



A comparative study of latex-producing tissues in genera of Liabeae (Asteraceae)

Diego Germán Gutiérrez^{a,*}, María Luján Luna^b

^a División Plantas Vasculares, Museo de La Plata, FCNyM, UNLP, Paseo del Bosque s.n., B1900FWA La Plata, Buenos Aires, Argentina

^b Cátedra de Morfología Vegetal, Facultad de Ciencias Naturales y Museo, UNLP (CICPBA), La Plata, Buenos Aires, Argentina

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ABSTRACT

Laticifers are highly specialized living plant cells which produce and contain latex. Occurrence of latex was used to establish morphological affinities (i) between Liabeae and other Asteracean tribes, (ii) among the Liabeae genera, and (iii) in order to obtain phylogenies within Liabeae. However, structures and types of latex-producing tissues in this tribe have not yet been studied anatomically. In the present paper latex-producing structures of aerial parts in species of *Microliabum*, *Munnozia*, and *Paranephelium* (Liabeae), from open areas in mid-elevation Andean forests and in Andean high-elevation habitats, were studied. In all the analyzed species, latex secretion was easily observed in stem and leaf blade hand sections. Laticifers accompanied vascular tissues in all the cases, throughout stems and leaves, and they were of the articulated anastomosed type, at least in fully developed stages. Laticifers were found facing both, the xylem and the phloem, except for *Paranephelium* stems, in which they occur merely next to the phloem. Leaf laticifers form a reticulum accompanying the vein system. The type of latex-producing tissue shared by *Microliabum* and *Munnozia* could be a character shared by common ancestry whereas the laticifer system of *Paranephelium* stems could represent an evolutionary novelty for this genus. The laticifer type described in this study in aerial parts of Liabeae may allow establishing morphological affinities with tribes Cichorieae and Arctoteae.

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Introduction

Laticifers are highly specialized living plant cells defined by their characteristic anatomy and distinctive cytoplasm, which produce and contain latex (Hagel et al., 2008). Latex is an aqueous suspension or emulsion kind of particles that is usually exuded following tissue damage (Agrawa and Konno, 2009; Fahn, 1979). Production of latex has been proposed to be involved in primary metabolism for storage and movement of plant nutrients, of waste, and for maintenance of water balance (Evert, 2006; Fahn, 1979; Kekwick, 2001). However, for none of the latter functions strong empirical support is existing yet (Agrawa and Konno, 2009; Farrell et al., 1991; Hunter, 1994; Mahlberg, 1993). Currently, laticifers are assumed to have a primarily defensive role against herbivores and pathogens by means of storage of secondary metabolites (Agrawa and Konno, 2009; Dussourd and Denno, 1991; Pickard, 2007).

Although there are evidence of latex producing cells in species belonging to conifers, aquatic ferns and fungi (Evert, 2006; Kekwick, 2001), latex is produced almost exclusively in species of flowering plants (near 12,500–20,000), belonging to 20–40 plant families,

among them Asteraceae being particularly prominent (Evert, 2006; Fahn, 1979; Hagel et al., 2008; Lewinsohn, 1991; Metcalfe, 1983).

Latex and latex-producing tissues in Asteraceae

According to recent studies, Asteraceae include 23,000–24,000, up to 30,000, species (Funk et al., 2009; Jeffrey, 2007a), 1800 of which are known to produce latex (Lewinsohn, 1991). Latex production has developed several times throughout the Asteracean history and this trait appeared in different evolutionary lineages, being characteristic of basal tribes, especially those belonging to Cichorioideae (Bremer, 1994; Funk and Chan, 2009; Funk et al., 2009; Jeffrey, 2007a; Ortiz et al., 2009).

Latex is common in Arctoteae, Cichorieae, Cardueae, Gochnatieae, Liabeae, and Stifftieae but it is rare in Heliantheae and Astereae, where it has been only noted in guayule (*Parthenium argentaum* A. Gray) and in goldenrod (e.g., *Solidago altissima* L.), respectively (e.g., van Beilen and Poirier, 2007). According to Lewinsohn (1991) and Robinson (2007, 2009) latex occurs in some Vernonieae.

Earlier comprehensive and detailed or general studies of latex-producing tissues in Asteraceae were made by different authors (e.g., Carlquist, 1958; Col, 1889, 1901, 1903, 1904; Small, 1919; Solereder, 1908). Latex-producing tissues have been found in

* Corresponding author.

E-mail addresses: dgutierrez@fcnym.unlp.edu.ar (D.G. Gutiérrez), lujanluna@fcnym.unlp.edu.ar (M.L. Luna).

vegetative (e.g., roots, stems, leaves, phyllaries, peduncles) as well as in reproductive organs (e.g., florets, fruits). Recently, Hagel et al. (2008) reported three main types of latex producing and containing histological compartments in Asteraceae: (1) parenchymatous cells, (2) laticiferous cells, and (3) articulated anastomosed laticifers. Parenchymatous latex-producing tissues are recorded until now only in *Parthenium argentatum* and in leaves of *Solidago* L. (Evert, 2006; Fahn, 1979; Metcalfe, 1983; Simpson, 2009). Laticiferous cells were reported in Cardueae and Stiffiteae (e.g., Duida S.F. Blake, *Gongylolepis* R.H. Schomb., *Neblinaea* Maguire and Wurdack, *Quelchia* N.E. Br. – Carlquist, 1958; Metcalfe, 1966; Susanna and Garcia-Jacas, 2007).

The most common type of latex-producing tissues in Asteraceae are the articulated laticifers, which consist in cells or series of connected cells forming systems that permeate various tissues of the plant body (Evert, 2006; Hagel et al., 2008; Jeffrey, 2007a). They have a compound origin, being anastomosed at maturity if a connection is established between the lateral cell walls of adjacent laticifers. This type have been reported in subterranean and/or aerial parts of Arctoteae (Carlquist, 1958; Funk et al., 2004; Karis et al., 2009; Karis, 2007; Kilian et al., 2009; Metcalfe, 1966), Cardueae (Carlquist, 1958; Kilian et al., 2009; Metcalfe, 1966), Cichorieae (Jeffrey, 2007a,b; Katinas et al., 2008; Kilian et al., 2009; Lack, 2007), Gochnatieae (Jeffrey, 2007a; Melo de Pinna and Menezes, 2003), Liabeae (Jeffrey, 2007a), and Vernoniaceae (Carlquist, 1958; Jeffrey, 2007a; Metcalfe, 1966).

Latex and latex-producing tissues in Liabeae

The Neotropical tribe Liabeae is distributed from Mexico to central Argentina and it is characterized by the frequent occurrence of white latex (Bremer, 1994; Dillon et al., 2009; Funk et al., 1996, 2007; Robinson, 1983). Liabeae has 20 genera (and approximately 160 species): Gutiérrez (2010), Robinson and Funk (2011), Funk et al. (2012) and Gutiérrez and Katinas (in press). 65% of them produce latex at least in one species (Table 1). Only Robinson (1978) associated the stems of some genera from Ecuador as latex producing organs in Liabeae.

Taking into account the three classical subtribes of Liabeae (Liabinae, Munnoziinae, and Paranepheleinae), the subtribes Munnoziinae and Paranepheleinae were characterized by the presence of latex, whereas Liabinae included genera both with and without latex (Funk et al., 2007). Recently, a new subtribe Sinclairiinae was proposed based on molecular research, together with several rearrangements of the existing subtribes (Dillon et al., 2009; Funk et al., 2012). Only the latex-producing genus *Cacosmia* Kunth was not included in any subtribe. According to these points of view, all the genera within Liabinae lack of latex, whereas Sinclairiinae and Munnoziinae show latex and Paranepheleinae includes genera with and without latex (Funk et al., 2012; our study).

Latex has been considered as an important trait to establish affinities between Liabeae and other tribes, as well as to characterize this tribe and its subtribes and to distinguish genera within Liabeae and to make cladistic analyses in order to obtain phylogenies (Bremer, 1994; Dillon et al., 2009; Funk and Robinson, 2001; Funk et al., 1996, 2007, 2012; Moran and Funk, 2006; Robinson and Funk, 2011; Robinson, 1983). Despite being a very important trait for understanding the diversity and evolution of Liabeae, latex-producing tissues in this tribe have not been studied until the present work.

The goals in this study are to describe and anatomically compare latex-producing tissues in the aerial parts of species from three of Liabeae genera: *Microliabum* Cabrera, *Munnozia* Ruiz and Pav., and *Paranepheleus* Poepp.

Materials and methods

Plant selection

Three representative genera of Liabeae, which were previously recorded as latex-producing, were selected: *Microliabum*, *Munnozia*, and *Paranepheleus* (Table 1). Each genus belongs to one of the three classical subtribes of Liabeae: Liabinae, Munnoziinae, and Paranepheleinae, respectively (Funk et al., 2007). According to molecular studies (Dillon et al., 2009; Funk et al., 2012), *Microliabum* and *Paranepheleus* are included in Paranepheleinae and *Munnozia* in Munnoziinae.

Microliabum contains five or six species from central Bolivia to central Argentina (Dillon et al., 2009; Funk et al., 2012; Gutiérrez et al., 2007; Gutiérrez, 2007; Robinson, 1990). Two species were studied: *M. mulgediifolium* (Muschl.) H. Rob. and *M. polymnioides* (R.E. Fr.) H. Rob.

Munnozia occurs from Colombia to northwestern Argentina, with more than 40 species (Dillon et al., 2009; Funk and Robinson, 2001; Funk et al., 2012; Robinson, 1983). *Munnozia hastifolia* (Poepp.) H. Rob. and Brettell was selected for this study.

Paranepheleus is distributed from Ecuador to northwestern Argentina, grouped into seven species (Dillon et al., 2009; Funk et al., 2012; Robinson, 1983; Soejima et al., 2008) from which *P. asperifolius* (Muschl.) H. Rob. and Brettell was chosen.

Living plants of each species were collected in natural populations from Argentina during 2007 up to 2011. Individuals were also cultivated in La Plata city, Buenos Aires province (Argentina). Voucher specimens have been deposited in LP herbarium.

Information on latex in Liabeae was obtained from bibliography, labels of herbarium specimens and personal observations in field trips. Bibliography (Table 1) and labels of herbarium specimens were sometimes contradictory about the presence of latex in the genera of this tribe. In this work, a Liabean genus is considered laticiferous if at least one species was clearly identified as presenting latex.

As the terminology used to describe and classify latex-producing tissues have varied greatly from early works to the present time (e.g., Bremer, 1994; Fahn, 1979; Mahlberg, 1993; Metcalfe, 1983) in this study we followed Hagel et al. (2008) because of usefulness for practical classification of laticifers for general analyses in Asteraceae.

Anatomy

Macroscopic observations were made on living plants during field trips as well as on those cultivated in La Plata by means of hand sections rendering latex release from cut surfaces.

For light microscopy (LM) studies, portions of blades and stems were fixed in formaldehyde–acetic acid–alcohol, dehydrated through an ethanol series and embedded in Paraplast. Sections (8–12 µm thickness) were double stained with safranin-fast green (Johansen, 1940). Material was also cut with a cryotome (20–30 µm thickness) and stained with safranin. Blades were processed as well according to Foster's diaphanization method. Anatomical observations and photographs were carried out with a Nikon Photolab 2 light microscope.

We use abbreviations TS and LS for transversal section and longitudinal section respectively.

Results

In all the species analyzed, latex secretion was easily observed in stem and leaf blade hand sections. Latex was milky, whitish and showed dense consistency (Fig. 1B, C, E, F, H, and I). Latex exudation was abundant and scarce, respectively, in natural populations and

Table 1

Presence/absence of latex in genera of Liabeae. Note: If at least one species presents latex, the genus is considered here with latex.

Genera	Latex present	Latex absent or not reported
<i>Bishopanthus</i> H. Rob. <i>Cacosmia</i> Kunth	Bremer (1994), Funk et al. (1996, 2007) Robinson (1978, 1983), Bremer (1994), Funk et al. (1996, 2007, 2012)	
<i>Chionopappus</i> Benth. <i>Chrysactinium</i> (Kunth) Wedd.	Funk et al. (1996, 2012) Funk et al. (1996), Funk and Zermoglio (1999), Funk et al. (2007) ^a , Funk et al. (2012)	Robinson (1983), Bremer (1994), Funk et al. (2007) Robinson (1983), Bremer (1994), Moran and Funk (2006)
<i>Dillandia</i> V.A. Funk and H. Rob. <i>Erato</i> DC.	Robinson (1983) (sub <i>Munnozia</i>) Robinson (1978, 1983), Bremer (1994), Funk et al. (1996), Moran and Funk (2006), Funk et al. (2007) ^a , Funk et al. (2012)	Funk and Robinson (2001), Funk et al. (2007, 2012)
<i>Ferreyranthus</i> H. Rob. and Brettell		Robinson (1978, 1983), Bremer (1994), Dillon and Sagástegui Alva (1994), Funk et al. (1996, 2007), Dillon et al. (2009), Funk et al. (2012)
<i>Inkaliabum</i> D.G. Gut. <i>Liabellum</i> Rydb.	Robinson (1983), Bremer (1994) (sub <i>Sinclairia</i>), Funk et al. (1996, 2007), Dillon et al. (2009), Funk et al. (2012)	Robinson (1983) (sub <i>Liabum</i>), Gutiérrez (2010) Turner (1989) (sub <i>Sinclairia</i>)
<i>Liabum</i> Adans.		Robinson (1978, 1983), Bremer (1994), Funk et al. (1996, 2007), Dillon et al. (2009), Funk et al. (2012), Gutiérrez and Katinas (in press)
<i>Microliabum</i> Cabrera	Robinson (1983) (sub <i>Austroliabum</i>), Robinson (1990), Bremer (1994), Funk et al. (1996), Cabrera et al. (1999), this work	
<i>Munnozia</i> Ruiz and Pav.	Robinson (1978, 1983), Bremer (1994), Funk et al. (1996), Cabrera et al. (1999), Moran and Funk (2006), Funk et al. (2007) ^a , Funk et al. (2012), this work	
<i>Oligactis</i> (Kunth) Cass.		Robinson (1983), Bremer (1994), Funk et al. (1996, 2007), Dillon et al. (2009), Funk et al. (2012) Soejima et al. (2008)
<i>Paranephelius</i> Poepp.	Robinson (1983), Bremer (1994), Funk et al. (1996), Cabrera et al. (1999), Funk et al. (2007) ^a , Funk et al. (2012), this work	
<i>Philoglossa</i> DC.	Robinson (1978, 1983), Bremer (1994), Funk et al. (1996, 2007) ^a , Funk et al. (2012)	Moran and Funk (2006)
<i>Pseudonoseris</i> H. Rob. and Brettell	Robinson (1983), Bremer (1994), Funk et al. (1996, 2007) ^a , Soejima et al. (2008), Funk et al. (2012)	
<i>Sampera</i> V.A. Funk and H. Rob.		Robinson (1978) (sub <i>Oligactis</i>), Robinson (1983) (sub <i>Oligactis</i>), Bremer (1994) (sub <i>Oligactis</i>), Funk et al. (1996) (sub <i>Oligactis</i>), Funk et al. (2007) (sub <i>Oligactis</i>), Funk and Robinson (2009), Funk et al. (2012) Turner (1989)
<i>Sinclairia</i> Hook. and Arn. (including <i>Megaliabum</i> Rydb.) <i>Sinclairiopsis</i> Rydb.	Robinson (1983), Bremer (1994), Funk et al. (1996, 2007), Dillon et al. (2009), Funk et al. (2012) Robinson (1983) (sub <i>Sinclairia</i>), Funk et al. (2007) (sub <i>Sinclairia</i>), Dillon et al. (2009), Funk et al. (2012)	Turner (1989) (sub <i>Sinclairia</i>)
<i>Stephanbeckia</i> H. Rob. and V.A. Funk		Robinson and Funk (2011), Funk et al. (2012)

^a Generalization under subtribes Munnoziinae and Paranepheleinae.

in cultivated plants. In cultivated plants, latex was scarce or even completely absent in some cases.

Microliabum

Microliabum mulgediifolium and *M. polymnioides* are herbs or somewhat subshrubs up to 1–2 m high with erect, sparsely branched, glabrescent, glandular-pubescent or densely white-tomentose stems (Fig. 1A and B). Under the light microscope (LM) stems show a cylindrical outline in transversal sections (TS). Fig. 2A illustrates the stem of *M. mulgediifolium* with a single-layered epidermis and pluricellular trichomes. The cortex contains 1–2 rows of collenchyma (up to 8 in *M. polymnioides*) and 5–6 rows of thin-walled parenchyma cells (Fig. 2A). A cylinder of collateral vascular bundles encloses the extensive parenchymatous pith (Fig. 2A and B) that breaks up in older stems (Fig. 1B). Secondary growth is also revealed in older stems by the presence of a cambial zone between xylem and phloem.

Laticifers are located next to the vascular tissues, on the phloem and the xylem sides (Fig. 2B–E). On phloem side, they are positioned outside the groups of fibers that limit this tissue (Fig. 2C and D). In TS laticifers (20–30 µm in diameter) are thin-walled and better distinguished from the cortical parenchyma cells when secretion products are present (Fig. 2C). In longitudinal sections (LS) they

turn out to be articulated, not ramified and anastomosed in older stems (Fig. 2F).

Leaves are opposite, distant, long petiolate with triangular-hastate blade, irregular dentate–mucronate margins, glabrescent or pubescent adaxial surface, and white-tomentose abaxial surface (Fig. 1A–C). The blades of *M. mulgediifolium* and *M. polymnioides* are illustrated in Fig. 3A–F. In TS blades consist of single layered adaxial and abaxial epidermes; the former with relatively enlarged cells, and stiff pluricellular trichomes (Fig. 3A and B). The abaxial epidermis bears the stomata and long coiled pluricellular trichomes (Fig. 3A and B). The mesophyll is dorsiventral, with one layer of palisade parenchyma and 4–5 layers of spongy parenchyma (Fig. 3A and B). The collateral vascular bundles are flanked by collenchyma.

Laticifers are noticeable in transversal and longitudinal views next to the vascular bundles (Fig. 3C and D). In paradermal view they run parallel to the veins forming a reticulum and they are not ramified (Fig. 3E and F). In some instances disintegration of contact walls was observed. Hydathodes are also observable in this sight (not shown).

Munnozia

Munnozia hastifolia is an herb or subshrub up to 2 m high, with decumbent, branched, glabrous or laxly arachnoid pubescent

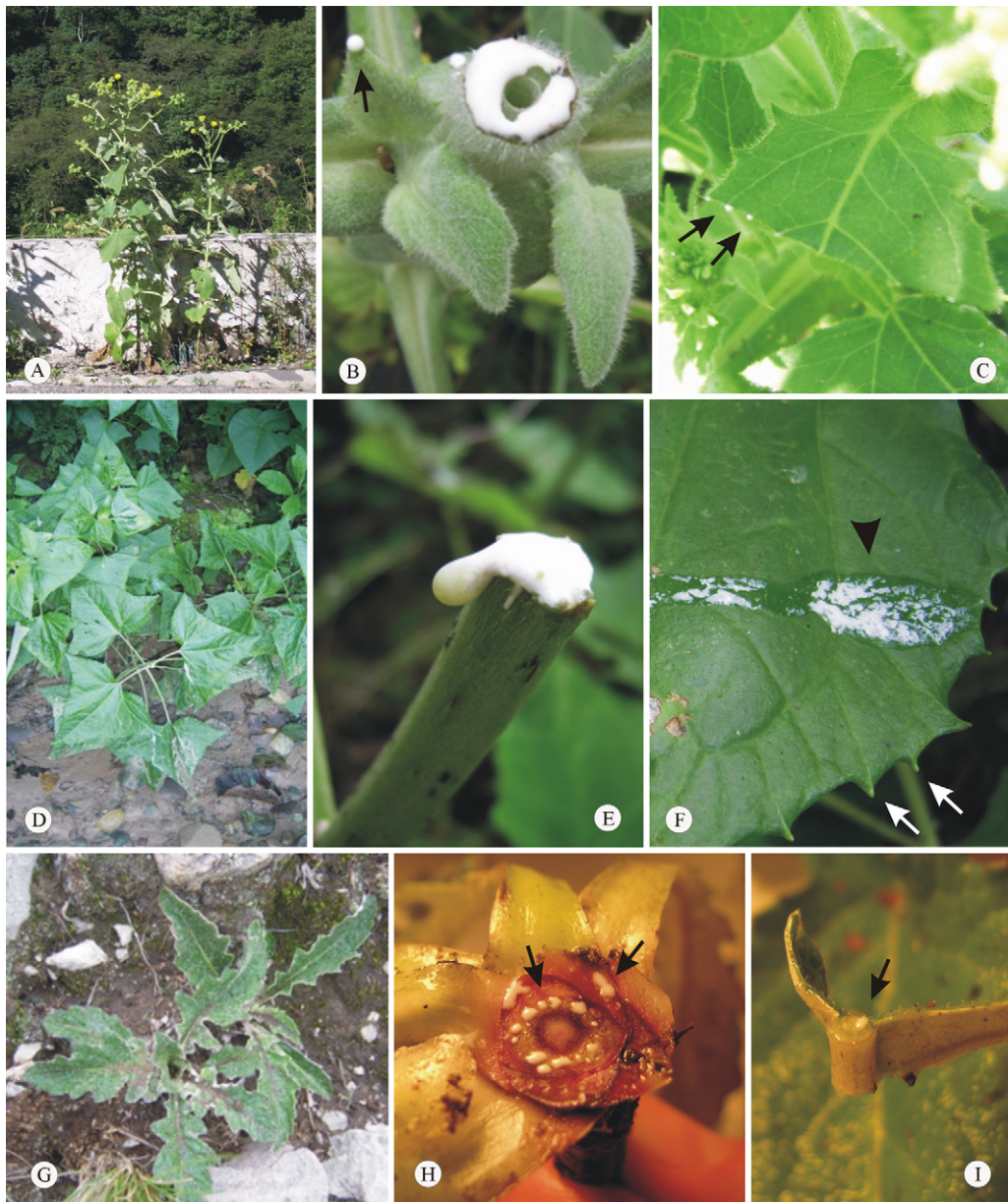


Fig. 1. (A–C) *Microliaabum polymnioides*. (A) General aspect of plants. (B) Latex exudation in stem and leaf (arrow). (C) Sectioned leaf showing latex droplets (arrows). (D–F) *Munnozia hastifolia*. (D) General view of plants. (E) Profuse latex exudation in stem. (F) Latex in leaf (arrowhead) and hydathodes in leaf margin (arrows). (G–I) *Paraneupheliu asperifolius*. (G) General aspect of plants. (H) Latex exudation in stem and leaf bases (arrows). (I) Detail of latex exudation at leaf midvein level (arrow).

stems (Fig. 1D and E). The stem is cylindrical in TS and it is surrounded by a single layered epidermis. The cortex consists of 8–10 layers of angular collenchyma and parenchymatous tissue. Secondary growth is evidenced by the occurrence of a cambial zone in the vascular bundles, between the phloem and the xylem (Fig. 4A). The vascular tissues encircle a parenchymatous pith.

Laticifers (30–40 μm in diameter) are found mainly outside the groups of fibers adjacent to the phloem, and they are similar in appearance to parenchyma cells (Fig. 4B). In LS laticifers appear articulated and not ramified (Fig. 4C). Dissolution of contact walls was observed in older stems. Longitudinal sections also show laticifers next to primary xylem elements (Fig. 4D).

Secretory structures lined by epithelia are also observed in close proximity to the vascular cambium, on the secondary xylem side (Fig. 4E).

Leaves are opposite, long petiolate, with hastate blades, with regularly dentate–mucronate margins, glabrescent adaxial surface and densely white-tomentose abaxial surface (Fig. 1D–F). The blade exhibits in TS single layered adaxial and abaxial epidermes; scarce stiff pluricellular trichomes adaxially and long coiled pluricellular trichomes abaxially (Fig. 5A and B). Stomata are located on the abaxial epidermis. The mesophyll is dorsiventral, with one layer of palisade parenchyma and 5–6 layers of spongy parenchyma. Colenchymatous tissue is present at the midvein level (Fig. 5B).

Laticifers occur at both sides of the veins, adjacent to the xylem and the phloem (Fig. 5C and D). In LS they run parallel to the veins and, as occurs in stems, they are articulated, not ramified and anastomosed in more mature conditions (Fig. 5D). Leaf laticifers form a reticulum because they follow the system of veins. This feature is observed in paradermal views (Fig. 5E), together with the presence of hydathodes (Fig. 5F).

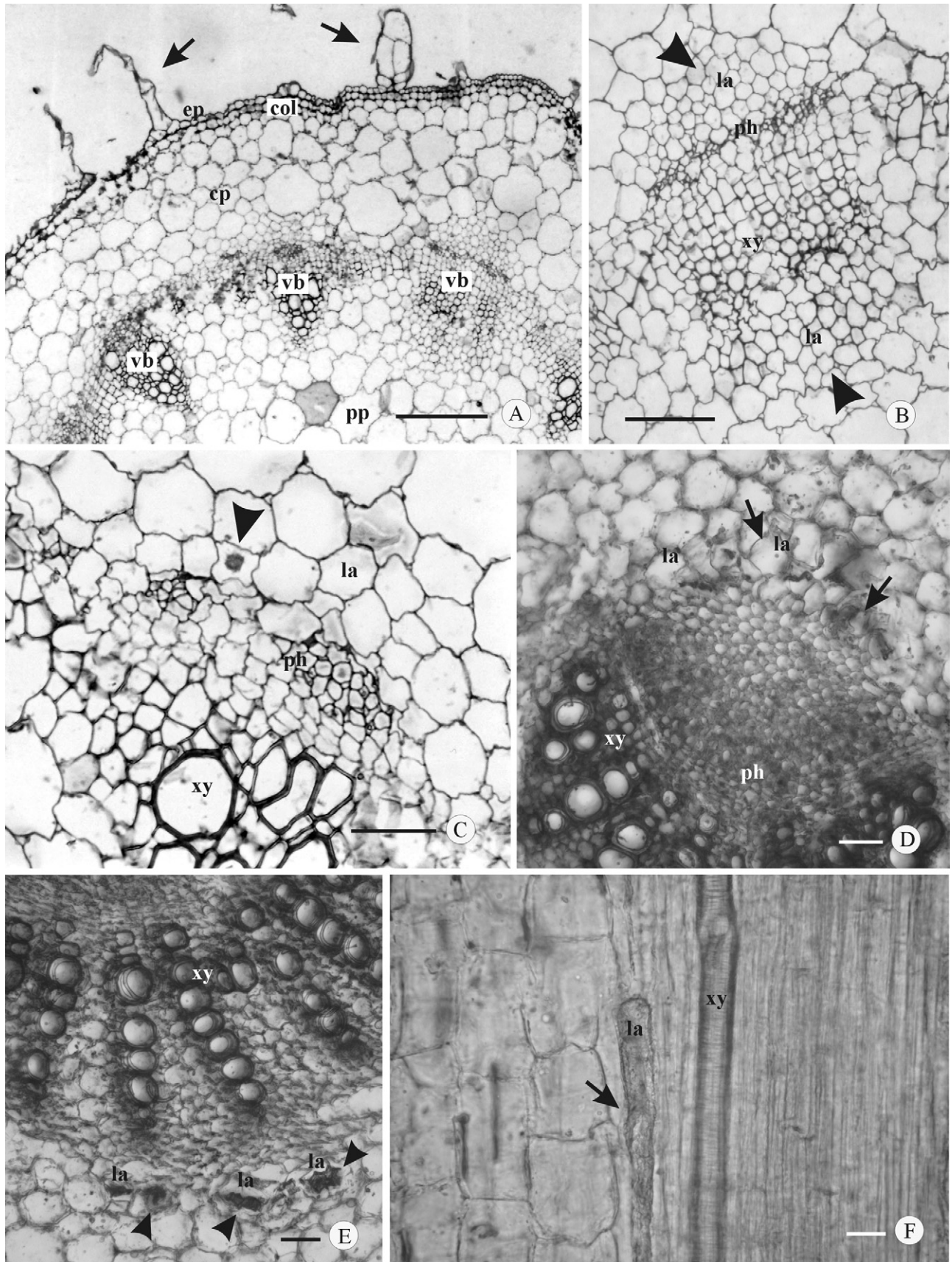


Fig. 2. (A–C) *Microliabum mulgediifolium* stems. (A) General view in TS showing epidermis (ep) with trichomes (arrow), collenchyma (col), cortical parenchyma (cp), vascular bundles (vb) and parenchymatous pith (pp). (B) Detail of vascular bundle with associated laticifers (la) (arrowheads). (C) Magnification of laticifers (la) and secretion products (arrowhead). (D–F) *Microliabum polymnioides*. (D) Detail of vascular bundle with laticifers (la) (arrows) adjacent to the phloem (ph). (E) Laticifers (arrowheads) adjacent to the xylem (xy). (F) LS showing no ramified laticifer (la and arrow) (col, collenchyma; cp, cortical parenchyma; ep, epidermis; la, laticifer; ph, phloem; pp, parenchymatous pith; vb, vascular bundle; xy, xylem). Bars: (A) 250 μm ; (B) 100 μm ; (C) 30 μm and (D–F) 20 μm .

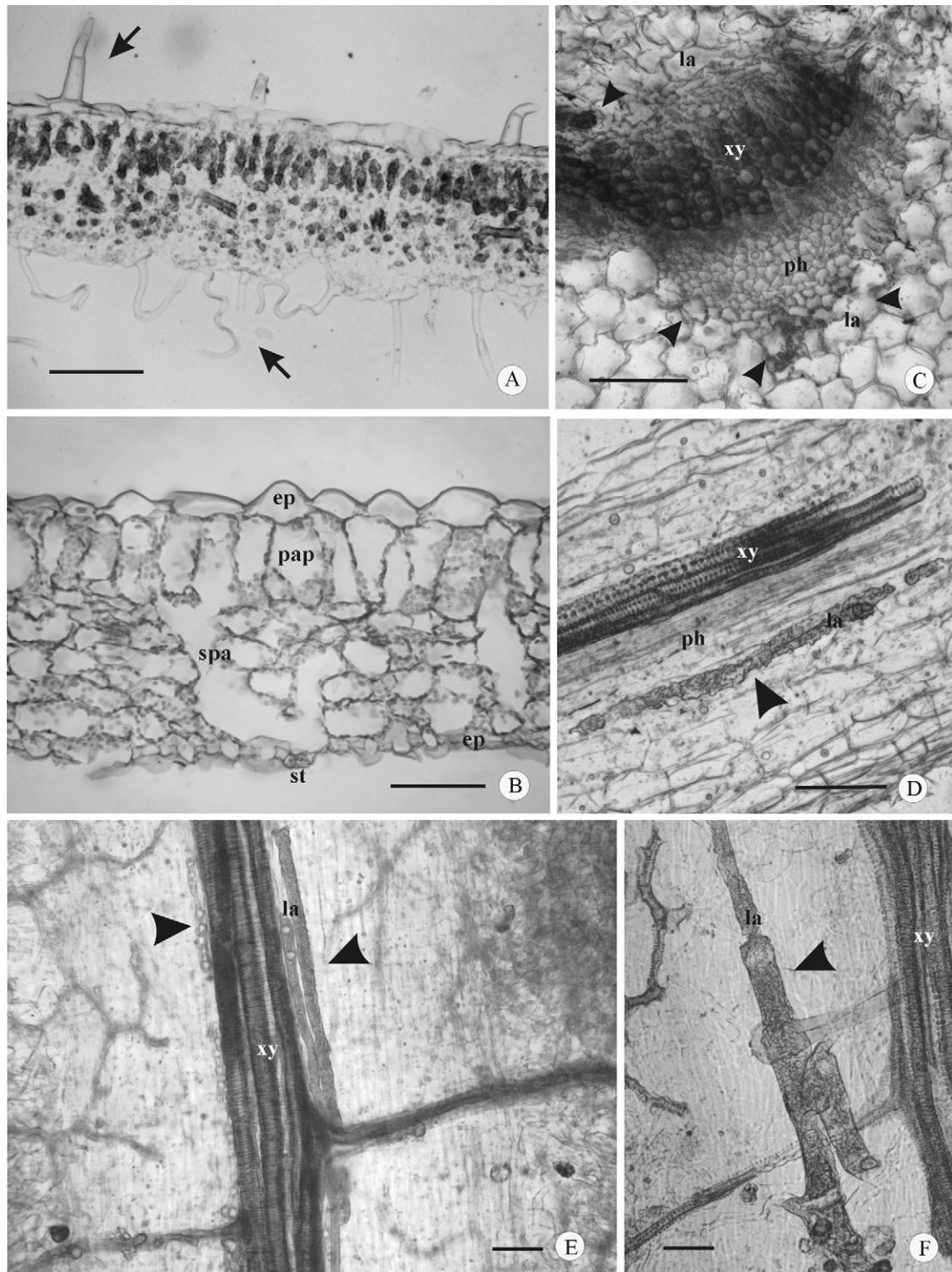


Fig. 3. (A, C, E–F) *Microlabium polymnioides* leaf. (B and D) *Microlabium mulgediifolium* leaf. (A) General view in TS displaying trichomes in both epidermis (arrows) and dorsiventral mesophyll. (B) Detail of blade section showing uniseriate epidermis (ep), dorsiventral structure with palisade (pap) and spongy (spa) parenchyma and stomata (st) on the abaxial surface. (C) Detail of vascular bundle of midvein flanked by laticifers (la) on phloem (ph) and xylem (xy) sides (arrowheads). (D) Longitudinal view of laticifers (la) adjacent to the phloem (ph and arrowhead). (E) Laticifers (la) running parallel to the veins in paradermal view (arrowheads). (F) Detail of articulated non ramified laticifer (la and arrowhead) (ep, epidermis; la, laticifer; pap, palisade parenchyma; ph, phloem; spa, spongy parenchyma; st, stomata; xy, xylem). Bars: (A) 100 μm and (B–F) 50 μm .

Paranephelius

Paranephelius asperifolius is an acaulescent herb (Fig. 1G). The stem is very short in length and cylindrical in TS (Fig. 1H).

Anatomically it consists of a single layered epidermis, parenchymatous cortex, and a cylinder of collateral vascular bundles that enclose the parenchymatous pith (Fig. 6A). A small amount of secondary growth is observable in older stems.

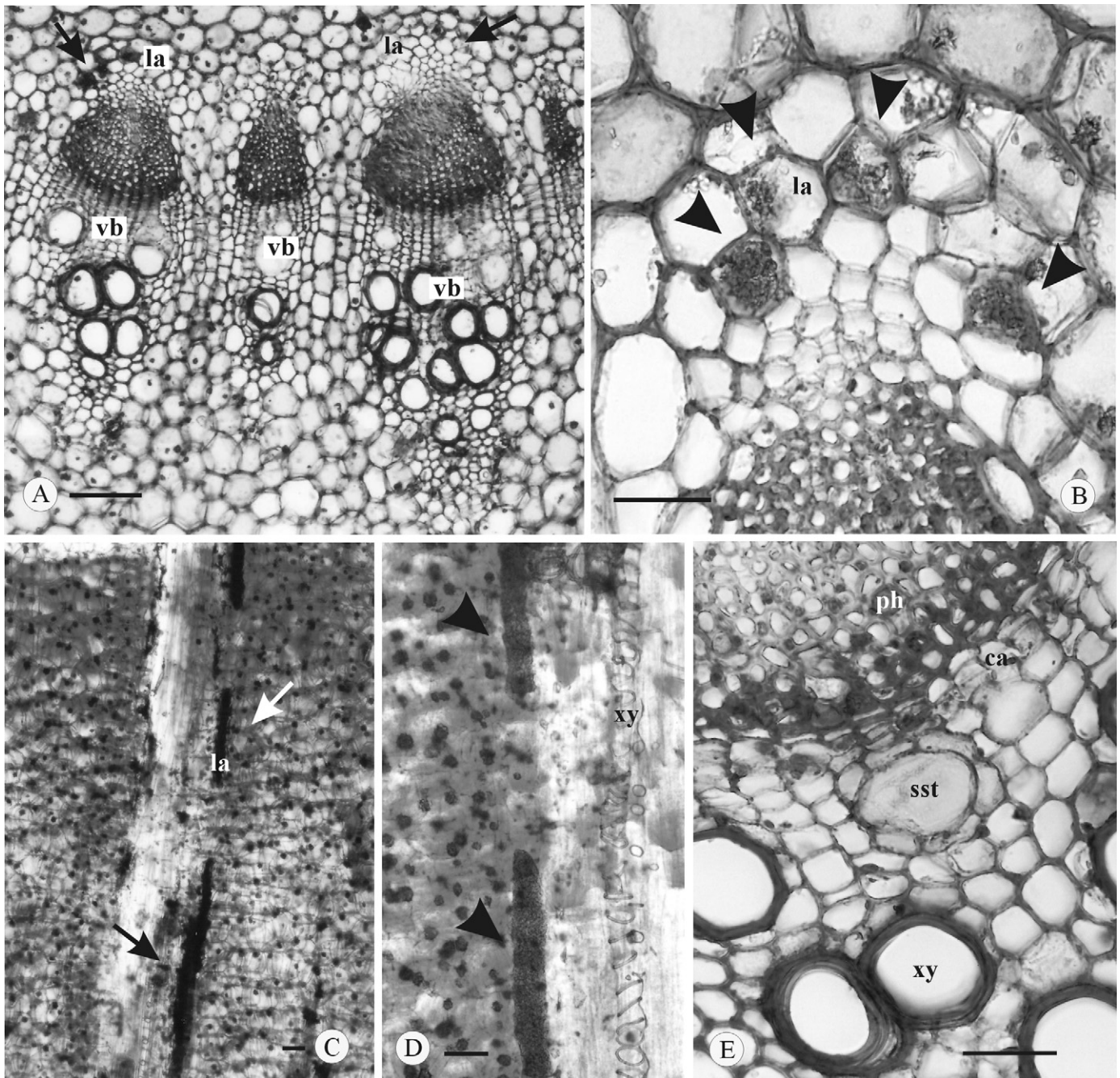


Fig. 4. *Munnozia hastifolia* stem. (A) General view in TS of vascular bundles (vb) with secondary growth and associated laticifers (la, arrows). (B) Detail of laticifers (la) adjacent to the phloem. Secretion products are observed in some laticifers (arrowheads). (C) LS showing no ramified laticifers (la, arrows). (D) Laticifers (arrowheads) next to the xylem (xy) in LS. (E) TS in detail showing secretory structures (sst) on the xylem (xy) side, probably derived from the vascular cambium (ca) (ca, vascular cambium; la, laticifer; ph, phloem; sst, secretory structure; vb, vascular bundle; xy, xylem). Bars: (A) 100 μ m; (B) 40 μ m; (C, E) 80 μ m and (D) 50 μ m.

Laticifers are developed in association with the phloem, next to groups of sclereids that border this tissue (Fig. 6A and B). In TS laticifers possess a diameter of 50–80 μ m. In LS it is apparent that they run parallel to the veins and are articulated, unbranched and initially not anastomosed (Fig. 6C and D). Advanced developmental stages of laticifers show dissolution of the terminal walls promoting anastomosis of neighboring cells.

Paranephelium asperifolius leaves are grouped in a basal rosette; they are pseudopetiolate with usually obovate blades, lobed and dentate–mucronate margins, with a hirsute adaxial surface, and a white–tomentose abaxial surface (Fig. 1G–I). The blade displays in TS a single layered adaxial epidermis of enlarged cells that

possess thick outer tangential cell walls and large stiff pluricellular trichomes (Fig. 7A). The abaxial epidermis shows thin walled cells and bears numerous long coiled pluricellular trichomes and stomata (Fig. 7A, C–E). A hypodermis is present at the mid-vein level (Fig. 7B). The dorsiventral mesophyll is represented by three layers of palisade parenchyma and 2–3 layers of spongy parenchyma (Fig. 7A and D). The vascular bundles are collateral (Fig. 7C).

Laticifers are located in association with the vascular bundles. In TS they occur adjacent to the phloem and the xylem and show different diameters, from 20 to 40 μ m (Fig. 7B and C). In LS they are tubular, articulated, unbranched and anastomosed (Fig. 7D and E).

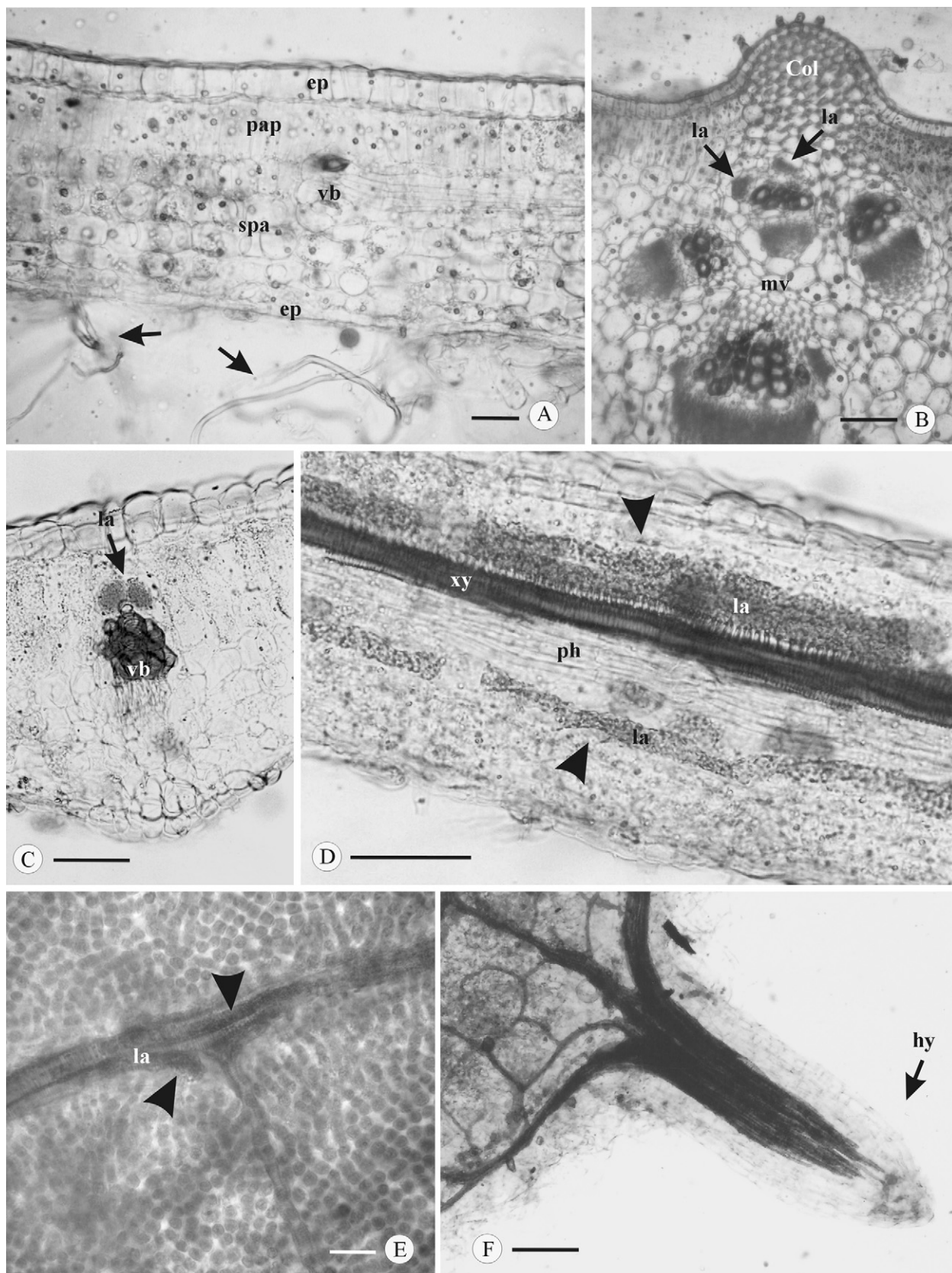


Fig. 5. *Munnozia hastifolia* leaf. (A) General view in TS showing epidermis (ep) with trichomes (arrows), and dorsiventral structure. (B) Midvein (mv) in TS with laticifers (la, arrows) associated to the vascular tissues. (C) Detail of vascular bundle (vb) with laticifers (la, arrow) on the xylem side. (D) Longitudinal view of laticifers (la, arrowheads) adjacent to the xylem (xy) and the phloem (ph). (E) No ramified laticifers (la, arrowheads) running parallel to the veins in paradermal view. (F) Hydatode (hy, arrow) in longitudinal view. (col, collenchyma; ep, epidermis; la, laticifer; pap, palisade parenchyma; ph, phloem; spa, spongy parenchyma; vb, vascular bundle; xy, xylem). Bars: (A) 30 μ m; (B) 100 μ m; (C–E) 50 μ m and (F) 200 μ m.

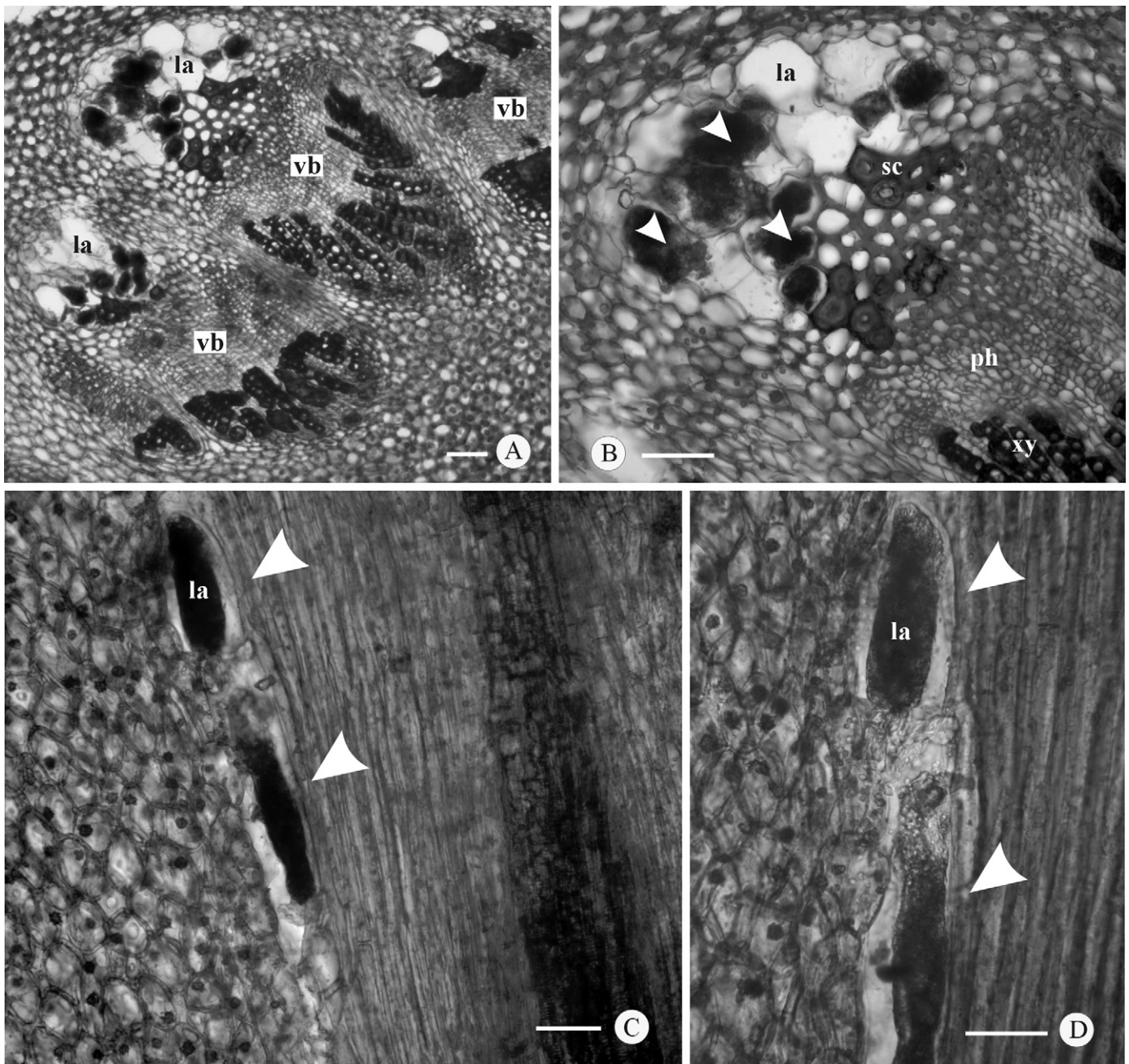


Fig. 6. *Paranephelius asperifolius* stem. (A) General view in TS of vascular bundles (vb) and associated laticifers (la) in TS. (B) Detail of laticifers (la) with secretion products (arrowheads) next to the phloem (ph). (C) LS showing no ramified laticifer (la) with dark secretion products (arrowheads). (D) Articulated laticifer (la, arrowheads) in detail (la, laticifer; ph, phloem; sc, sclereids; vb, vascular bundle; xy, xylem). Bars: (A and B) 100 μm and (C and D) 50 μm .

In larger laticifers (more mature condition) perforation of end walls occurs leading to the formation of continuous cytoplasm. In paradermal views leaf laticifers form a reticulum because they follow the system of veins (Fig. 7F and G).

Discussion

Our findings corroborate the presence of latex in the leaves and stems of Liabeae genera *Microliabum*, *Munnozia*, and *Paranephelius*. Laticifers accompany vascular tissues in all species, throughout stems and leaves, and they are of the articulated anastomosed type, at least when fully developed. The laticifers are formed by series of

superimposed cells with perforated end walls and frequently also lateral walls, leading to anastomoses between adjacent laticifers.

In stems, laticifers are located in groups intimately associated with vascular bundles, not entering the cortical or medullary parenchyma. In the leaf blades, laticifers run parallel from the main veins up to veins of smaller size, forming a reticulum.

According to Karis et al. (2009), latex and laticifers probably evolved in parallel in Arctoteae, Cichorieae, and Liabeae. The presence of articulated laticifers in the aerial parts of the species focussed upon in the present study allow us to establish morphological affinities among the tribe Liabeae with the tribes Cichorieae and Arctoteae (Funk et al., 2004).

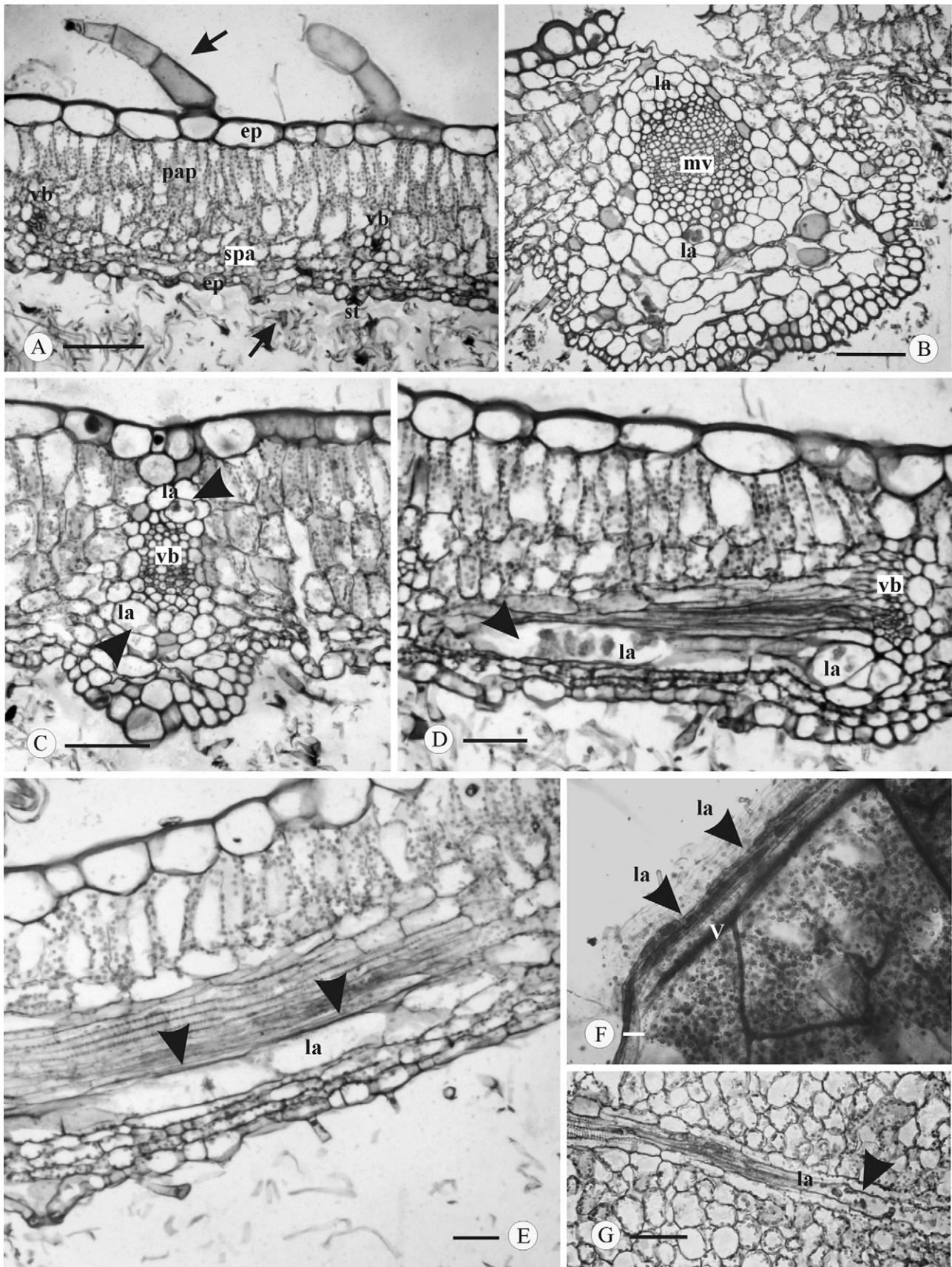


Fig. 7. *Paranephelius asperifolius* leaf. (A) General view in TS showing pluricellular trichomes (arrows) in epidermis (ep) and dorsiventral mesophyll. (B) Midvein (mv) in TS and associated laticifers (la). (C) Detail of vascular bundle (vb) in TS with laticifers (la, arrowheads) on xylem and phloem sides. (D) Longitudinal view of laticifers (la) with secretion products (arrowhead). (E) Anastomosing laticifers (la, arrowheads) in longitudinal view. (F) Laticifers (la, arrowheads) running parallel to the veins (v) in paradermal view. (G) Anastomosing laticifers (la) with secretion products (arrowhead) in paradermal view (ep, epidermis; la, laticifers; mv, midvein; pap, palisade parenchyma; spa, spongy parenchyma; st, stomata; v, vein; vb, vascular bundle). Bars: (A) 150 μm and (B–G) 50 μm.

Anatomical studies on laticifers in Asteraceae stated that they are mainly associated to the phloem or with the surrounding sclerenchymatic sheath in both, primary and secondary tissues (Katinas et al., 2008; Metcalfe, 1983). In the species analyzed here, laticifers are found facing both, the xylem and the phloem, except for *Paranephelium* stems, in which they occur merely next to the phloem.

According to Robinson (1983), the presence of latex seems fundamental to the Liabeae. However, phylogenetic studies based on morphological data (including the presence/absence of latex) are contradictory regarding basal and derived groups within this tribe (Bremer, 1994; Funk et al., 1996). Recent phylogenies based on DNA sequence data also found the same difficulty (Dillon et al., 2009; Funk et al., 2012; Gutiérrez et al., 2007; Kim et al., 2003). For purposes of discussion, if we take into account the last phylogeny proposed for Liabeae (Funk et al., 2012), the presence of latex could be regarded as a primitive character within the tribe, where *Cacosmia* is considered as basal. Thus, absence of latex in any genus of Liabinae would be associated to the loss of this character throughout the evolution from the ancestors of this subtribe. The most derived genera belonging to Sinclariinae, Paranepheliinae, and Munnoziinae, which are characterized mostly or entirely by having latex, would have retained the primitive character of the tribe. On the other hand, considering DNA sequences, Gutiérrez et al. (2007) proposed the genus *Microliabum* preliminary as basal of the classical Liabinae (Funk et al., 1996, 2007; Robinson, 1983). In this case, the presence of latex would be a character common to all evolutionary lines of Liabeae, even Liabinae that could have lost it recently in the more derived genera of this subtribe (e.g., *Liabum* Adans., *Sampera* Funk & H. Rob.).

The present work contributes to establish, still at a preliminary level, morphological affinities within Liabeae, since a similar type of laticifers was found in all three analyzed genera. However, *Microliabum* and *Munnozia* are morphologically more close to each other, because their laticifers are located beside phloem and xylem sides in aerial organs, and they look like parenchymatic cells in TS when secretion products are not observed. By contrast, *Paranephelium* laticifers are located only on the phloem side in stems and they are wider even in younger stems. The type of latex-producing tissue shared by *Microliabum* and *Munnozia* could be a character shared by their common ancestor and the laticifer system of *Paranephelium* stems an evolutionary novelty for this genus.

In addition, other anatomical traits agree with this morphological affinity: *Microliabum* and *Munnozia* share the dorsiventral type of mesophyll, with only one layer of palisade parenchyma. On the other hand, the dorsiventral mesophyll in *Paranephelium* was commonly represented in the investigated samples by three layers of palisade parenchyma. This feature contrasts with the most recent phylogeny based on DNA sequences (Funk et al., 2012) where *Microliabum* is placed closer to *Paranephelium*, and *Munnozia* is phylogenetically more distant. However, since the phenotypic character may be variable according to the environmental conditions, more studies are required to corroborate the affinity. *Munnozia hastifolia*, *Microliabum mulgediifolium*, and *M. polymnioides* occur in similar habitats: open areas in mid-elevation Andean forests. *Paranephelium asperifolium* inhabits Andean high-elevation habitats around 4000 m.

In the case of *Munnozia*, the origin and function of the secretory structures adjacent to the cambial zone remain to be investigated, and as consequence the question, whether they constitute laticifers or another type of secretory ducts. Both, secretory ducts and laticifers have been reported, for example, in the roots of few genera of Cichorieae (Kilian et al., 2009, and references here cited) and in the genus *Richterago* Kuntze of Gochnatieae (Melo de Pinna and Menezes, 2003). It was mentioned that involuclral

hairs of the Cichorieae become directly connected with the laticifers and that these trichomes actually correspond to terminations of the laticiferous system (Evert, 2006; Metcalfe, 1966). Since several latex-producing species of Liabeae have also glandular hairs and a very similar laticiferous system, it seems to be interesting and promising to investigate in the future if any kind of connection of these structures occurs in these species.

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