Revista Brasileira de Farmacognosia 28 (2018) 393–401

Brazilian Journal of Pharmacognosy
REVISTA BRASILEIRA DE FARMACOGNOSIA
www.elsevier.com/locate/bjp

Original article

Morphoanatomical and histochemical characterization of *Larrea* species from Northwestern of Argentina

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ARTICLE INFO

Article history:
Received 10 March 2018
Accepted 30 May 2018
Available online 23 June 2018

Keywords:
Larrea
Morphology
Anatomy
Histochemistry
Leaf
Stem

ABSTRACT

Larrea divaricata Cav., *L. cuneifolia* Cav. and *L. nitida* Cav., Zygophyllaceae, are evergreen xerophytic shrubs occurring in Northwestern Argentina used in traditional medicine. The aim of this work was to characterize the morphology, anatomy and histochemistry of the vegetative organs of three *Larrea* species by light and scanning electron microscopy in order to provide supporting data for their correct identification and to determine the site of synthesis and accumulation of its main active compounds. The shape, number and percentage of coalescence of leaflets, presence or absence of mucrones and rachis and the shape of the stipules represented the main botanical differences between the studied *Larrea* species. Anatomically three species presented amphystomatic leaves, with thick resinous slightly striated cuticle with resinous deposits, polygonal epidermal cells with straight anticlinal walls, ciclocytic, brachy-paracytic and paracytic stomatal types, non-glandular trichomes and isolateral mesophyll. The position and abundance of the sclerenchyma at the mid vein and petiole transection allows the differentiation of the three species, been more abundant in *L. cuneifolia.* Secondary phloem and parenchyma cells presented abundant calcium oxalate druses and solitary rhomboidal crystals. Epidermal cells and cuticle layer of leaflets and stipules of the three species presented amber resin deposits and content which stained positively for polysaccharides, phenolic compounds, flavonoids and tannins, while mesophyll palisade cells showed small refracting droplets stained positively for lipophilic substances.

Introduction

Zygophyllaceae family is represented approximately by 280 species almost restricted to tropical and subtropical areas. In Argentina the family is represented by seven genera (*Bulnesia* Gay, *Kallstroemia* Scop., *Larrea* Cav., *Plectrocarpa* Gill. ex Hook. & Arn., *Porlieria* Ruiz & Pav., *Tribulus* L. and *Zygophyllum* L.), and typically they are often dominant in the landscapea of Chaco and Monte regions (Cabrera and Willink, 1973; Cabrera, 1976; Zuloaga and Morrone, 1999; Flora Argentina, 2018). In Brazil it is represented by three genera (*Kallstroemia*, *Larrea* and *Tribulus*) (Engler, 1827).

The genus *Larrea*, Zygophyllaceae, comprises five species with amphitropical distribution in dry regions of South America (Argentina, Chile, Bolivia, Peru, Brazil) and North America (Mexico to Utah, United Stated of North America) (Engler, 1827; Hunziker et al., 1972, 1977; Flora Argentina, 2018). *L. divaricata* (common names: "jarilla", "jarilla hembra", "chamanilla", "jarilla del cerro", "yarilla"), *L. cuneifolia* (common names: "jarilla", "jarilla macho", "jarilla crespa", "jarilla norte-sur", "jarilla del campo") and *L. nitida* (common names: "jarilla", "jarilla de la montaña", "crespa", "pispa o pispita", "jarilla fina"), are represented in Northwestern Argentina forming shrubby associations called "jarillales". Among these, *L. divaricata* is the only *Larrea* species cited in the Brazilian flora (Engler, 1827).

Larrea species are evergreen, xerophytic, erect aromatic shrubs, 1–4 m with opposite, pubescent, sub-sessile and stipulate compound leaves which show a resinous yellowish appearance. The main botanical difference between *Larrea* species resides in their

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https://doi.org/10.1016/j.bjp.2018.05.012

morphology, phenological patterns, and mating systems (Barbour et al., 1977; Simpson et al., 1977; Ezcurra et al., 1991). Ragonese (1960) noted some differences based on anatomical characters mainly the proportion and disposition of sclerenchyma tissues.

Larrea divaricata, *L. cuneifolia* and *L. nitida* are used in Argentinean traditional medicine as anti-inflammatory, antirheumatic, hypotensive, rubefacient, diaphoretic, febrifuge, oxytocic, emenagogo, odontalgic, antitussive and to treat fungal and bacterial infections (Alonso and Desmarchelier, 2006; Alonso, 2007; Barboza et al., 2009).

A wide range of pharmacological activities were previously described for these species indicating their potential usage as alternative or complementary medicine. Aqueous and/or alcoholic extracts from *L. divaricata* showed antibacterial (Stege et al., 2006; Zampini et al., 2007), antitumoral (Anesini et al., 1996a, 2001; Davicino et al., 2010, 2011; Martino et al., 2016), antioxidant (Carabajal et al., 2017) and inmunomodulatory (Anesini et al., 1996b; Davicino et al., 2007) activity. Organic solvent extracts were active against phytopathogenic fungi (Quiroga et al., 2001; Svetaz et al., 2010; Vogt et al., 2013). Whereas *L. cuneifolia* showed larvicidal (Batallán et al., 2013) and antioxidant properties (Torres et al., 2003; Carabajal et al., 2017). A synergistic antifungal effect of *L. nitida* and *Zuccagnia punctata* Cav. was also reported (Butassi et al., 2015).

Martino et al. (2016), Carabajal et al. (2017), Agüero et al. (2011) and Blecja et al. (2007) reported the presence of nordihydroguaiaretic acid, essential oils and flavonoids as main constituents in some *Larrea* species. Several flavonoids including quercetin, apigenin and kaempferol derivatives were identified in organic extracts of *L. cuneifolia* (Valesi et al., 1972).

Bioactive compounds of *Larrea* species are presumably found in the resin that covers their leaves and stems. Ragonese (1960) observed a gradual decline in the resin content of older leaves and stems, and suggested that the resin is synthesize in the stipules from where it spills on nearby organs.

Pointing to the great potential of these species and their traditional use, the aim of this study was to characterize the morphoanatomy and histochemistry of the vegetative organs of *L. divaricata, L. cuneifolia* and *L. nitida* utilized in folk medicine of Northwestern Argentine, to identify anatomical diagnostic characters for their correct identification, and to determine the site of synthesis and accumulation of its main active compounds.

Materials and methods

Plant material

Aerial parts of *Larrea cuneifolia* Cav. and *L. divaricata* Cav., were collected in April 2015 at Amaicha del Valle, Tucumán, Argentina at 2000 m.a.s.l. Samples of *L. nitida* Cav. were collected in April 2015 at Vinchina, La Rioja, Argentina at 3485 m.a.s.l. Voucher specimens of each collection were deposited at the Herbarium of Fundación Miguel Lillo (LIL). Herbarium numbers of specimens are as follows: *L. cuneifolia*: LIL 614829; *L. divaricata*: LIL 614299 and *L. nitida*: LIL 615845.

Light microscopy

Samples of leaves and stems of five plants of each species were fixed in FAA (formalin, acetic acid, 50% ethanol, 5:5:90 v/v/v) and stored during one week after processing. Sections (10–25 μm) were obtained with a rotation microtome, subsequently treated with 50% NaClO solution, washed with distilled water and stained with astra blue-safranin and then mounted in 50% glycerol (Zarlavsky, 2014). Sections were visualized with a Zeiss Axiolab optic microscope equipped with a polarized light filter and a Zeiss Axiocam ERc 5s digital camera.

Measurements were made using AxioVision software version 4.8.2 (Carl Zeiss Ltd, Herts, UK).

Histochemistry

The main classes of chemical compounds of the leaves and stipules were investigated in transverse microtome sections of fresh material. Fresh leaves and stipules were place between dental wax supports and sectioned at 20–25 μm with a rotation microtome.

Vanillin–sulphuric acid (Gaucher et al., 2013) and Neu's reagent (2-aminoethyl-diphenylborinate, Sigma) 10% in absolute methanol (Neu, 1957), were used to visualized flavonoids. Sections stained with Neu's reagent were analyzed under a fluorescence microscope (Nikon Optiphot) with UV light (filter UV-1A: 365 nm excitation filter, 400 nm barrier filter). Under these conditions, flavonoids were detected by a yellowish fluorescence (Mondolot-Cosson et al., 1997). Photographs were taken with a digital Nikon Coolpix 4500 camera. Nadi reagent was used to detect terpenoids, essential oil and oil resins (David and Carde, 1964). Ferric chloride (10%) in methanol (Zarlavsky, 2014) and Vainillin–HCl (Gardner, 1975) were used to visualize phenolic compounds and tannins respectively. Toluidine blue O was used for the detection of polysaccharides (Heslop-Harrison and Heslop-Harrison, 1981).

Some of the sections were treated with 50% sodium hypochlorite and washed with distilled water, prior to dyeing with Sudan IV for the detection of lipids (Zarlavsky, 2014; D'Ambrogio de Argüeso, 1986) and ruthenium red for pectins (Johansen, 1940; Zarlavsky, 2014). Iodine potassium iodide (IKI) (Johansen, 1940) was employed for the detection of starch. Standard control procedures were carried out simultaneously.

Scanning electron microscopy

Samples of leaves were fixed in 2.5% glutaraldehyde in 0.1 M sodium phosphate buffer, pH 7.2, for 4–6 h at 4 °C. Following rinsing in the same buffer, the material was dehydrated in a graded acetone series and sputter coated with gold. Observations were carried out on a field emission scanning electron microscope (FESEM-ZEISS SUPRA-55 VP). Electronic microscopy observations were performed at the Centro Integral de Microscopía Electrónica (CIME), CONICET, Tucuman, Argentina.

Results

Morphology and anatomy

Larrea species are evergreen xerophytic, erect aromatic shrubs 1–4 m with opposite, composite, sub-sessile, pubescent, and stipulate leaves, with a succulent, resinous yellowish appearance.

Larrea cuneifolia presents leaves (4.5–13.2 × 2.5–16.0 mm) formed by two acute asymmetric oblong-ovate leaflets (2–4 × 1–2 mm) joined along two thirds of their internal edge culminating in a reflex apex with a filiform, vascularized mucro (0.3–0.5 mm) (Fig. 1A and D). Two stipules (squamous, subtriangular, reddish) (1.2–3.1 mm) are inserted at the base of the leaves (Fig. 1A and J).

Larrea divaricata shows divaricated leaves (7.3–17.8 × 2.5–4.5 mm) formed by two oblong-acute divergent leaflets joined at the base in a third of its total length (Fig. 1B), apex reflex with a short and, vascularized mucro (0.3–0.4 mm). The stipules are obtuse and rounded similar to those previously described for *L. cuneifolia* (Fig. 1E and F).

Larrea nitida presents odd pinnately compound leaves (7.2–13.1 × 3.5–5.0 mm) (Fig. 1C), with 11–17 sub-opposite,

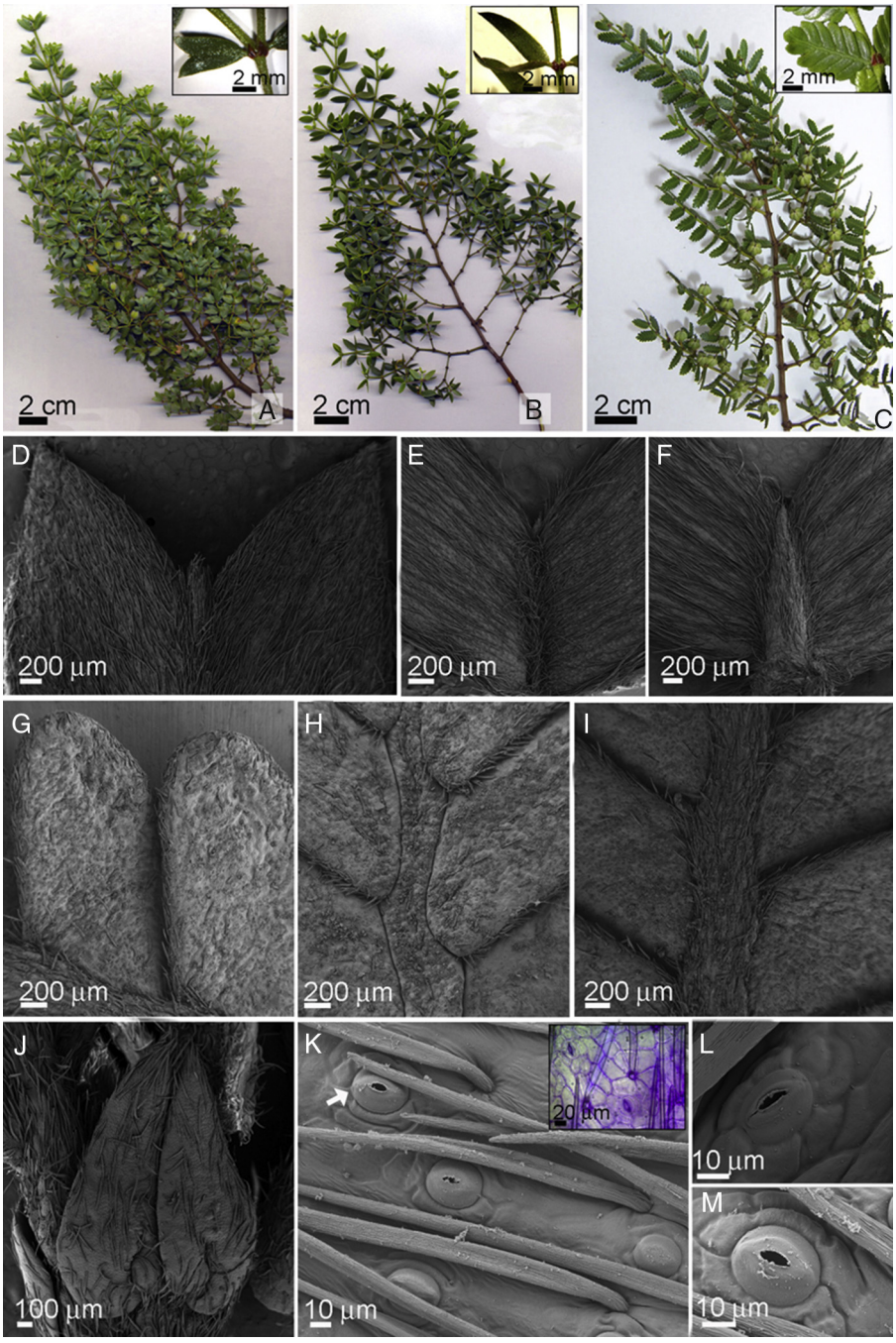

Fig. 1. Photographs of the plant material. (A) *Larrea cuneifolia*. Leaf detail leaflets joined along two thirds of their internal edge. (B) *L. divaricata.* Divaricated leaf detail. (C) *L. nitida*. Odd pinnately compound leaves detail. (D–M) SEM images of leaf surface of *Larrea* sp. with abundant non glandular unicellular antrorse-appressed trichomes. (D) *L. cuneifolia* reflex apex with mucro. (E and F) *L. divaricata* adaxial and abaxial leaf surfaces respectively. (G) *L. nitida* adxial leaflet surface. (H and I) *L. nitida* raquis adaxial and abaxial surfaces, respectively. (J) *L. cuneifolia* stipules. (K) *L. cuneifolia* adaxial surface with slightly striated cuticle, non glandular unicellular trichomes, arrow shows brachy-paracytic stomata. Detail of trichome base with 4–6 cells with actinocytic appearance. (L) Ciclocytic stomata. (M) Paracytic stomata.

sessile, oblong leaflets with rounded to convex apex (3.7–7.5 × 1.4–3.5 mm); the terminal leaflet is sometimes larger with acuminated to emarginated apex (Fig. 1G). Pubescent rachis (Fig. 1H and I) and acuminated stipules were observed (1–3 mm).

The three species presented amphistomatic leaves, with thick resinous slightly striated cuticle (Fig. 1K), polygonal epidermal cells with straight anticlinal walls, ciclocytic (Fig. 1L), brachy-paracytic (Fig. 1K) and paracytic stomatal types (Fig. 1M). Unicellular non glandular trichomes, antrorse-appressed with thick wall, striated ornamentation, acute-rounded apex (241.31 ± 56.84 µm) and surrounded by 4–6 cells at their base were observed (Fig. 1K). Trichomes appeared bi-refringent under polarized light. *L. nitida* appeared less pubescent than the other two species.

In transection leaflets presented thick cuticle, uniseriate epidermis with thick walled epidermal cells. Trichomes bases were inserted in depressions between the epidermal cells (Fig. 2H). Stomata appeared raised above the epidermal surface, with strongly projecting cutinized outer ledge (Fig. 2I). Mesophyll is isolateral, with 3–4 layers of tightly packed adaxial palisade cells; 1–2 layers of shorter abaxial palisade cells and a narrow central zone of spongy mesophyll interrupted by collateral vascular bundles. Parenchymatous sheath and sclerenchyma caps at phloem poles

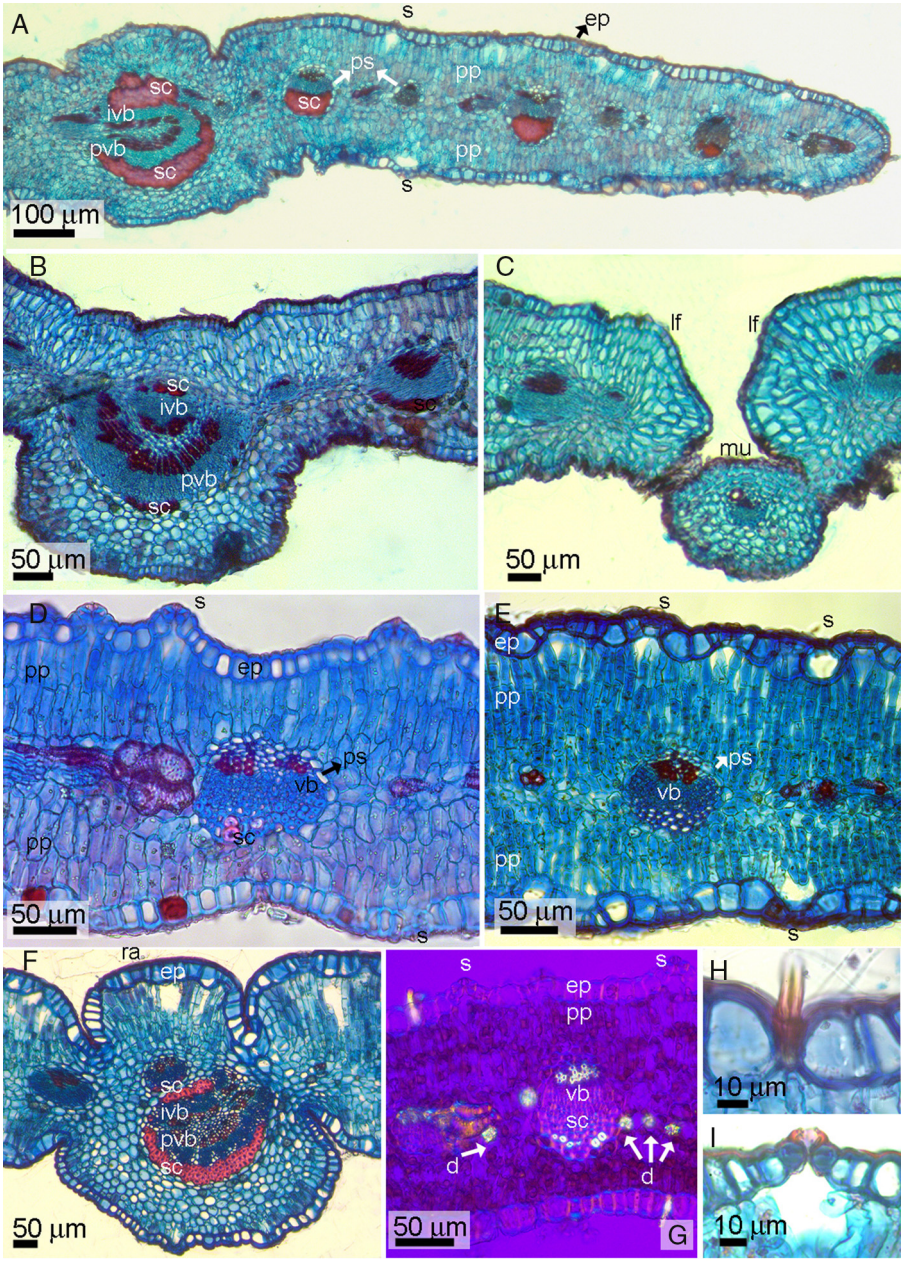

Fig. 2. Photomicrographs of examined *Larrea* species leaves. (A) *L. cuneifolia*. (B) *L. divaricata* proximal leaf at region where the leaflets are fused, near the petiole. (C) *L. divaricata* leaf at mucro level, where leaflets become separated. Note mucro vascularization. (D) *L. divaricata* distal region, free leaflet at the mid vein region. (E) *L. nitida* leaflet. (F) *L. nitida* raquis. (G) Druses, thick walled trichomes and vessels show characteristic bi-refringence under polarized light. (H) Trichome insertion. (I) Raised stomata with strongly projecting cutinized outer ledge. *Abbreviations*: d, druse; ep, epidermis; ivb, inverted vascular bundle; lf, leaflet; mu, mucro; pp, palisade parenchyma; ps, parenchymatous sheath; pvb, primary vascular bundle; ra, raquis; s, stomata; sc, sclerenchyma cap.

were observed in the vascular bundles of higger order (Fig. 2A–E). The sclerenchymatous sheaths of the vascular bundles were extensively developed in *L. cuneifolia* (Fig. 2A) and *L. divaricata* (Fig. 2B–D) and less developed or absent in *L. nitida* (Fig. 2E). Numerous calcium oxalate druses, refractive under polarized light, were observed in the mesophyll of all studied species (Fig. 2G).

An abaxial collateral vascular bundle and an adaxial smaller inverted vascular bundle, with a parenchymatic pith between them, sometimes accompanied by one or two small latero-adaxial collateral bundles was observed in *L. cuneifolia* and *L. divaricata* mid vein in the region where the leaflets are fused near the petiole, and in *L. nitida* raquis (Fig. 2F). The vascular bundles of the mayor order veins (primary and secondary) presented sclerenchyma cups at the phloem poles (Fig. 2A, B and F). *L. cuneifolia* and L. *divaricata* transection at mucro level, presented a small collateral vascular bundle (Fig. 2C).

Larrea divaricata and *L. cuneifolia* leaflets at their free endings, presented midvein with a single collateral vascular bundle with a less or more developed sclerenchymatous cap at phloem pole, surrounded by a parenchymatous sheath (Fig. 2D and E).

The stipule anatomy was similar in the three species. They presented navicular shaped stipules, sessile, with obtuse apex, pubescent on the abaxial surface with unicellular non glandular trichomes identical to those previously described for the leaflet. In transection thick cuticles and deposits of resin were observed. The uniseriate adaxial or inner epidermis consisted of elongated, columnar cells whereas the uniseriate abaxial or outer epidermis was formed by more isodiametric polyhedric cells. In both,

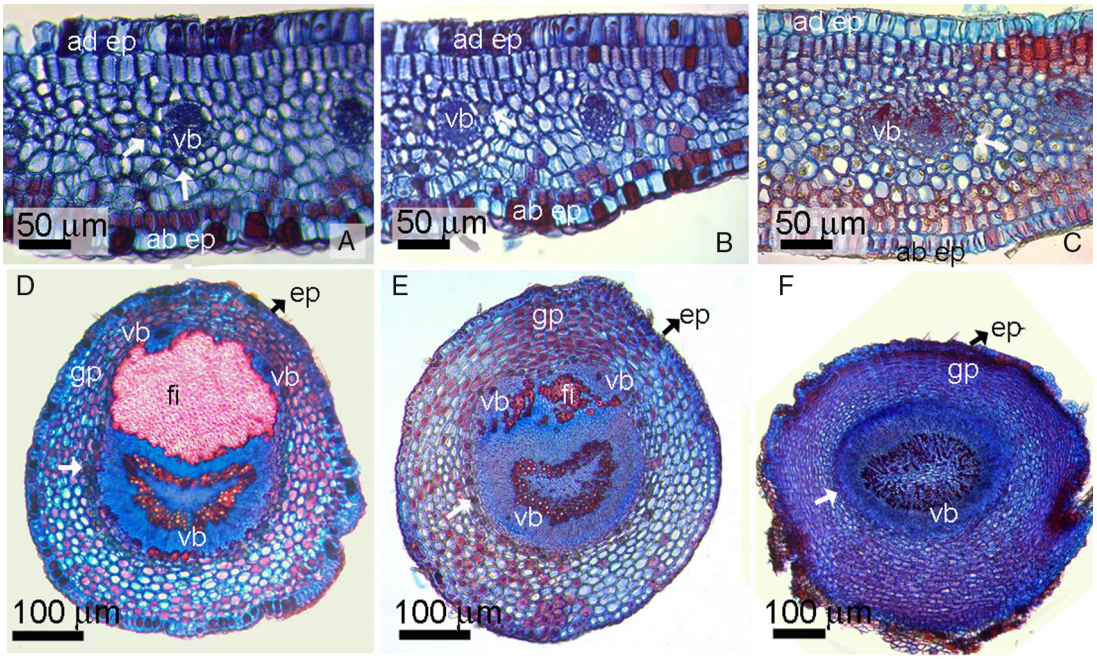

Fig. 3. (A–C) Photomicrographs of the stipules transection. (D–F) Photomicrographs of the petiole transection. (A, D) *Larrea cuneifolia.* (B, E) *L. divaticata.* (C, F) *L. nitida. Abbreviations*: ab ep, abaxial epidermis; ad ep, adaxial epidermis; arrows indicate druses; fi, fibers; gp, ground parenchyma; vb, vascular bundle.

epidermis and mesophyll idioblasts are evidenced by their amber content. Mesophyll presented 1–2 adaxial layers and a single abaxial layer of short palisade cells and homogeneous compact rounded central parenchyma with thick walls and abundant druses, interrupted by collateral vascular bundles (Fig. 3A–C).

The petiole outline of the three species was oval to circular, occasionally truncated adaxially (Fig. 3D–F). Transection showed uniseriate epidermis, three adaxial layers and seven abaxial layers of ground parenchyma form by elliptical to round cells with abundant calcium oxalate druses, which sometimes form a ring around the vascular tissues. The vascular bundle resembles an ectophloic siphonostele, accompanied in *L. cuneifolia* and *L. divarivata* by two small latero-adaxial bundles. *L. cuneifolia* presented a strong adaxial cup of fibers and some abaxial clusters of lignified cells (Fig. 3D); *L. divaricata* showed a less developed adaxial cup of fibers and isolated abaxial clusters of fibers or sclereids in some cases not lignified (Fig. 3E), finally *L. nitida* presented no lignified tissue or sometimes some isolated abaxial fibers or sclereids (Fig. 3F).

Primary stem transection showed, elliptic outline, sometimes with prominent ribs in *L. divaricata*, thick cuticle, uniseriate epidermis form by quadrangular cells with thick walls. Non glandular trichomes were observed. Stomata raised above the epidermal surface. Cortex with 1–2 layers of chlorenchymatous cells tangentially elongated and 10–14 layers of rounded parenchymatous cells with druses and rhombic crystals. A complete or incomplete ring of fibers was observed; occasionally associated with brachysclereids clusters, internally a parenchymatous ring formed by 4–5 layers of rounded parenchymatic cells rounds the vascular cylinder. The vascular system is a continuous cylinder of phloem, follow by cambium and xylem and a cross shaped pith form by isodiametric parenchymatous cells with druses (Fig. 4A–C).

In older stems (Fig. 4D–F) a complete cylinder of sclerenchymatous fibers rounds the phloem. Periderm begins to differentiate in the outer cortex, followed by 2–3 layers of large quadrangular, thin-walled parenchymatous cells and 4–5 layers of smaller rounded parenchyma cells with abundant druses and rhomboidal crystals (Fig. 4G and H). The secondary phloem presented abundant druses and solitary rhomboidal calcium oxalate crystals, 1 seriated heterocelullar rays with square marginal cells (Fig. 4I). The secondary xylem was constituted by thick walled solitary vessels, rarely in pairs; accompanied by many fiber-tracheids and 1–2 seriated rays (Fig. 4J). The pith becomes lignified (Fig. 4K).

Histochemistry

Epidermal cells and cuticle layer of leaflets and stipules of the three species presented an amber content and resin deposits, while mesophyll palisade cells showed small refracting droplets. Cellulose and pectates in the cellular walls (Fig. 5A–C) was detected with ruthenium red and toluidine blue O. Toluidine blue O also stained positively the content of the epidermal cells of the stipules (Fig. 5D).

The resin deposits and the content of palisade and epidermal cells stained positively (black) with $FeCl_3$ for phenolic compounds (Fig. 5E and F) and vainillin–HCl for tannins (Fig. 5G and H) both in leaflets as in stipules.

Flavonoids were detected with vanillin–sulfuric acid (stained bright yellow to orange) in resin deposits in the cuticles and droplets at the palisade and mesophyll cells of the leaflets and stipules (Fig. 6A and B). These results were also confirmed for the resin deposits of the leaflets stained with Neu's reagent which under UV light induced a bright yellow fluorescence (Fig. 6C). Nadi reagent for terpenoids resulted in a faint violet staining of the leaflet mesophyll droplets (Fig. 6D) and did not react in the stipules.

Mesophyll droplets and cuticle stained positively (red) for lipophilic substances with Sudan IV (Fig. 6E and F). Iodine potassium iodide test carried out to detect starch gave negative results (not shown).

Discussion and conclusion

The morphological characters observed in the present work are coincident with those cited in the Flora of Brazil, Chile, Argentina and North America Flora for the genus and the particular species (Engler, 1827; Reiche, 1896; Flora Argentina, 2018; North America Flora, 2018). *Larrea divarivata* presents similar morphological characters than North American *Larrea*, *L. tridentata* Sessk & Moc. ex DC

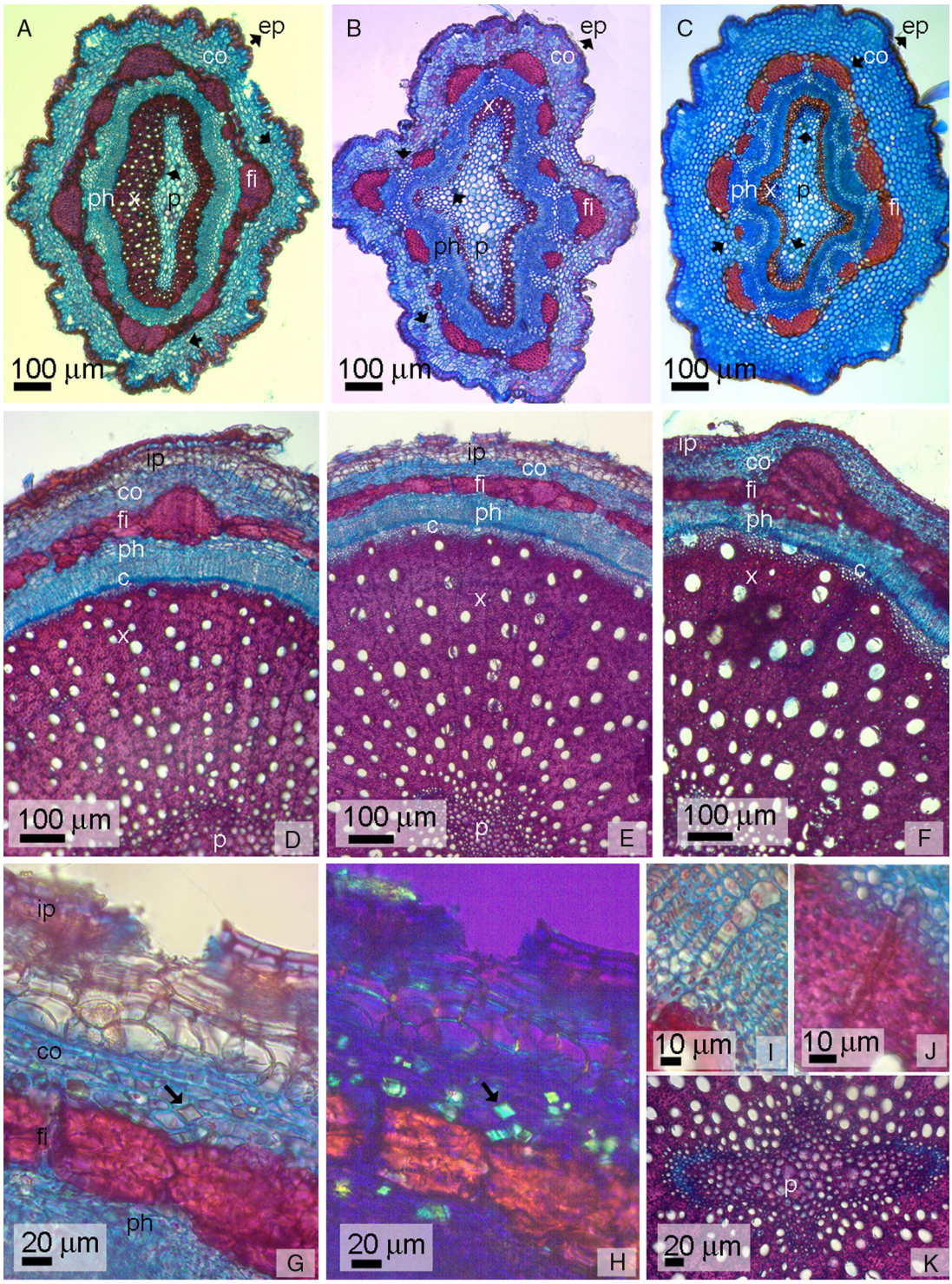

Fig. 4. Photomicrographs of stem transection. (A–C) Early stage of secondry growth. (D–F) Secondary growth. (A, D) *Larrea cuneifolia*. (B, E) *L. divaricata*. (C, F) *L. nitida*. (G) Complete cylinder of schlerified cells rounds the phloem, follow centrifugally by 2–3 layers of large quadrangular parenchymatous cells and centripetally by 4–5 layers of smaller rounded parenchyma cells with abundant druses and rhomboidal crystals. (H) Crystals under polarized light. (I) Secondary phloem 1 seriated rays. (J) Secondary xylem 1–2 seriated rays weakly storied. (K) Lignified pith. *Abbreviations*: c, cambium; co, cortex; ep, epidermis; fi, fibers; ip, incipient periderm; p, pith; ph, phloem; x, xylem.

(Yang et al., 1977; Laport and Ramsey, 2015). There has been much controversy as to whether the North American species is a separate species from the diploid South American *L. divaricata*, and in some papers it has been called *L. divaricata* subsp. *tridentata*. However, *L. tridentata* seems to be derived by long distance dispersal from South America, cytogenetic, isozyme and molecular studies confirm that they are closely related, but separate, species (Lia et al., 2001; Laport and Ramsey, 2015). *Larrea ameghinoi* Speg. and *L. nitida* are similar in their compound leaf morphology, the flower anatomy, and the merocarp texture and size (Hunziker et al., 1977, Simpson et al., 1977) but with marked differences in their growth habit (Ezcurra et al., 1991). Barbour et al. (1974, 1977), Barbour and Díaz (1973), and Ezcurra et al. (1991) analyzed the physiological behavior and shrub architecture of South and North American *Larrea* species and

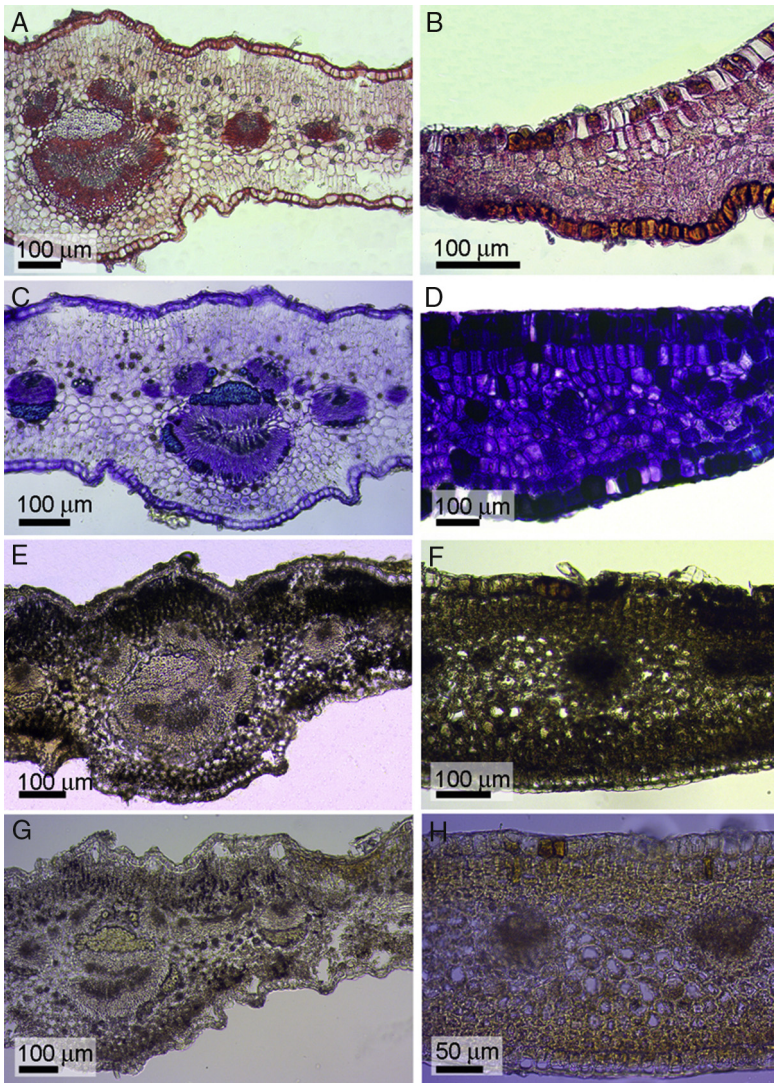

Fig. 5. Leaf and stipule histochemical characterization. (A, B) Ruthenium red revealed pectates in leaf and stipules cell walls respectively. (C, D) Toluidine blue O stain positively blue-violet cellulose and lignin in leaf and stipules, respectively. (E, F) Resin deposits, and palisade cells stained positively (black) with ferric trichloride for phenolic compounds in leaf and stipules respectively. (G, H) Vanillin–HCl stained positively black for tannins in the leaflets and negative in the stipule.

they stated that the mayor differences between species could be the shrub architecture rather than shrub physiology or leaf anatomy. In the present work we find that the main botanical differences between examined *Larrea* species from Northwestern Argentine resides in their leaf morphology, shape of leaf, leaflets and stipules; presence or absence of mucro and rachis, and percentage of coalescence of the leaflets.

Anatomical characteristics such as non-glandular trichomes, stomata types, thick striated cuticle with resinous deposits, isolateral mesophyll type, the presence of large parenchyma sheath in the minor vascular bundles and calcium oxalate crystals are common features for the examined species and other *Larrea* species such as *L. tridentata* (Ragonese, 1960; Pyykko, 1966; Metcalfe and Chalk, 1972; Meyer and Meola, 1978).

In accordance with Ragonese (1960), the position and abundance of the sclerenchymatic tissue at the mid vein and petiole transection allows the differentiation of these three species that coexist. *L. cuneifolia* possess fibers associated to the vascular bundles, while *L. divaricata* and *L. nitida* present little or undeveloped sclerified tissues. In the same way, Sheahan and Cutler (1993) indicate that the vascular bundles of the petiole of *L. tridentata* are surrounded by collenchymatous tissue, whereas Meyer and Meola (1978) indicate the presence of fibers that almost completely encircle the leaf midrib vascular tissues.

Stem characteristics were coincident with those described for the genera by Metcalfe and Chalk (1972) and for *L. tridentata* by Meyer and Meola (1978), however, unlike these authors who describes that the phellogen originates deep in the phloem, we observed periderm originating from the outer cortex as stated for *L. tridentata* by Sheahan and Chase (1996) and Sheahan and Cutler (1993). For *L. ameghinoi* there are few references on its anatomy, only referred to the presence of root nodules (Medan and Tortosa, 1983).

In *L. tridentata*, Meyer and Meola (1978), observed a thin cuticle on both surfaces of the leaflet lamina and numerous epidermal cells on both surfaces containing dark-stain which they suggest may be resins and tannin idioblasts. Ragonese (1960) suggested that the stipules were the responsible of the secretion of the resinous substance deposited in stems and leaves, however in this work we demonstrate the presence of phenolic compounds and other chemical constituents at the epidermis and the cuticle of the stipules and at the epidermis and palisade cells of the leaflets mesophyll, all these cells may act as glandular structures where the resin is synthesize and subsequently excreted to the surface.

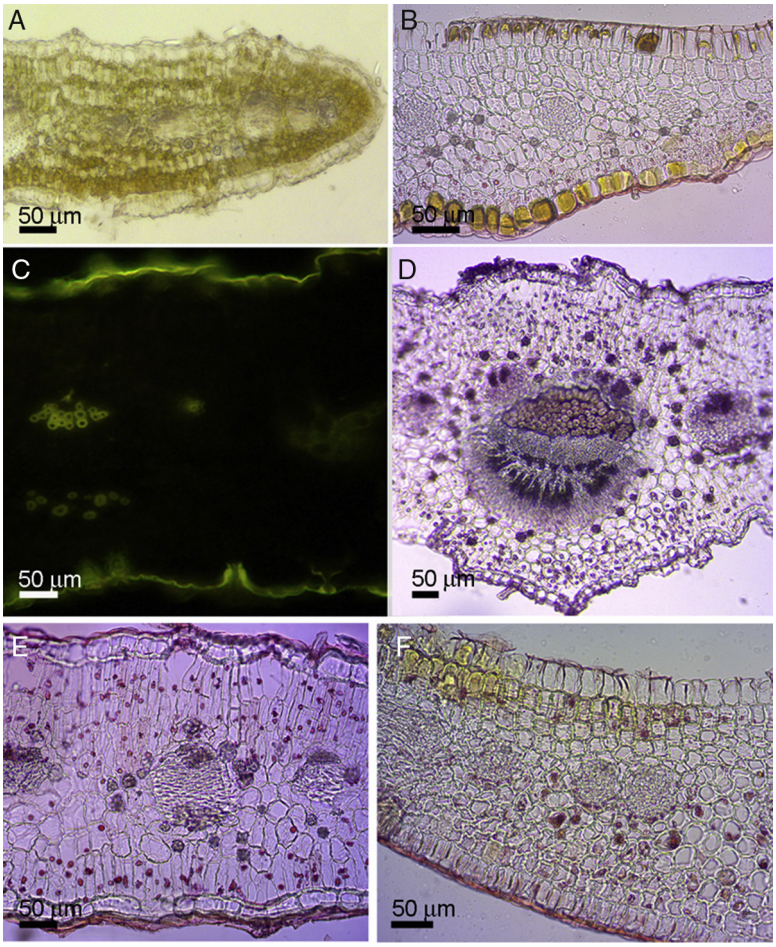

Fig. 6. Leaves and stipules histochemical characterization. (A, B) Resin deposits in the cuticles and droplets in the palisade and mesophyll cells of leaflets and stipules, stained bright yellow to orange with vainillin–sufuric acid, indicating the presence of flavonoids. (C) Resin deposits and cuticle stain positively for flavonoids revealing bright yellow under fluorescence with Neu's reagent. (D) Nadi reaction for terpenoids resulted in a faint violet staining of the leaflet mesophyll droplets. (E, F) Mesophyll refrigent droplets and cuticles stained positively for lipophilic substances staining red with Sudan IV in leaflet and stipules respectively.

Our study contributes to the knowledge of *Larrea* species anatomy, identifying morphoanatomical characters of diagnostic value such as leaflets and stipules shape; presence or absence of mucro and rachis, percentage of coalescence of the leaflets and the position and abundance of the sclerenchymatic tissue at the mid vein and petiole transection. It also allowed determining that both stipules and leaves are the site of synthesis and accumulation of secondary metabolites of interest and will lend support to further studies on their chemical constituents and its functional role in nature.

Authors' contributions

MAM, IFR, ICZ and MII collected the plant material. MIM, AIR and GIP carried out the laboratory work. MII and GIP identified the plant material. ICZ, MII and GIP provided critical reading and insightful recommendations of the manuscript. MII and GIP created the project. MII and GIP supervised the laboratory work and MIM wrote the manuscript. All the authors have read the final manuscript and approved the submission.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgments

We acknowledge the Electron Microscopy Center CIME, Centro Integral de Microscopía Electrónica for assistance in preparing SEM images.

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