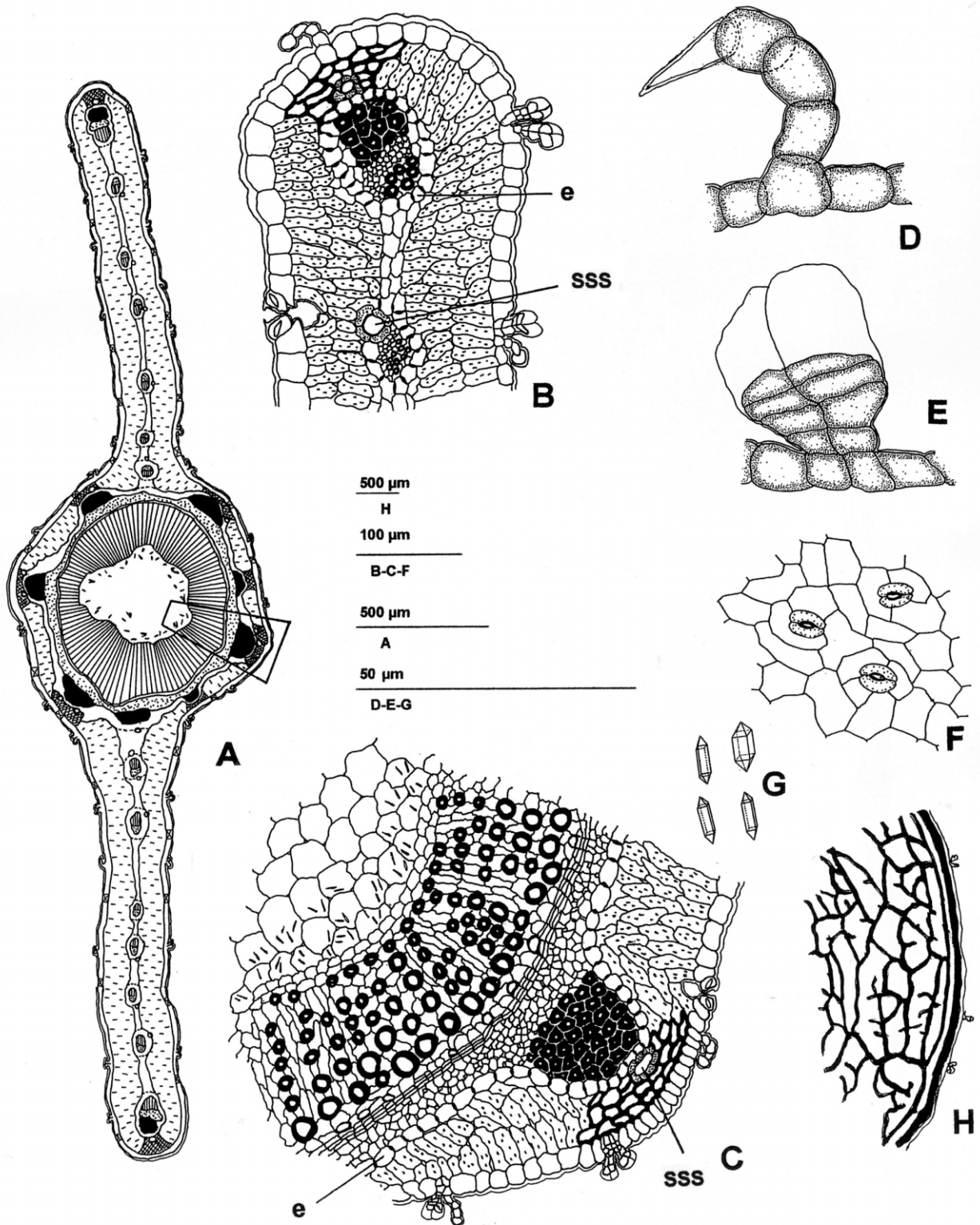
The presence of subepidermal collenchyma in the wing margin for *B. articulata* has been previously reported (Ariza Espinar, 1973; Müller, 2006). It is interesting to note that Müller (2006) also reported the presence of collenchyma in *B. genistelloides* in this position; however our results are not in agreement with previous reports as we did not observed any collenchyma in *B. crispa* and *B. trimera*. Cortadi *et al.* (1999) reported the presence of 1 row of collenchyma in *B. trimera* and its absence in *B. crispa*.

## Conclusion

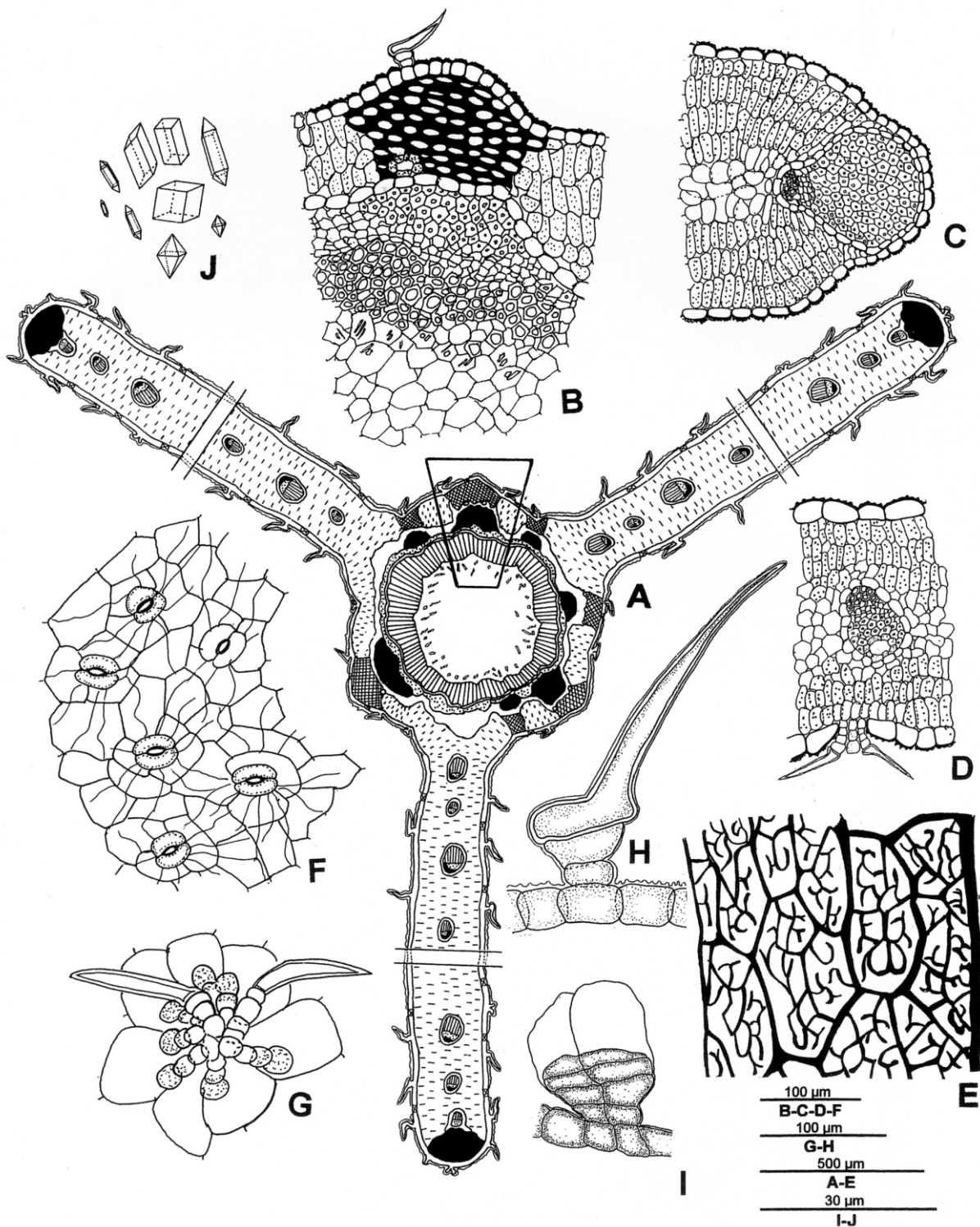
According to these results, we conclude that we can characterize the five winged stem species of sect. Caulopterae using the selected qualitative and quantitative variables determined in this study.

As part of quality control of herbal medicines, five micrographic monographies of the studied species were elaborated (Figures 4, 5, 6, 7 and 8).

Figure 4.- *Baccharis articulata*

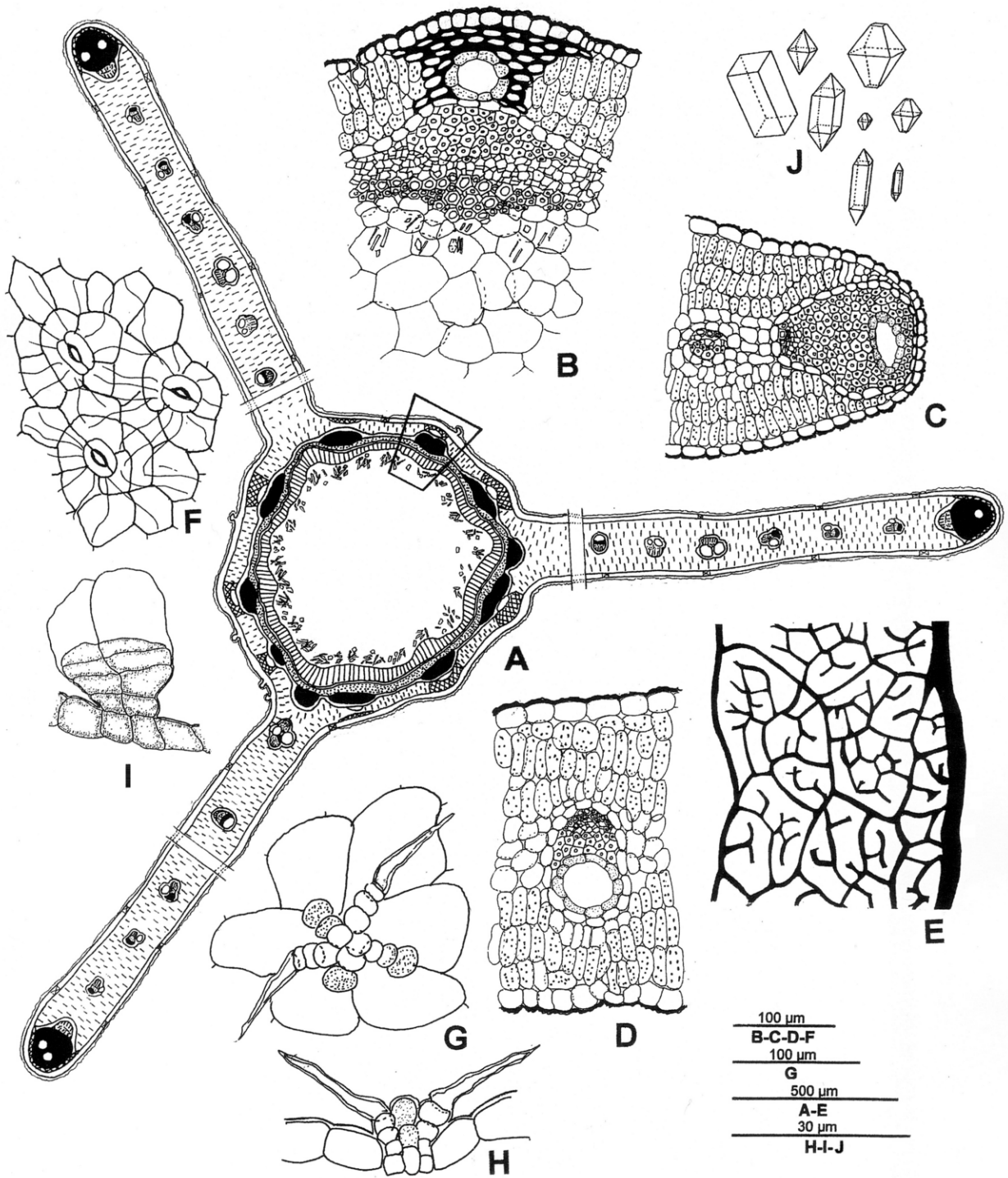


A: 2-winged stem representative diagram; B: detail of the wing margin with collenchyma and a secreting schizogenous structure; C: Stem detail, as indicated in A, with a secreting schizogenous structure; D-E: trichomes, D: non-glandular; E: glandular. F: Surface view of the wing epidermis showing anomocytic stomata; G: polyhedral crystals of calcium oxalate; H: wing vascularization; e: endodermis; sss: secreting schizogenous structure.

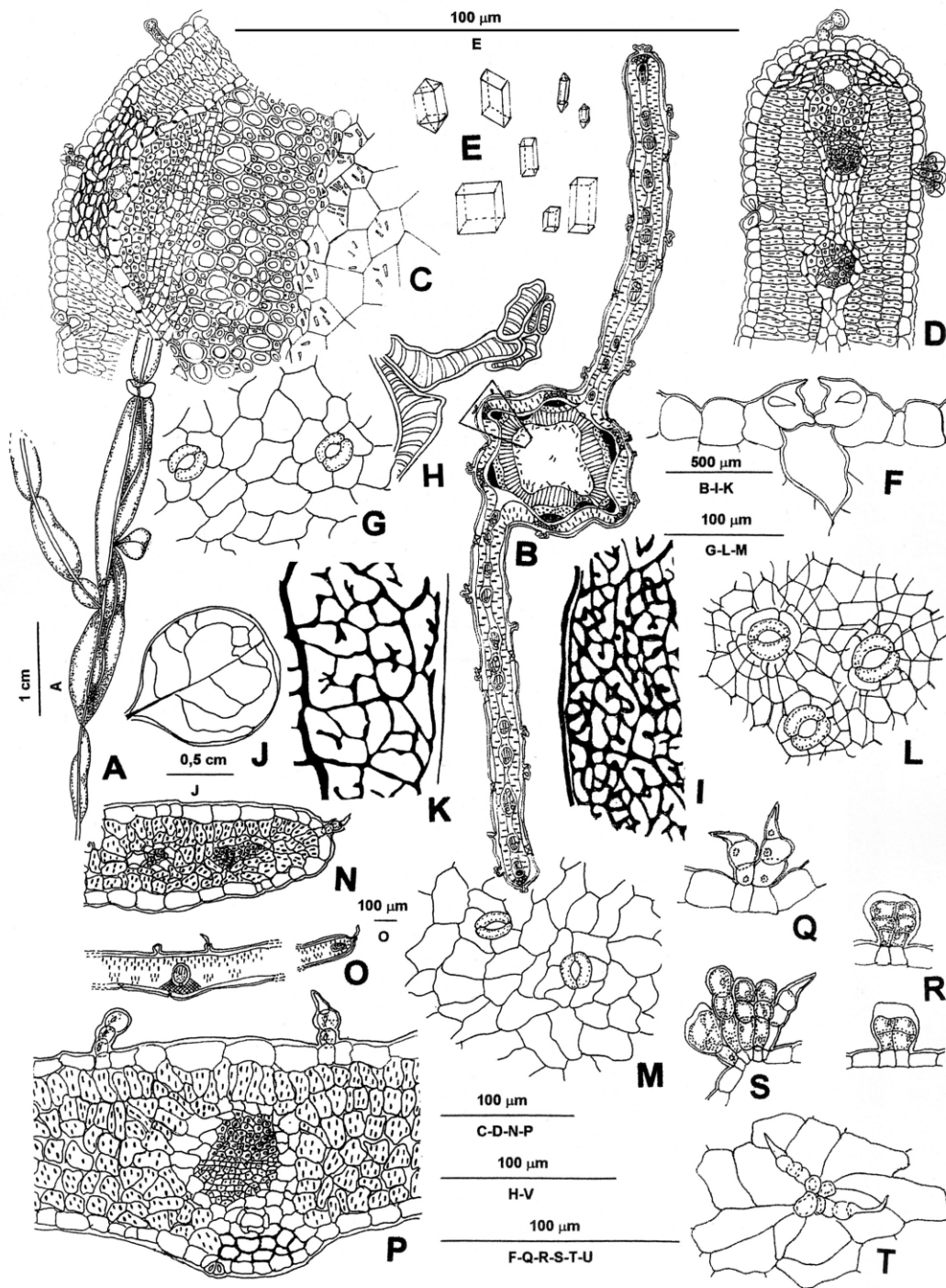
Figure 5.- *Baccharis crispera*

**A:** 3-winged stem representative diagram; **B:** Stem detail, as indicated in A, with a secreting schizogenous structure; **C:** detail of the wing margin with fibres and a secreting schizogenous structure; **D:** detail of wing vascular bundles with a secreting schizogenous structure; **E:** wing vascularization; **F:** Surface view of the wing epidermis showing aniscytic stomata and striated cuticle; **G:** Surface view of glandular and non-glandular tuft of trichomes; **H:** non-glandular trichome; **I:** glandular trichome; **J:** crystals of calcium oxalate.

Figure 6.- *Baccharis trimera*

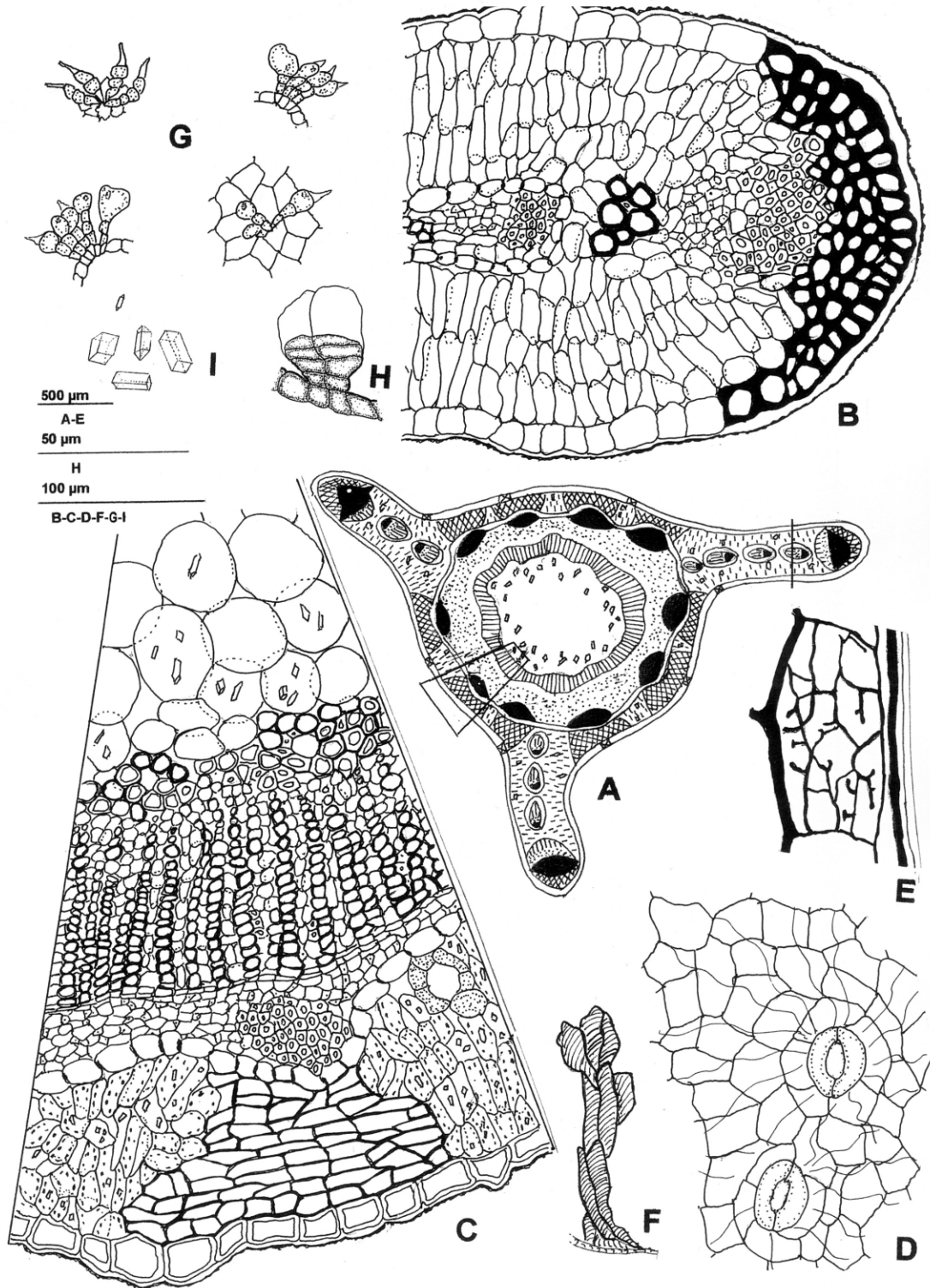


A: 3-winged stem representative diagram; B: Stem detail, as indicated in A, with a secreting schizogenous structure; C: detail of the wing margin with fibres and a secreting schizogenous structure; D: detail of wing vascular bundles with a secreting schizogenous structure; E: wing vascularization; F: Surface view of the wing epidermis showing aniscytic stomata; G: Surface view of glandular and non-glandular tuft of trichomes; H: cross section of G; I: glandular trichome; J: crystals of calcium oxalate.

Figure 7.- *Baccharis gaudichaudiana*

A: exomorphology of winged stem; B, C, D, F: winged stem cross section; B: 2-winged stem representative diagram; C: stem detail, as indicate in B, with a secreting schizogenous structure; D: wing margin detail, as indicate in B, with a secreting schizogenous structure; F: raised estomata; G-H: wing surface view; G: epidermis with anomocytic stomata; H: vascular endings with dilated tracheids; I: wing vascularization; J-P: leaf; J: external morphology; K-P: internal morphology; K-M: surface view; K: architecture; L-M: epidermis; L: adaxial; M: abaxial, both with anomocytic stomata; N-P: cross section; N: margin detail; O: lamina, representative diagram; P: middle nerve detail; Q-T: trichomes; Q: non-glandular; R-T: glandular; E: polyhedral crystals of calcium oxalate.

Figure 8.- *Baccharis microcephala*



**A:** 3-winged stem representative diagram; **B:** wing margin detail with collenchyma, as indicate in A; **C:** stem detail, as indicate in A, with a secreting schizogenous structure; **D-F:** wing surface view; **D:** epidermis with anomocytic stomata and striated cuticle; **E:** wing vascularization; **F:** dilated vascular endings detail; **G:** glandular and non-glandular tuft of trichomes; **H:** glandular trichome; **I:** polyhedral crystals of calcium oxalate.

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