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Original article

Morphological diversity of glandular trichomes in Urticalean Rosids

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ABSTRACT

Glandular trichomes are epidermal structures that produce, store and release a variety of chemical compounds. Their high morphological diversity allows their taxonomic use at various levels. Therefore, their description and standardization of terminology become challenging. Cannabaceae is a well known family of Urticalean Rosids because of the presence of cannabinoid-secreting trichomes in *Cannabis sativa*. Other Urticalean Rosid families (Moraceae, Ulmaceae and Urticaceae) are neglected in this regard. Thus, the morphology and distribution of glandular trichomes were studied in Cannabaceae and Ulmaceae species and compared with published data for Moraceae and Urticaceae. Surface and anatomical analyses were performed on vegetative and floral organs. Urticalean Rosids show at least 15 types of glandular trichomes, 12 of them capitate and three filiform. In particular, *Trema micrantha*, a Cannabaceae species, has six different types. A trichome with biseriate stalk and pluricellular head, found in *Trema micrantha* and *Pteroceltis tatarinowii*, was not previously reported. Glandular trichomes are widely distributed in the plant body, which may be related to protection against herbivory. The greatest difficulty was the large quantity of terms and the lack of morphological details of the glandular trichomes in previous studies. A standardization of the terminology of glandular trichomes was therefore proposed.

Keywords: anatomy, Cannabaceae, Moraceae, terminology, Ulmaceae, Urticaceae

Introduction

Glandular trichomes are epidermal appendages responsible for the production, storage and release of a variety of chemical compounds (Esau 1977; Fahn 1979). They are formed by a portion inserted into the epidermis, called base, and a segment that is above the surface of the epidermis, called stalk, which may be single or pluricellular, single or multiseriate, or even absent (Fahn 1979; Dickinson 2000; Cutler *et al*. 2008). In the apical portion of the trichome there is a secretory head, which may be uni- or pluricellular (Fahn 1979; Cutler *et al*. 2008).

Glandular trichomes are of great importance for the systematic investigation of angiosperms, with a few species being devoid of this type of structure (Fahn 1988; Metcalfe & Chalk 1950; Wagner 1991; Duke 1994; Beck 2005). The number and arrangement of the cells forming the stalk and the head result in several morph types of trichomes that are important for plant systematics (Payne

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1978; Tobe & Takaso 1996; Azizian 2002; Marquiafável *et al*. 2009; Vargas *et al*. 2019). Proper identification of glandular trichomes requires investigation of important aspects such as macromorphology, anatomy, and chemical composition of the exudate (Theobald *et al*. 1979, Azizian 2002; Marquiafável *et al*. 2009; Vargas *et al*. 2019).

Cannabaceae is a prominent family known for its medicinal potential regarding cannabinoid production in the glandular trichomes of *Cannabis sativa* (Furr & Mahlberg 1981). These glandular trichomes have been extensively studied for morphology, distribution and exudate composition. However, the wide diversity of glandular trichomes in the family (Hammond & Mahlberg 1973; 1977; Gangadhara & Inamdar 1977; Tobe & Takaso 1996; St-Laurent *et al*. 2000) has not yet been cataloged. Ulmaceae, the elm family closely related to Cannabaceae, also exhibits glandular trichomes (Tobe & Takaso 1996; Leme *et al*. 2018), which, however, have been little studied in terms of morphology, distribution and exudate composition.

The circumscription of Cannabaceae and Ulmaceae was changed by molecular data, with genera previously belonging to Ulmaceae being transferred to Cannabaceae (Sytsma *et al*. 2002). These two families, together with Urticaceae and Moraceae, form the Urticalean clade of rosids (Sytsma *et al*. 2002), a group with some registers of glandular trichomes (Gangadhara & Inamdar 1977; Tobe & Takaso 1996; Schnetzler *et al*. 2017). Different terms are often used (see Briosi & Togni 1894; Dayanandan & Kaufman 1976; Gangadhara & Inamdar 1977; Hammond & Malhberg 1977; Oliveira *et al*. 1988; Tobe & Takaso 1996), with the study of the morphology of trichomes in this group being a challenge.

Thus, the objective of the present study was to investigate the distribution and morphology of the glandular trichomes of three species of Cannabacae and four species of Ulmaceae (see Tab. 1) and compare them with published data for Moraceae and Urticaceae. We intended to elaborate a set of taxonomic characteristics for this group of plants and also to contribute to the standardization of a more appropriate terminology for glandular trichomes in general. The lack of standardization of terms for the glandular trichomes prevents comparative studies, thus hampering the use of these structures as support for taxonomic studies.

Materials and methods

Samples of the vegetative and reproductive organs of seven species of Cannabaceae and Ulmaceae were collected at different locations (Tab. 1). Two to three individuals were sampled for each species. Vouchers were deposited in the SPFR herbarium (FFCLRP/USP, Ribeirão Preto, Brazil) and in the CGMS herbarium (UFMS, Campo Grande, Brazil) (Tab. 1).

The material was fixed in FNT (buffered formalin; Lillie 1965) or in FAA (formalin, acetic acid, and 50 % ethanol) (Johansen 1940) for 24 hours, washed in water or 50 % ethanol, respectively, dehydrated in an ethanol series up to 70 % and then processed for analysis of distribution and external morphology of the trichomes by scanning electron microscopy (SEM), and of anatomy by light microscopy (LM).

For surface analysis (SEM), samples were dehydrated in an ethanol series up to absolute alcohol and dried at CO_2 critical point in a Bal-Tec CPD 030 apparatus. Next, they were mounted on metal supports with carbon adhesive tape, coated with gold with a Bal-Tec SCD 050 sputter-coater and observed with a Zeiss EVO-50 scanning electron microscope at 15 kv.

For the anatomical study (LM), samples were dehydrated in an ethanol series up to absolute alcohol, embedded in histological resin (Leica) and cut into 3 to 6 µm-thick sections on transverse and longitudinal planes using a rotary microtome (Leica RM2245). The sections were stained with 0.05 % toluidine blue, pH 4.4 or 5.8 (O'Brien *et al*. 1964), Sudan III, and Sudan Black B (Pearse 1972) for observation of cutin and suberin in the cell wall.

Table 1. Information on the Cannabaceae and Ulmaceae species analysed in the present study.

Family	Species	Organ analysed	Sample source	Voucher
Cannabaceae	*Celtis pubescens* (Kunth) Spreng.	Stem, leaf, sepal, stamen and pistil	USP campus, Ribeirão Preto, SP, Brazil.	F. M. Leme 98 (SPFR)
	Pteroceltis tatarinowii Maxim.	Stem, leaf, sepal, stamen and pistil	Botanical Garden, University of Vienna, Vienna, Austria.	F. M. Leme 128 (CGMS)
	Trema micrantha (L.) Blume	Stem, leaf, sepal, stamen and pistil	USP campus, Ribeirão Preto, SP, Brazil.	F. M. Leme 94, 97, 101(SPFR)
Ulmaceae	*Ampelocera glabra* Kuhlm.	Stem, leaf, sepal, stamen and pistil	Reserva Particular do Patrimônio Natural (RPPN), Serra do Teimoso, Jussari, BA, Brazil.	F. M. Leme 102, 112 (SPFR)
	Phyllostylon rhamnoides J. Poiss.) Taub.	Stem and leaf	Assentamento Andalúcia, Nioaque, MS, Brazil.	F. M. Leme 109 (SPFR)
	Ulmus parvifolia Jacq.	Stem and leaf	Horto medicinal of the FCFRP, USP campus, Ribeirão Preto, SP, Brazil.	F. M. Leme & I. C. Nascimento (Spirit Collection of FCFRP/USP)
	Zelkova serrata (Thunb.) Makino	Stem, leaf, sepal, stamen and pistil	Botanical Garden, University of Vienna, Vienna, Austria.	F. M. Leme 124 (CGMS)

We used the functions prcomp and autoplot (ggfortify package) in the R computational environment (R Development Core Team 2020) to run the principal component analysis (PCA). The dataset consisted of 56 lines representing the species and 15 columns representing the glandular trichome types. For each species we computed the number of organs each glandular trichome type occurred (*i.e.*, sepal, leaf or stem).

The glandular trichomes were classified according to Payne's glossary (1978) and by comparison with trichomes described in the literature for Cannabaceae and Ulmaceae species (Briosi & Togni 1894; Dayanandan & Kaufman 1976; Gangadhara & Inamdar 1977; Hammond & Malhberg 1977; Oliveira *et al.* 1988; Tobe & Takaso 1996; Leme *et al.* 2018; Leme *et al.* 2020).

In an attempt to standardize their terminology, glandular trichomes were denoted as capitate if there was a distinction between head and stalk, and filiform if there was no such distinction. Capitate trichomes were defined as uni-, bi-, or pluricellular and as uni-, bi-, or multiseriate considering the number of stalk cells, and as uni- or pluricellular considering the number of head cells. In addition, the filiform type of trichome was defined as uni- or biseriate considering the number of cell series and the total number of cells. Descriptions, schemes and images available in the literature were checked for such standardization.

Results

The data collected in the present study and those found in the literature accounted for 15 different glandular trichome morph types in Cannabaceae, Moraceae, Ulmaceae and Urticaceae, described and organized in Table 2. Among these 15 types of glandular trichomes, five types were found in the sampled species of Cannabaceae and Ulmaceae in this study (Tab. 3) and were first assigned to two large groups, *i.e.*, capitate (Fig. 1) and filiform (Fig. 2).

Capitate glandular trichomes

Three types of capitate glandular trichomes are found and classified according to descriptions of Table 2.

Capitate trichome with a uniseriate, four- to eight-celled stalk, multiseriate and pluricellular head (Figs. 1A, 3A-C). It

Table 2. Types and distribution of glandular trichomes in the analysed species of Cannabaceae and Ulmaceae. Symbols: + = present; - = absent; empty cell = without information.

Trichome type and description	Family	Species	Organ analysed	Reference
1. Capitate: unicellular stalk, unicellular head 	Cannabaceae	*Cannabis sativa* L.	Leaf	Gangadhara & Inamdar (1977)
	Moraceae	*Ficus carica* L.	Leaf	Gangadhara & Inamdar (1977)
		Ficus heterophylla L. f.	Leaf	Gangadhara & Inamdar (1977)
		Ficus hispida L.f.	Leaf	Gangadhara & Inamdar (1977)
		Ficus religiosa L.	Leaf	Gangadhara & Inamdar (1977)
		Maclura pomifera (Raf.) C.K.	Leaf	Hardin (1981)
		Morus alba L.	Leaf	Hardin (1981)
		Morus microphylla Buckley	Leaf	Hardin (1981)
		Morus nigra L.	Leaf	Hardin (1981)
		Morus rubra L.	Leaf	Hardin (1981)
	Urticaceae	*Boehmeria caudata* Sw.	Leaf	Fernandez *et al.* (2011)
		Fleurya interrupta (L.) Gaudich.	Leaf	Gangadhara & Inamdar (1977)
		Urtica dioica L.	Leaf	Gangadhara & Inamdar (1977)
2. Capitate: uniseriate filiform stalk, unicellular head 	Cannabaceae	*Celtis* sp.	Leaf	Gangadhara & Inamdar (1977)
		Trema orientalis (L.) Blume	Leaf	Gangadhara & Inamdar (1977)
	Ulmaceae	*Holoptelea integrifolia* Planch.	Leaf	Gangadhara & Inamdar (1977)
3. Capitate: uniseriate stalk, pluricellular head 	Cannabaceae	*Celtis pubescens* Spreng	Stem, leaf, flower	This study
		Trema orientalis (L.) Blume	Leaf	Gangadhara & Inamdar (1977)
	Moraceae	*Morus alba* L.	Leaf	Shah & Kachro (1975)

Table 2. Cont.

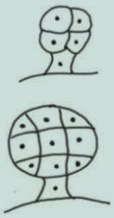
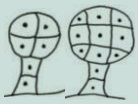
Trichome type and description	Family	Species	Organ analysed	Reference
4. Capitate: uniseriate stalk, bicellular head 	Cannabaceae	*Trema orientalis* (L.) Blume	Leaf	Gangadhara & Inamdar (1977)
	Ulmaceae	*Holoptelea integrifolia* Planch.	Leaf	Gangadhara & Inamdar (1977)
5. Capitate: unicellular stalk, bicellular head 	Moraceae	*Dorstenia cayapia* Vell.	Leaf	Schnetzler *et al.* (2017)
		Dorstenia indica Wight	Leaf	Gangadhara & Inamdar (1977)
		Ficus asperrima Roxb.	Leaf	Gangadhara and Inamdar (1977)
		Ficus heterophylla L. f.	Leaf	Gangadhara & Inamdar (1977)
		Ficus hispida L. f.	Leaf	Gangadhara & Inamdar (1977)
		Ficus racemosa L.	Leaf	Gangadhara & Inamdar (1977)
		Maclura pomifera (Raf.) C.K. Schneid.	Leaf	Azizian (2002)
		Morus alba L.	Leaf	Azizian (2002)
		Morus nigra L.	Leaf	Azizian (2002)
		Streblus asper Lour.	Leaf	Gangadhara & Inamdar (1977)
	Urticaceae	*Boehmeria caudata* Sw.	Leaf	Fernandez *et al.* (2011)
		Fleurya interrupta (L.) Gaudich.	Leaf	Gangadhara & Inamdar (1977)
		Girardinia diversifolia (Link) Friis	Leaf	Fu *et al.* (2003)
		Pouzolzia zeylanica (L.) Benn. & R.Br.	Leaf	Gangadhara & Inamdar (1977)
6. Capitate: unicellular stalk and pluricellular head constituted from four cells or more 	Moraceae	*Artocarpus heterophyllus* Lam.	Leaf, inflorescence peduncle	Schnetzler *et al.* (2017)
		Broussonetia papyrifera (L.) L'Hér. ex Vent.	Leaf	Shah & Kachroo (1975)
		Ficus carica L.	Leaf	Azizian (2002)
		Ficus heterophylla L. f.	Leaf	Gangadhara & Inamdar (1977)
		Ficus hispida L. f.	Leaf	Gangadhara & Inamdar (1977)
		Ficus racemosa L.	Leaf	Gangadhara & Inamdar (1977)
		Ficus repens Hook. Ex Miq.	Leaf	Gangadhara & Inamdar (1977)
		Maclura tinctoria (L.) D. Don ex Steud.	Stem, leaf	Schnetzler *et al.* (2017)
		Morus alba L.	Stem, leaf	Shah & Kachro (1975)
		Morus alba L.	Leaf	Gangadhara and Inamdar (1977); Azizian (2002)
		Sorocea bonplandii (Baill.) W.C. Burger, Lanj. & Wess. Boer	Stem, leaf, flower	Schnetzler *et al.* (2017)
		Streblus asper Lour.	Leaf	Gangadhara & Inamdar (1977); Shah & Kachoroo (1975)
	Ulmaceae	*Ampelocera glabra* Kuhlm.	Stem, leaf, bract	Leme *et al.* (2018); This study
		Phyllostylon rhamnoides (J. Poiss) Taub.	Stem, leaf	This study
		Ulmus parvifolia Jacq.	Stem, leaf, bract	This study
		Zelkova serrata (Thunb.) Makino	Stem, leaf, flower	This study
	Urticaceae	*Boehmeria caudata* Sw.	Leaf	Fernandez *et al.* (2011)
		Dendrocnide meyeniana (Walp.) Chew	Leaf	Fu *et al.* (2003)
		Urtica dioica L. (Ur)	Leaf	Gangadhara & Inamdar (1977)
		Urtica thumbergiana Siebold & Zucc.	Leaf	Fu *et al.* (2003)
7. Capitate: uniseriate and bicellular stalk, and pluricellular head 	Moraceae	*Ficus racemosa* L.	Leaf	Gangadhara & Inamdar (1977)
8. Capitate: biseriate stalk with two cells in each series, and head from four to eight cells (bulbous) 	Cannabaceae	*Cannabis sativa* L.	Bract	Hammond & Malhberg (1977); Dayanandan & Kaufman (1976); Briosi & Togni (1894)
		Humulus lupulus L.	Leaf, bract	Oliveira *et al.* (1988)

Table 2. Cont.

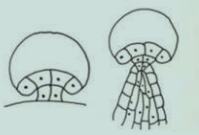
Trichome type and description	Family	Species	Organ analysed	Reference
9. Capitate: biseriate stalk with two cells in each series and disc-shaped head formed by a layer of cells (peltate) 	Cannabaceae	*Cannabis sativa* L.	Bract, flower (stamen)	Dayanandan & Kaufman (1976); Hammond & Malhberg (1977); Leme *et al.* (2020)
			Leaf	Gangadhara & Inamdar (1977)
		Humulus lupulus L.	Leaf, bract	Oliveira *et al.* (1988)
10. Capitate: peltate head (cells radially arranged) 	Moraceae	*Artocarpus altilis* (Parkinson) Fosberg (*syn. Artocarpus incisa*)	Leaf	Shah & Kachroo (1975)
		Artocarpus heterophyllus Lam.	Leaf	Schnetzler *et al.* (2017)
		Brosimum gaudichaudii Trécul	Bract	Jacomassi *et al.* (2010)
11. Capitate: uniseriate and bicellular stalk, and head with three to seven cells (peltate) 	Moraceae	*Broussonetia papyrifera* (L.) L'Hér.ex Vent.	Leaf	Shah & Kachroo (1975)
12. Capitate: biseriate stalk with several cells in each series and pluricellular head 	Cannabaceae	*Pteroceltis tatarinowii* Maxim.	Leaf	This study
		Trema micrantha (L.) Blume	Leaf	This study
13. Filiform short (short clavate): up to four linear cells 	Cannabaceae	*Lozanella enantiophylla* (Donn. Sm.) Killip & C.V. Morton	Leaf, flower (ovary)	Tobe & Takaso (1996)
		Lozanella permollis Killip & C. V. Morton	Leaf, flower (ovary)	Tobe & Takaso (1996)
	Ulmaceae	*Hemiptelea davidii* (Hance) Planch.	Leaf, flower (ovary)	Tobe and Takaso (1996)
		Holoptelea integrifolia Planch.	Leaf, flower (ovary)	Tobe & Takaso (1996)
		Phyllostylon brasiliensis Capan. Ex Benth. & Hook. f.	Leaf, flower (ovary)	Tobe & Takaso (1996)
		Planera aquatica J. F. Gmel.	Leaf, flower (ovary)	Tobe & Takaso (1996)
		Ulmus davidiana Planch.	Flower (ovary)	Tobe & Takaso (1996)
		Ulmus laciniata (Trautv.) Mayr	Flower (ovary)	Tobe & Takaso (1996)
		Ulmus parvifolia Jacq.	Leaf, flower (ovary)	Tobe & Takaso (1996)
		Zelkova schneidriana Hand. Mazz.	Flower (ovary)	Tobe & Takaso (1996)
		Zelkova serrata (Thunb.) Makino	Flower (ovary)	Tobe & Takaso (1996)
14. Filiform long (long clavate): uniseriate, with more of four linear cells 	Cannabaceae	*Aphananthe aspera* (Thunb.) Planch.	Leaf, flower (ovary)	Tobe & Takaso (1996)
		Celtis sp.	Leaf	Gangadhara & Inamdar (1977)
		Celtis pubescens Spreng	Stem, leaf, flower	This study
		Celtis boninensis Koidz	Leaf	Tobe & Takaso (1996)
		Celtis sinensis Pers.	Leaf, flower (ovary)	Tobe & Takaso (1996)
		Celtis spinosa Spreng.	Leaf	Tobe & Takaso (1996)
		Lozanella permollis Killip & C. V. Morton	Leaf, flower (ovary)	Tobe and Takaso (1996)
		Parasponia rigida Merr. & Perry	Leaf, flower (ovary)	Tobe & Takaso (1996)
		Pteroceltis tatarinowii Maxim.	Leaf, flower (ovary)	Tobe & Takaso (1996)
		Trema lamarkiana (Roem. & Schult.) Blume	Leaf	Tobe & Takaso (1996)
		Trema micrantha (L.) Blume	Leaf	Tobe & Takaso (1996)
	Ulmaceae	*Ampelocera ruzzi* Klotzsch	Leaf	Tobe & Takaso (1996)
15. Filiform: biseriate (long clavate biseriate) 	Cannabaceae	*Chaetachme aristata* E. Mey. Ex Planch.	Leaf	Tobe & Takaso (1996)
		Trema micrantha (L.) Blume	Stem, leaf, flower	This study
		Trema politoria (Planch.) Blume	Leaf	Bhat & Kachroo (1979) apud Tobe & Takaso (1996)

Table 3. Types of glandular trichomes found in the present study and described in the literature for other Urticalean Rosids.

Trichome type	Species	Stem	Petiole	Leaf blade	Pedicel	Sepal	Stamen	Pistil
capitate with uniseriate stalk (type 3)	*Celtis pubescens*	+	+	+	+	+	-	-
capitate with biseriate stalk (type 12)	*Pteroceltis tatarinowii*	+	+	+	+	+	-	+
	Trema micrantha	+	+	+	+	+	-	-
capitate with unicellular stalk (type 6)	*Ampelocera glabra*	+	+	+	-	+	-	+
	Phyllostylon rhamnoides	+	+	+				
	Ulmus parvifolia	+	+	+				
	Zelkova serrata	+	+	+	-	-	-	+
uniseriate filiform (type 14)	*Celtis pubescens*	+	+	+	+	+	-	+
biseriate filiform (type 15)	*Trema micrantha*	+	+	+	+	+	-	-

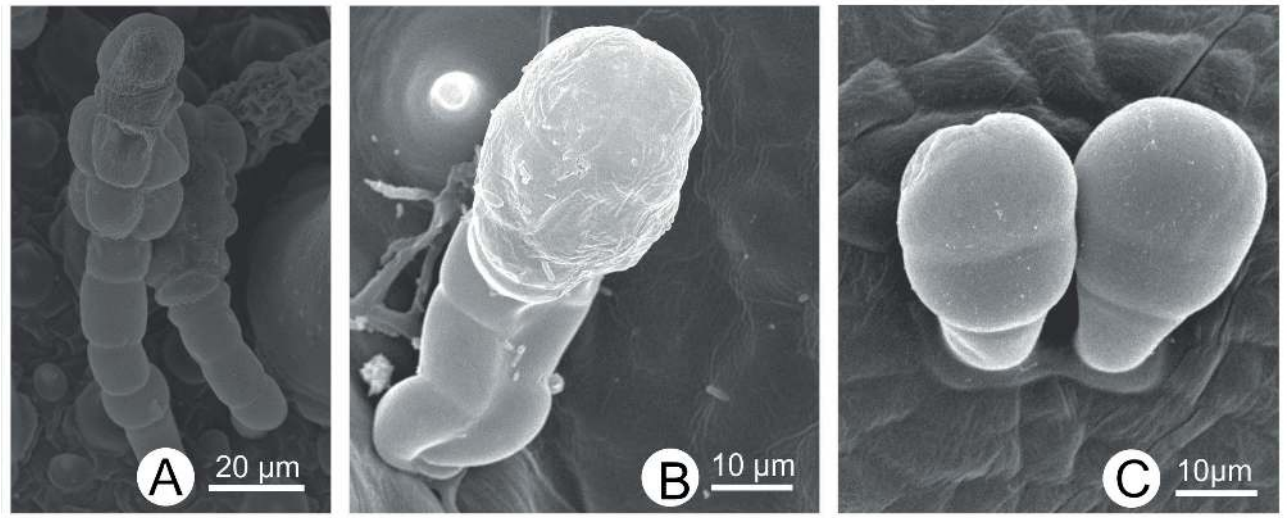


Figure 1. Capitate trichomes of Cannabaceae and Ulmaceae species (SEM). **A**. Capitate trichome with a uniseriate and pluricellular stalk and pluricellular head, *Celtis pubescens* (type 3). **B**. Capitate trichome with a biseriate stalk and pluricellular head, *Trema micrantha* (type 12). **C**. Capitate trichome with a unicelular stalk and pluricellular head, *Ampelocera glabra* (type 6).

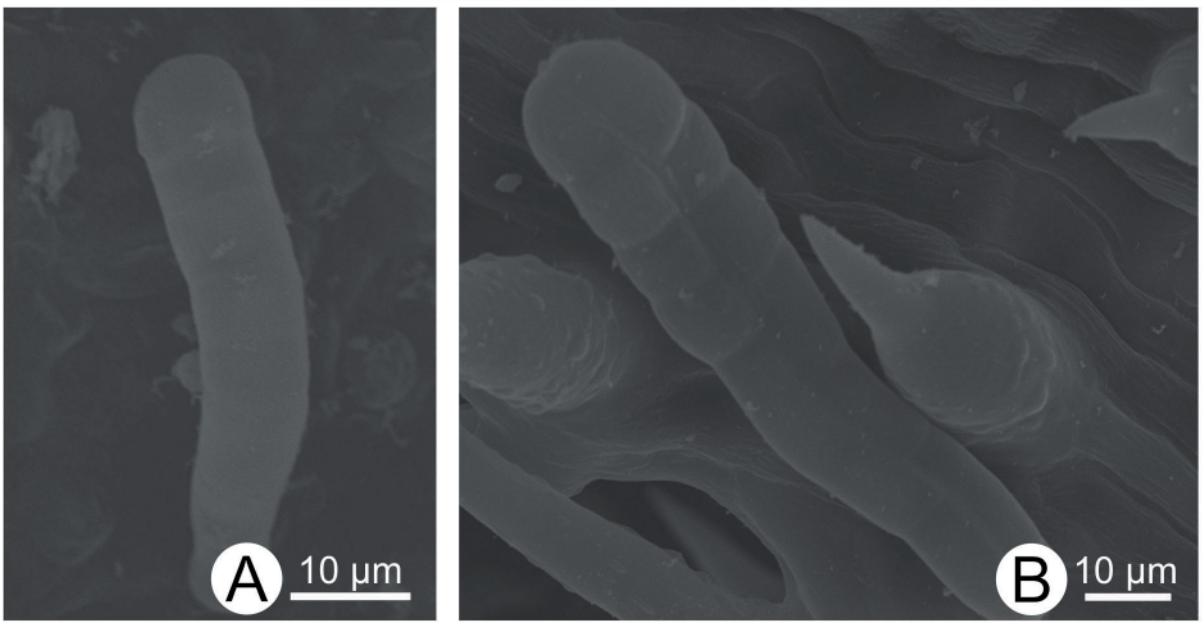


Figure 2. Filiform trichomes of Cannabaceae species (SEM). **A**. Filiform uniseriate trichome, *Celtis pubescens* (type 14). **B**. Filiform biseriate trichome with six to eight cells, *Trema micrantha* (type 15).

occurs on the young stem, on the petiole, on both surfaces of the leaf blade (Fig. 3E), on the pedicel (Fig. 3F), and on the abaxial surfaces of the sepals only in *Celtis pubescens* (Cannabaceae) (type 3 - Tabs. 2, 3).

Capitate trichome with a biseriate stalk, each series with four to six cells, and a six- to eight-celled head (Figs. 1B, 4A-B). It occurs on the young stem, on the petiole, and on both sides of the leaf blade, predominantly on the abaxial side and on the midrib (Fig. 4D) in *Trema micrantha* and *Pteroceltis tatarinowii* (Cannabaceae). In the floral organs, it occurs on the pedicel only in *T. micrantha* (Fig. 4E), on the abaxial side of the sepals of *T. micrantha* and *P. tatarinowii*, and on the pistil of *P. tatarinowii* (type 12 – Tabs. 2, 3).

Capitate trichome with a unicellular stalk and a four-celled head (Figs. 1C, 5A, C-G). It occurs on the young stem, petiole, and on both sides of the leaf blade, predominantly on the abaxial side and on the midrib of *Ampelocera glabra*, *Phyllostylon rhamnoides*, *Ulmus parvifolia* and *Zelkova serrata* (Fig. 5B, D, F, H). In the floral organs it occurs on the sepals of *A. glabra* and on the pistil of *A. glabra* and *Z. serrata* (type 6 – Tabs. 2, 3).

Filiform glandular trichomes

Two types of filiform glandular trichomes are found and classified according to descriptions of Table 2.

Uniseriate, six- to eight-celled filiform trichome (Figs. 2A, 6A). It occurs only in *Celtis pubescens* (Cannabaceae) on

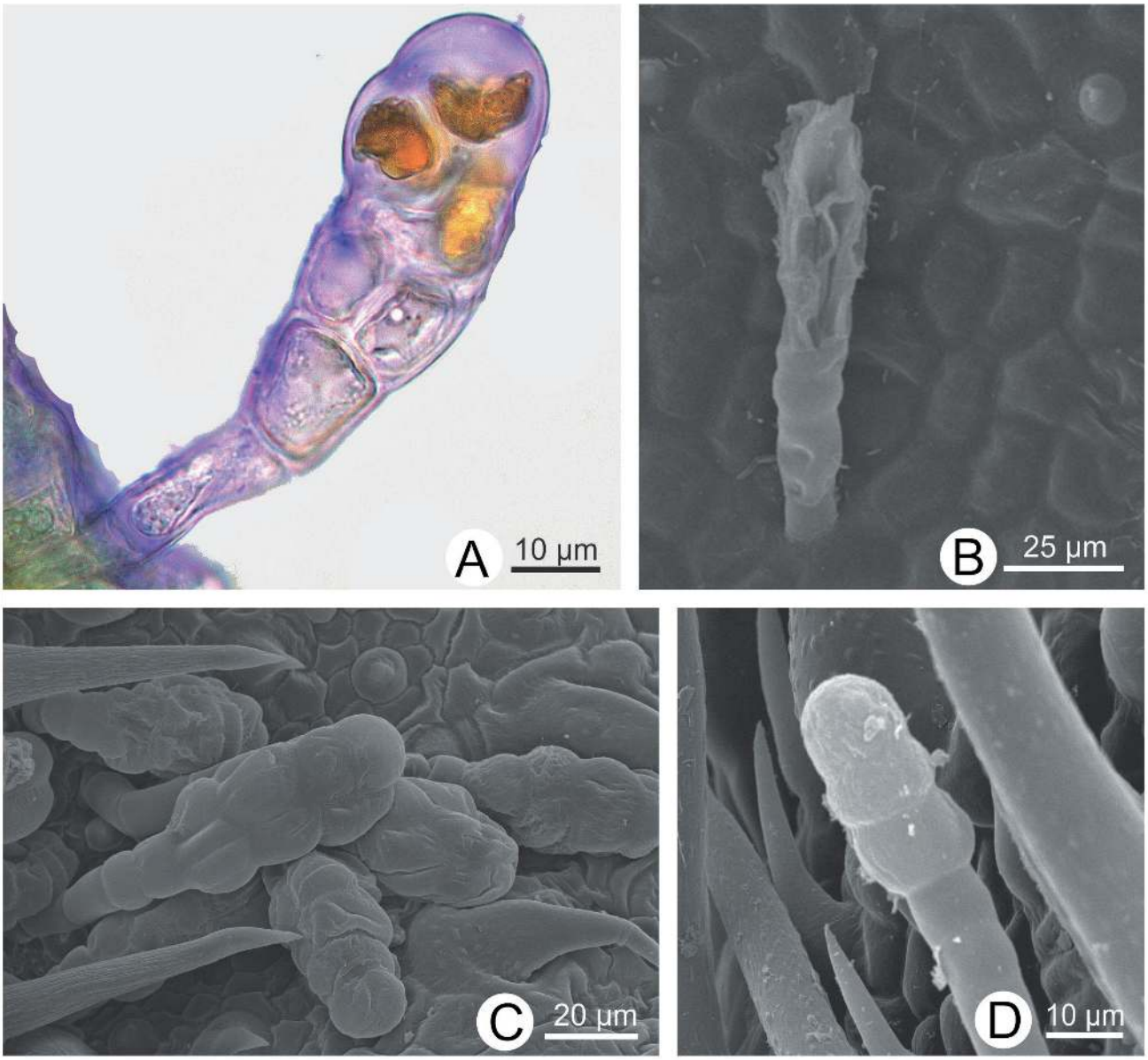


Figure 3. Capitate glandular trichomes (type 3) of *Celtis pubescens* (Cannabaceae). **A**. Longitudinal section showing the uniseriate stalk and pluricellular head (LM). **B**. Head cells after exudate release (SEM). **C**. Distribution of trichomes on the abaxial side of the leaf blade (SEM). **D**. Distribution of trichomes on the pedicel (SEM).

the young stem, on the petiole, and on both sides of the leaf blade (Fig. 6C), more densely distributed on the abaxial side and midrib, on the pedicel (Fig. 6D), and on the abaxial side of the sepals (type 14 – Tabs. 2, 3).

Biseriate filiform trichome, with six to eight cells in each series (Figs. 2B, 7A). It occurs only in *Trema micrantha* (Cannabaceae) on the young stem, petiole, on both sides of the leaf blade, more densely distributed on the abaxial side and midrib (Fig. 7C), on the pedicel (Fig. 7D), and on the abaxial side of the sepals (type 15 – Tabs. 2, 3).

All types of glandular trichomes found in the sampled species release the exudate with no evidence of disintegration of the secretory cells but with loss of turgidity characterizing a granulocrine type of secretion (Figs. 3B, 4C, 4D, 5G, 6B).

Principal Component Analysis (PCA)

The first three PCA axes explained 42.1 % of the data variance. The first axis separated Moraceae and Urticaceae species from the others especially by the presence of glandular trichomes of types 1 (capitate with a unicellular

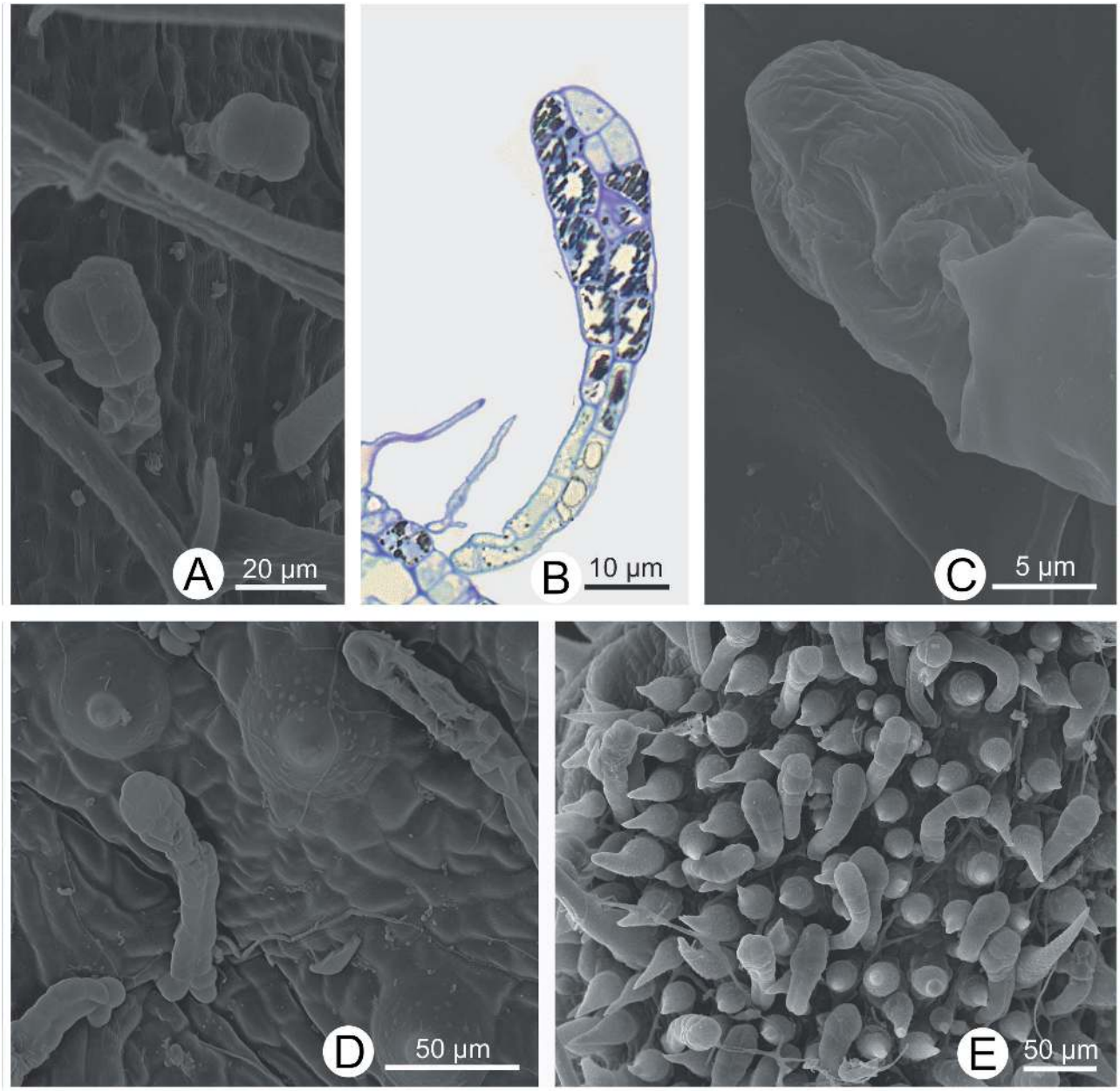


Figure 4. Capitate glandular trichomes (type 12) of Cannabaceae species. **A**. Biseriate and pluricellular stalk and pluricellular head, *Pteroceltis tatarinowii* (SEM). **B-E**. *Trema micrantha*. **B**. Longitudinal section of the capitate trichome with a biseriate stalk and pluricellular head (LM). **C**. Detail of the trichome head after exudate release; note that there is no cell disruption (SEM). **D**. Distribution of trichomes on the adaxial side of the leaf blade (SEM). **E**. Distribution of glandular trichomes on the pedicel among numerous simple trichomes (SEM).

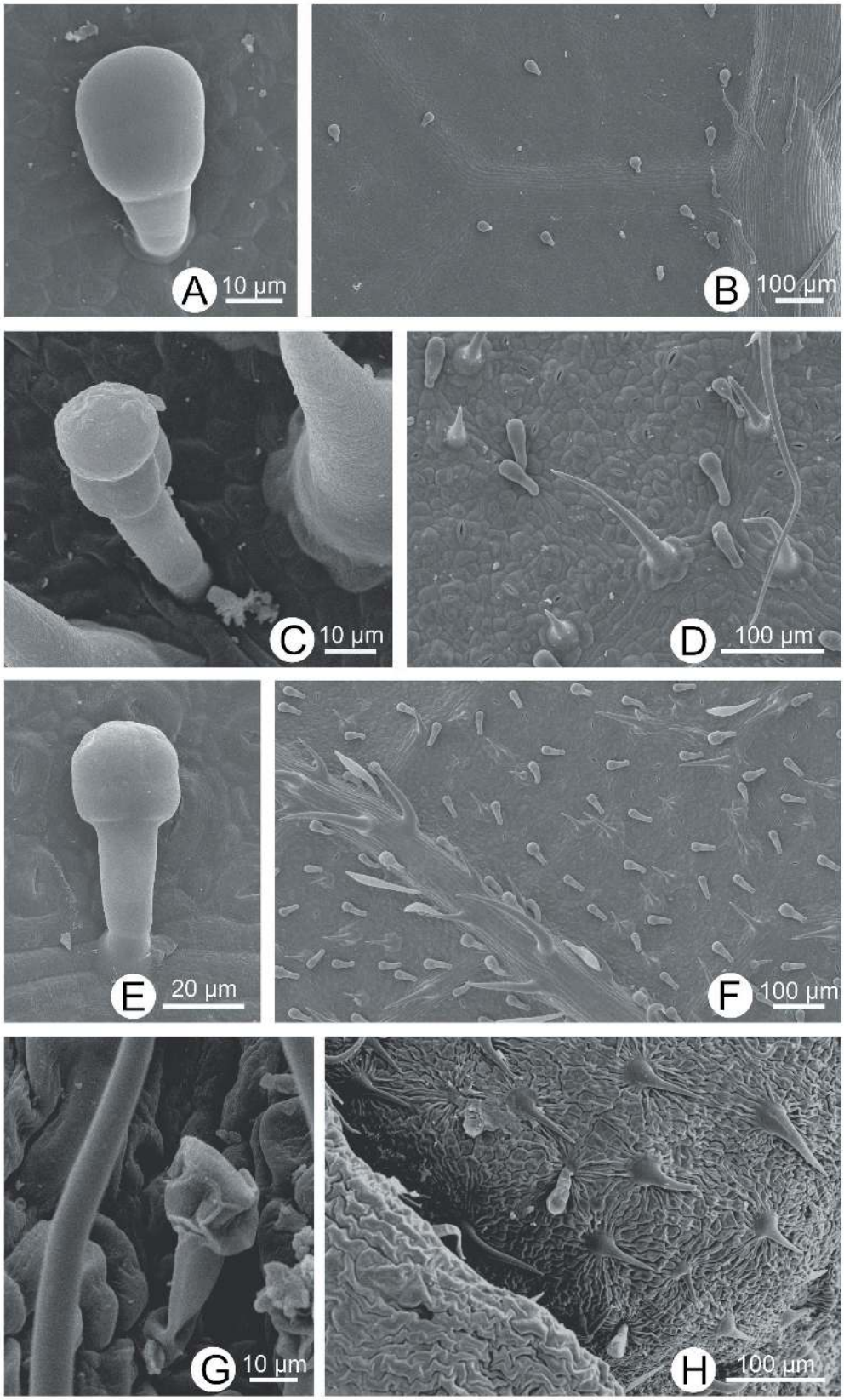


Figure 5. Capitate glandular trichomes (type 6) with a unicellular stalk and pluricellular head of Ulmaceae species (SEM). **A-B**. *Ampelocera glabra*. **A**. Detail. **B**. Distribution of trichomes on the abaxial side of the leaf blade. **C-D**. *Phyllostylon rhamnoides*. **C**. Detail. **D**. Distribution of trichomes on the abaxial side of the leaf blade. **E-F**. *Ulmus parvifolia*. **E**. Detail. **F**. Distribution of trichomes on the abaxial side of the leaf blade. **G-H**. *Zelkova serrata*. **G**. Trichome after release of the exudate; note that there is no disruption of cells. **H**. Distribution of trichomes on the ovary.

head), 5 (capitate with a bicellular head) and 6 (capitate with a pluricellular head) (Fig. 8, Tab. 2). The second and third axes separated Cannabaceae species from the others especially by the presence of glandular trichomes of types 8 (capitate with a four to eight-celled head), 9 (capitate with a disc-shaped head), 12 (capitate with a biseriate pluricellular stalk and pluricellular head), 14 (uniseriate filiform) and 15 (biseriate filiform) (Fig. 8, Tab. 2).

Discussion

Urticalean Rosids exhibit a great morphological diversity of glandular trichomes, with at least 15 different morph types described and grouped into capitate and filiform types (see Tab. 2). Cannabaceae, the most economically important family in the group, is also the most diverse family in terms of types of glandular trichomes (10 morph types), seven of the capitate type and three of the filiform type (Tab. 3). This is probably due to the greater number of studies carried out with *Cannabis sativa* and *Humulus lupulus* (Briosi & Togni 1894; Dayanandan & Kaufman 1976; Gangadhara & Inamdar 1977; Hammond & Malhberg 1977; Oliveira *et al.* 1988; Tobe & Takaso 1996; present study). The least diverse family is Urticaceae with three morph types, an unexpected finding compared to the number of species included in each family (1422 spp. in Urticaceae versus 110 spp. in Cannabaceae, Yang *et al.* 2013; The Plant List 2013). We must consider that this family has been subsampled, as shown in Table 3 (only seven species with

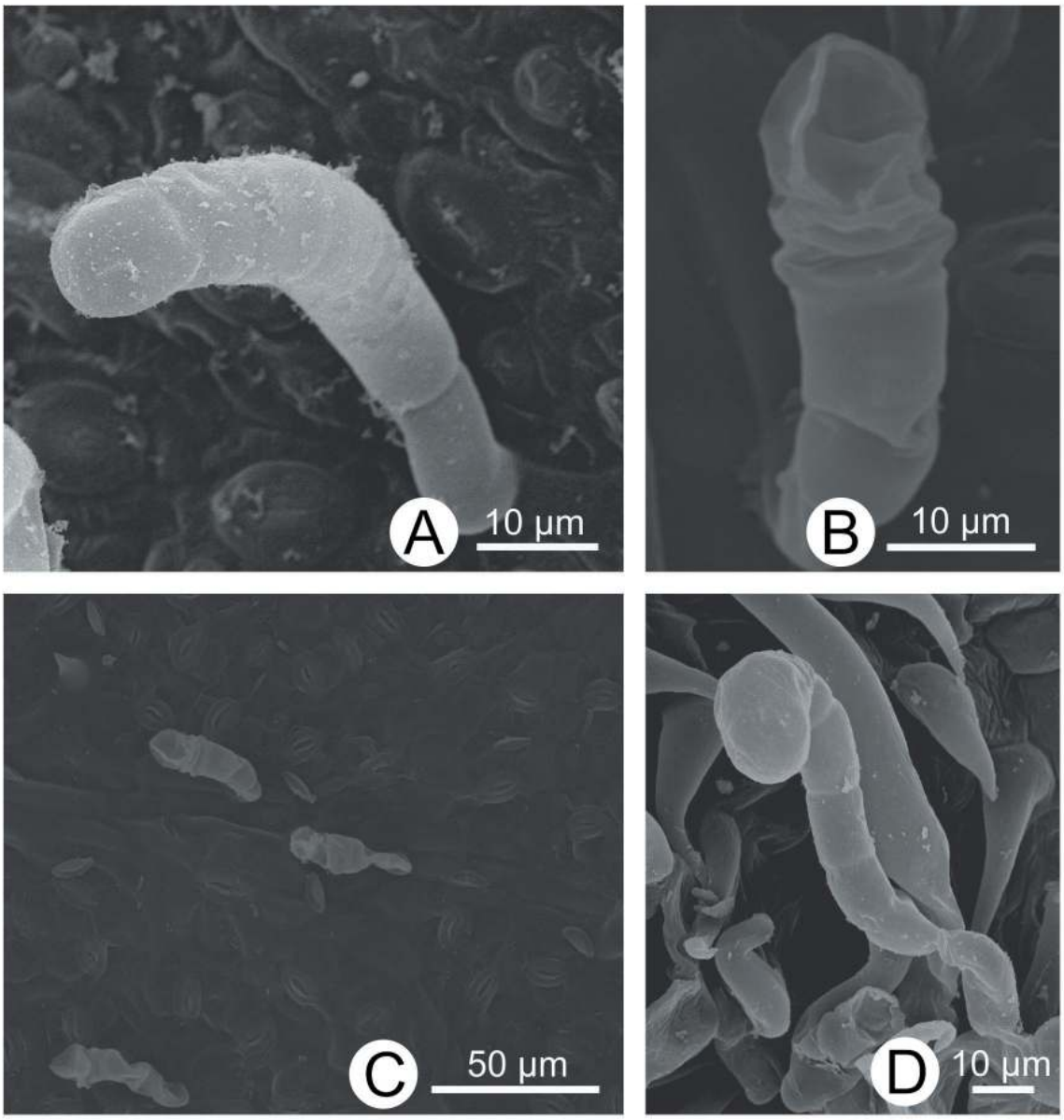


Figure 6. Uniseriate filiform trichomes (type 14) of *Celtis pubescens*, Cannabaceae (SEM). **A**. Apical cells before exudate release. **B**. Apical cells after exudate release. **C**. Distribution of trichomes on the midrib of the leaf blade on the abaxial side. **D**. Trichome on the pedicel

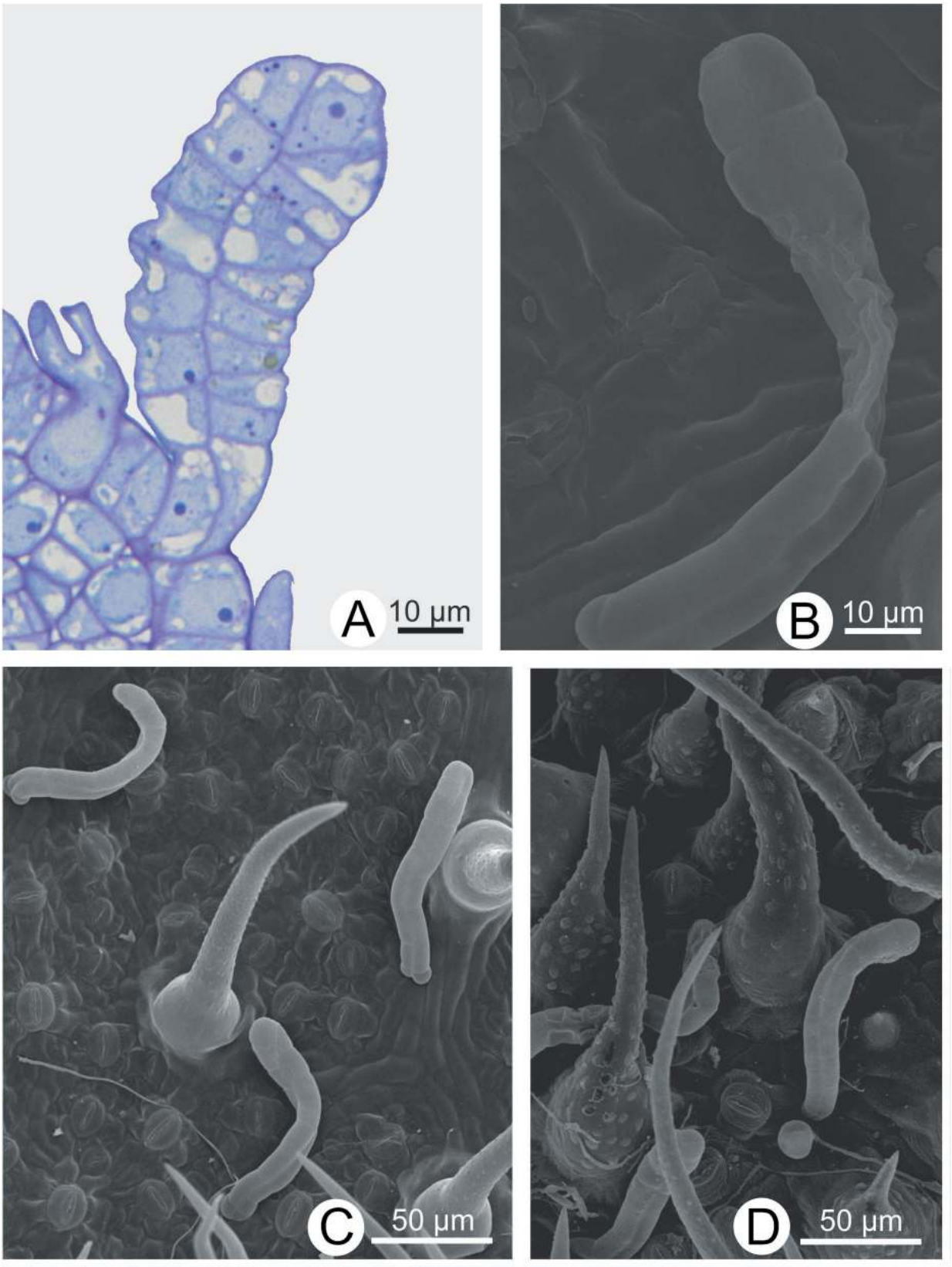


Figure 7. Biseriate filiform trichomes (type 15) of *Trema micrantha*, Cannabaceae. **A**. Longitudinal section of a trichome showing apical cells with densely stained cytoplasm and a large central nucleus (LM). **B**. Trichome in the process of exudate release; note that the cells lose their turgidity (SEM). **C**. Distribution of trichomes on the leaf blade among simple trichomes (SEM). **D**. Distribution of trichomes on the flower pedicel (SEM).

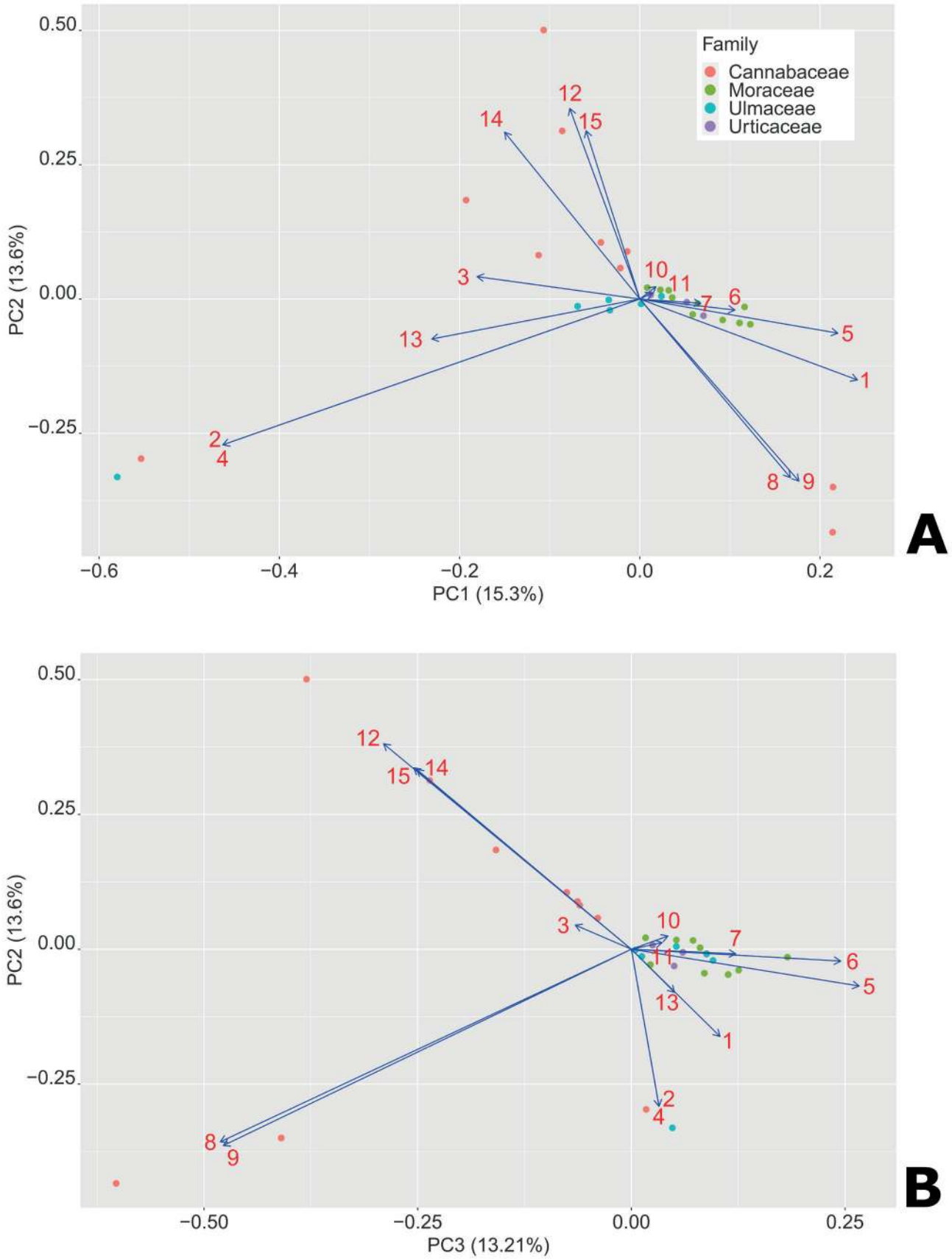


Figure 8. Biplot of axes 1 and 2 (**A**), and of axes 2 and 3 (**B**) of the species ordenation according to glandular trichome types and their distribution in the plant body.

records of glandular trichomes), or that it is noteworthy by the presence of "stinging trichomes" (Fu *et al*. 2003) that actually are stinging emergences (Evert 2006). In Ulmaceae, a family comprising 64 species (The Plant List 2013), five morph types of glandular trichomes were found, three of them of the capitate type and two of the filiform type (Tabs. 2, 3). Morph type 6 (capitate trichome with a unicellular stalk and pluricellular head) had already been reported for other species of this family (Tobe & Takaso 1996; Tab. 2) and could be considered to be a unifying feature for the group. However, taking into account that species of Moraceae exhibit similar glandular trichomes with pluricellular heads (Schnetzler *et al*. 2017), this morph type cannot be considered a diagnostic characteristic for Ulmaceae.

Trema Lour. was the Cannabaceae genus with the highest number of morph types (six) found, followed by *Celtis* (three) and *Cannabis* (three). Other genera such as *Humulus, Lozanella, Chaetachme*, *Parasponia* and *Pteroceltis* have one or two trichome morph types (Tab. 2). The previously unreported result of our study is the description of the capitate glandular trichome (morph type 12) found in *Trema micrantha* and *Pteroceltis tatarinowii*, which has a biseriate stalk and a pluricellular head. A glandular trichome with a biseriate stalk is a rare condition found in Cannabaceae (Tobe & Takaso 1996) or even throughout the Urticalean Rosids, with records only for *Cannabis sativa* (Dayanandan & Kaufman 1976; Hammond & Mahlberg 1977) and *Humulus lupulus* (Oliveira *et al*. 1988). The two morph types found in *Celtis pubescens* (Gangadhara & Inandar 1977; Tobe & Takaso 1996; present study), as well as the filiform morph type found in *Trema micrantha* (Bhat & Kachroo 1979, *apud* Tobe & Takaso 1996) had been previously reported.

It is noteworthy that the different glandular trichome morph types of Cannabaceae species can occur in the same organ (Tabs. 2, 3). This wide distribution in the plant body (leaf, stem, pedicel, ovary), also observed in other Urticalean Rosids (Tobe & Takaso 1996; Shah & Kachroo 1975; Tobe & Takaso 1996; Sugiyama *et al*. 2006; Schnetzler *et al*. 2017; Tab. 3), may be related to plant protection against herbivory (Loe *et al*. 2007) since many of these species are wind pollinated (Judd *et al*. 2009), meaning that such glandular trichomes would not be involved in the attraction of pollinating animals. The defense against herbivory has been previously reported for *Cannabis sativa* in which the action of chemical compounds secreted by trichomes occurs when the herbivore disrupts trichome head cells (Small & Naraime 2016). In some cases, the released compounds may trap small insects and, in the case of larger insects, trichomes may release sticky substances with an unpleasant odor and/or taste, which would discourage herbivory (Small & Naraime 2016).

Interestingly, the capitate types of glandular trichome described here for species of Cannabaceae and Ulmaceae are very different; those of Ulmaceae are smaller (around 40 to 50 μm vs. 40 to 100 μm in Cannabaceae species), mainly due to their uni- or bicellular stalk. However, it is difficult to propose explanations for such a difference that could be related to: (a) different chemical composition of the exudate in each family and (b) need for organ protection in a family whose members are diverse in terms of habits, as occurs in Cannabaceae (trees, shrubs, herbs or lianas – Judd *et al*. 2009).

The differences observed in the types of glandular trichomes found in the species of Cannabaceae and Ulmaceae (see Fig. 8) support the new circumscription of *Celtis, Pteroceltis* and *Trema* in Cannabaceae (previously inserted in Ulmaceae) (Sytsma *et al*. 2002). These genera stand out for being the species-richest in the family (Yang *et al*. 2013). Similar inferences can be found in Metcalfe & Chalk (1950), Narayana (1979), Adedeji *et al*. (2007). The diverse morphology and distribution of glandular trichomes allow their use in plant systematics, especially in the diagnosis of genera (Narayana 1979; Adedeji *et al*. 2007) or even of families, such as Lamiaceae (Metcalfe & Chalk 1950). However, the lack of homogeneity in terms and descriptions makes it difficult to infer about the taxonomic value of glandular trichomes, which can be exemplified by Cannabaceae. Thus, our proposed standardization of terminology for glandular trichomes reported for Cannabaceae and Ulmaceae (see Tab. 2) is essential. Some examples are given below.

Capitate trichomes as considered in the present study based on an evident distinction between the stalk and head cells have been reported in the literature as bulbous trichomes (spherical head) or as peltate trichomes (flat head) (Sugiyama *et al*. 2006). Bulbous trichomes have been reported to have different cell numbers: 1-2 stalk cells, 1-2, 4 head cells (Hammond & Mahlberg 1977; Oliveira & Pais 1988), one-celled stalk and head (Gangadhara & Inamdar 1977), or two-celled stalk and four-celled head (Dayanandan & Kaufman 1976). We classified these trichomes into two morph types for *C. sativa* (see trichomes 1 and 8 in Tab. 2). Most surprisingly, the term bulbous is also used for the stinging trichome of Urticaceae, not discussed in this study because it is actually a secretory emergence (Evert 2006). The peltate trichome has been described for *Cannabis sativa* and *Humulus lupulus* as two different morph types based on their distribution on the plant organs (Hammond & Mahlberg 1977; Sugiyama *et al*. 2006). Nevertheless, these morph types are morphologically similar in terms of stalk and head composition. Another issue to be considered is the origin of the stalk in the peltate trichome of *Cannabis sativa,* formed from hypodermal and epidermal cells (Dayanandan & Kaufman 1976). Thus, we unified these two types into one morph type based on their ontogeny and final structure (Dayanandan & Kaufman 1976) (see trichome 9 in Tab. 2). Peltate trichomes have also been described for Moraceae as having a head with radially arranged cells and a unicellular (see trichome 10 in Tab. 2, Jacomassi *et al*. 2010; Schnetzler

et al. 2017) or bicellular stalk (see trichome 11 in Tab. 2, Shah & Kachroo 1975).

Other types of capitate trichomes comprise those with a multicellular, uniseriate stalk (see trichomes 2, 3 and 4 in the Tab. 2), occurring in Cannabaceae, Ulmaceae (Gangadhara & Inamdar 1977; present study) and in Moraceae species (Shah & Kchroo 1975). Our analysis showed that capitate trichomes with a uniseriate head and stalk are typical of Cannabaceae and Ulmaceae species (see Fig. 8). Similar data were obtained for filiform trichomes (see trichomes 13 and 14 in Tab. 2).

Filiform trichomes, here described as having no apparent distinction between the stalk and the head, have been reported as short or long clavate trichomes: short when they have up to four linearly arranged cells and long when they have more than four cells (Tobe & Takaso 1996). The short clavate trichome is usually found in Ulmaceae species while the long clavate trichome is found more frequently in Cannabaceae species (see Tab. 2). The exceptions are *Ampelocera* (Ulmaceae) and *Lozanella* (Cannabaceae), which have long clavate and short clavate trichomes, respectively, probably because both genera are positioned most basally in the phylogeny. *Ampelocera* is a sister group of the rest of Ulmaceae species (Neubig *et al.* 2012) and *Lozanella* is among the most basal genera of Cannabaceae species (Yang *et al.* 2013).

It is important to emphasize that Gangadhara & Inamdar (1977) published a very good anatomical description of trichomes in Urticalean Rosids. However, their terminology is confused because they categorized a similar morphological trichome into two morph types, for example: "Capitate uniseriate filiform stalk with unicellular, bicellular or multicellular head" and "Uniseriate glandular head with uniseriate filiform stalk". Tobe & Takaso (1996) also published an excellent study using terms such as capitate, peltate, long-clavate and short-clavate for glandular trichomes. We added some information to those descriptions and also demonstrated a greater diversity of trichomes in Cannabaceae than in Ulmaceae.

Considering that new morph types of glandular trichomes continue to be described for Cannabaceae, it is important to improve efforts regarding this topic for Urticalean Rosids as a whole.

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