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Comparative Analysis of Leaf Architecture and Histochemistry in *Schinus fasciculatus* and *S. gracilipes* (Anacardiaceae)

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HIGHLIGHTS

- Secondary metabolites were localized in leaves of *Schinus* shrubs.
- Terpenoids and phenolic compounds were widely distributed in the leaves.
- Phloem ducts contained lipids and alkaloids.
- Glandular trichomes produce phenolics, terpenes and polysaccharides
- Ecophysiological roles are inferred from distribution of the secondary metabolites

Abstract: *Schinus fasciculatus* (Griseb.) I.M. Johnst and *S. gracilipes* I.M. Johnst are plants rich in secondary metabolites traditionally used for dye, fodder, and medicinal purposes. This work is a comprehensive comparative analysis of leaf architecture and histochemistry between the two species to determine the *in situ* localization of their secondary metabolites. Leaf anatomy was characterized by classical histological methods. Fresh leaf cross-sections were treated with ferric chloride, Fast Blue B, aluminium chloride, vanillin-HCl, 1% KOH, Sudan IV, Neu´s, NADI, Liebermann-Burchard, PAS, and lugol. The leaves of both species shared morphological traits suitable for survival in water-limited environments, such as amphistomacy and anomocytic stomata. Glandular and non-glandular trichomes were abundant in *S. gracilipes* suggesting that they have a protective role against biotic and abiotic stresses. Some features like mesophyll structure and thickness indicate *S. fasciculatus* leaves respond better to the selective pressure of extreme environments.

The histochemical analysis revealed a widespread distribution of phenolic compounds and terpenoids in the mesophyll tissue of both species. Glandular trichomes contained polysaccharides, terpenoids and phenolic compounds, including flavonoids. Numerous schizogenous phloem ducts containing terpenoids were observed in both species, with alkaloids only present in the phloem ducts of *S. fasciculatus*. These findings suggest that terpenoids and phenolic compounds in both *Schinus* species serve as plant defenses and protect against environmental stresses. The distribution and abundance of tannins and flavonoids suggest they protect against excessive UV radiation and reactive oxygen species. The ecophysiological significance of the results are discussed in relation to other Anacardiaceae species.

Keywords: Alkaloids; lipids; phenolic compounds; terpenoids.

INTRODUCTION

The genus *Schinus* (tribe Rhoeae, Anacardiaceae family) comprises 29 shrubs and tree species native from South America [1]. In Northwest Argentina, it includes *Schinus fasciculatus* (Griseb.) I.M. Johnst and *S. gracilipes* I.M. Johnst. The aerial parts of these plants are useful as dye, fodder and in traditional medicine [2]. Their berries were consumed as food spice for centuries [3]. In the case of *S. fasciculatus*, its hard wood is used in rustic buildings and to make small tools [4]. Leaf decoctions of both *Schinus* species are popularly advised against stomach pain and cough. Chewing of *S. fasciculatus* leaves is popularly recommended as antirheumatic, purgative, analgesic, vulnerary and antidisenteric [5]. Extracts from the aerial parts of *S. fasciculatus* and *S. gracilipes* showed antimicrobial activity on a wide range of microbial phytopathogens. The ethanolic and ethyl acetate leaf extracts of *S. fasciculatus* and *S. gracilipes* suppressed the growth of toxigenic *Fusarium* species [6] while essential oils from both species had a moderate antifungal activity [7]. Alcoholic and hydroalcoholic extracts from aerial parts of *S. fasciculatus* killed phytopathogenic bacteria [8] and in some cases also exerted anti-biofilm activity [9]. Active principles involved in these effects were identified as flavonoids, other phenolic compounds and hydrocarbonated monoterpenes [6,7,8]. The functional *in vivo* role of these compounds in the *Schinus* plants is currently unknown.

Leaf architecture and anatomy features have been reported as distinguishing factors between *S. fasciculatus* from *S. gracilipes* [1,4,5]. However, a comprenhensive comparative reassessment of these traits focusing on their ecological adaptations is still needed. This analysis would greatly contribute to understand the pattern of secondary metabolite accumulation in leaves of both *Schinus* species, which has not been previously reported. Histochemical studies performed in the Rhoeae tribe suggest interspecific variations in the distribution of secondary metabolites within the tissues. For instance, *Schinopsis balansae* and *S. lorentzii* accumulated flavonoids only in the mesophyll cells [10] whereas *Spondias tuberosa*, *S. mombin* and *Schinus terebinthifolius* also exhibited accumulation in the epidermal and parenchyma cells near the midvein [11]. Hence, the aim of this work was to compare the leaf architecture and *in situ* localization of flavonoids, terpenes and other secondary metabolites in both *Schinus* species.

MATERIAL AND METHODS

Leaves were collected from shrubs of *Schinus fasciculatus* and *S. gracilipes* during may 2019. The collection sites were located in Tafí del Valle department (Tucumán province) at 26°42'14.6"S 65°47'57.7"W for *S. fasciculatus* and 26°55'59.6"S 65°40'55.1"W for *S. gracilipes*. Fresh samples were obtained from three shrubs per species and collections were performed at the middle of the shrub tops, at north orientation. Dr. Nora Muruaga, curator of the LIL herbarium, confirmed the identity of the sampled shrubs by comparison with voucher specimens already deposited in the Herbarium of the Miguel Lillo Foundation (Tucumán, Argentina).

Expanded leaves of the *S. fasciculatus* and *S. gracilipes* were detached of the third and fourth nodes counted from the branch tips. Then, leaves were fixed in FAA (formalin: ethanol: acetic acid: water; 100:500:50:350, v/v) or dried at room temperature (15-20ºC) in a dark and ventilated place during 2-3 weeks. Then, they were sectioned with a Thermo Scientific™ HM 325 Rotary Microtome in a thickness range of 30-35 µm [12]. The cuts were clarified with sodium hypochlorite prepared at concentration of 50%, washed with distilled water, sequentially stained with astra blue-safranin [13] and mounted into 50% glycerol. The analyses of leaf architecture and surface leaf features were performed on whole leaves (3 per Schinus species). Each leaf was diaphonized according to Dizeo de Strittmatter [14], washed with distilled water, stained in cresyl violet 1% [13] and mounted in 50% glycerol. Stomata types were described according to Dilcher [15].

Expanded dry leaves detached from third and fourth nodes were rehydrated in distilled water for 10 min and sectioned as described in the anatomical analysis. A part of the rehydrated material was took apart as

control and remained without staining. The remaining sections were treated with staining reagents suitable for visualization of secondary metabolites. Total phenolic compounds were visualized with 10% ferric chloride in methanol [13] and 0.5% Fast Blue B ½ZnCl2 in 5% acetic acid [7] while flavonoids were observed after treatment with 5% aluminum chloride in methanol [16].

Flavonoids and hydroxycinnamic derivatives were detected after incubation of the sections with 1% Neu's reagent (2-aminoethyl-diphenylborinate, Sigma) [17] in EtOH: H2Od (1:1, v/v) to prevent washout of alcohol-soluble compounds. A KOH solution at 1% was used to screen for presence of phenylpropanoids [18]. Section treated with Neu´s reagent, $AlCl_3$ and the KOH solution were observed under a fluorescence microscope equipped with UV filter [17] and compared against control untreated sections.

Triterpenes and steroids were identified by using Liebermann-burchard reagent [19], terpenes and non-terpene lipids with Sudan IV [13], terpenoids and essential oils with NADI reagent (1-naphthol and N,N-dimethyl-p-phenylene diamine, Sigma), tannins with Vainillin-HCl [20], and starch with lugol. The PAS reaction was applied to test the presence of polysaccharides o ther than starch [21].

Light microscopy of coloured sections was made in a Zeiss Axiolab optic microscope coupled with a polarized light filter and a stereomicroscope Zeiss Stemi 305 both fitted with a Zeiss Axiocam ERc 5s digital camera. Fluorescence microscopy was performed in a Nikon Optiphot provided with a 365 nm excitation filter and a 400 nm barrier filter. Axio Vision software version 4.8.2 (Carl Zeiss Ltd, Herts, UK) was used for tissue measurements (n=30 for each parameter, 10 repetitions by individual). Statistical summary measures were calculated with the statistical package InfoStat V1.1.2.2 Text).

RESULTS

General features and leaf architecture of the *Schinus* plants

S. fasciculatus is an evergreen shrub of about 2-3 m high (Figure 1A) with spinescent branches. It showed single lineal-lanceolate leaves (0.8-3.0 x 0.5-1.0 cm), with pubescent petiole (1.0-2.0 mm length), subcoriaceous-subglabrous lamina, entire margins and obtuse or emarginated tips (Figure 1C). Leaf arrangement was alternated in young branches and fasciculated in old branches. In the case of *S. gracilipes*, its individuals were 1.6-3.5 m high evergreen shrubs or small trees (Fib. 1B) with thornless stems, alternate single oblong-obovate or obovate-lanceolate leaves (3.6-10.0 x 1.0-3.5 cm), pubescent petiole (5-17 mm length), subchartaceous pubescent lamina and rounded obtuse apex sometimes acute or retuse. The leaf base was mostly oblique-attenuate or cuneate while the leaf margin was crenate, except at the base where it was entire (Figure 1D). Both *Schinus* species had a primary pinnate venation followed by a secondary cladodromous pattern, with type I in *S. gracilipes* and type II in *S. fasciculatus* (Fig 1C-D). Tertiary veins irregularly branched, diverged in consistent straight to obtuse angles from the secondary veins (Fig. 1C, E). Minor secondary veins joined to form an incomplete intramarginal vein (Figure 1C, F). Areoles were absent in *S. fasciculatus* and poorly developed in *S. gracilipes*. Quaternary vein fabric constituted the higher venation order and ramifies freely into highly branched ending veinlets (Figure 1C, E-F). The marginal ultimate venation formed incomplete loops (Figure 1C, F).

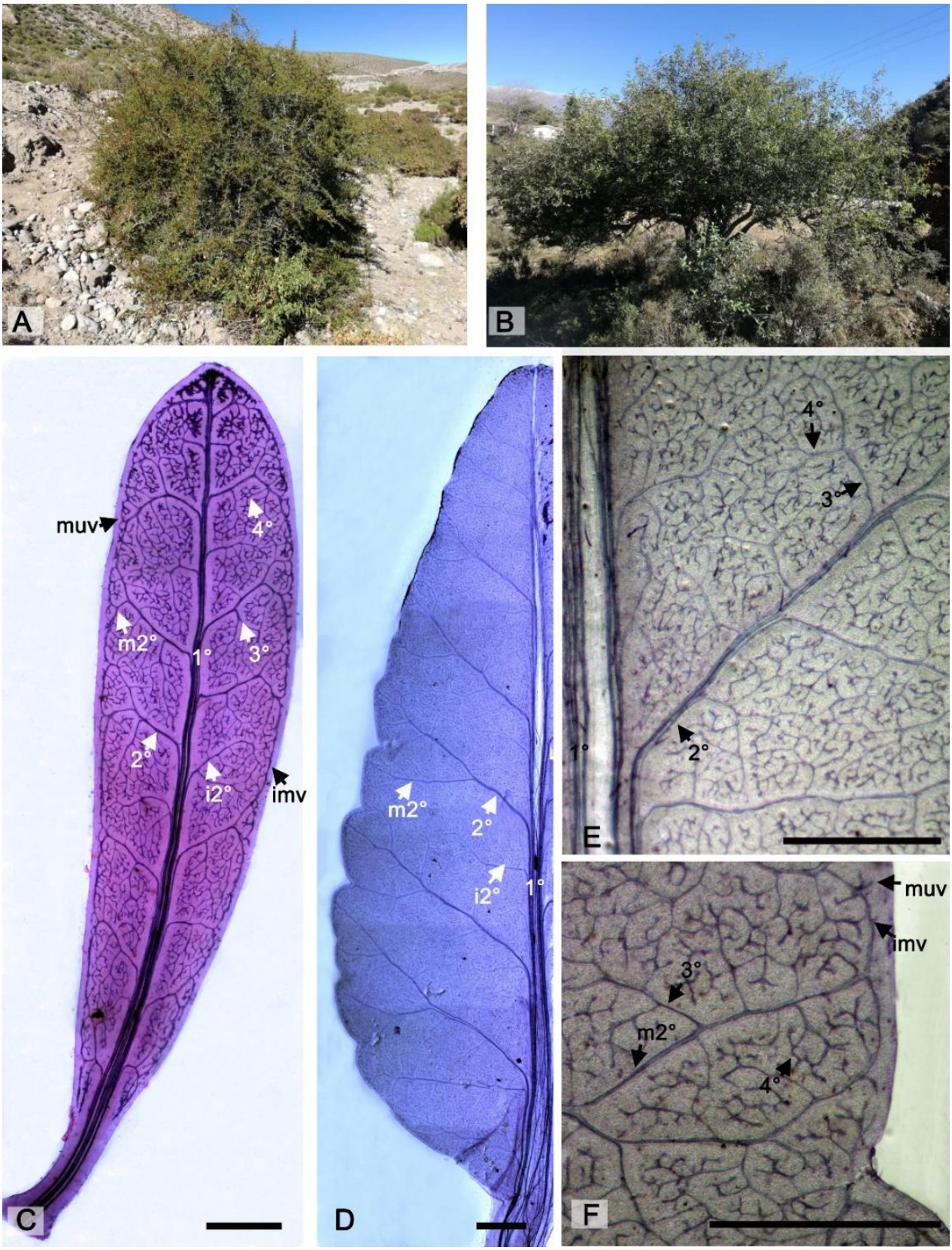

Figure 1. General view of *Schinus fasciculatus* and *S. gracilipes* shrubs. (A,B), and paradermal views of leaf surface showing a primary pinnate venation pattern extended into a type I (C) or a type II cladodromous venation (D). Veins of lower order can be seen in augmented sections of the leaf surface (E,F). Pictures belong to *S. fasciculatus* (A, C, E) and *S. gracilipes* (B, D, F). References: 1°, primary vein; 2°, secondary vein; m2°, minor secondary vein; 3° tertiary vein; 4°, quaternary vein; i2°, intersecondary; imv, incomplete marginal vein; muv, marginal ultimate venation. Scales: C-D, 1 mm.

Leaf anatomy

Both *Schinus* species presented amphystomatic leaves. Stomata were randomly distributed on the leaf surfaces of *S. fasciculatus* with the highest abundance in the adaxial surface (Table 1). In *S. gracilipes*, stomata were fewer on the adaxial surface where they were located close to the middle and higher order veins, while they were abundant and randomly distributed on the abaxial side. Superficial views of the leaves revealed that both *Schinus* species had polygonal cells with straight thickened anticlinal walls and anomocytic stomata (Figure 2A-D). Similar average stomata sizes were recorded for *S. fasciculatus* on both leaf sides, while *S. gracilipes* showed bigger stomata in the upper leaf side (Table 1). Foliar indument of both species consisted of unicellular erect non-glandular trichomes (Figure 2B, E, G), claviform glandular trichomes (Figure 2C-D, F, H-O), and capitate trichomes with a short pedicel and a pluricellular multiseriated head. In *S. fasciculatus*, non-glandular trichomes and glandular trichomes were more frequent towards the base of the leaf and on the petiole. Trichomes of *S. gracilipes* were more abundant between veins of higher order, towards the apex and the margin. In the latter species, the glandular trichomes were observed at different maturation stages, varying from unicellular and uniseriate to claviform (Figure 2H-O), mostly located towards the apex on the abaxial leaf side. Figure 3A-B shows transverse sections of leaf midribs exhibiting a biconvex shape in both *Schinus* species. However, *S. fasciculatus* also revealed flat-convex and flat-flat shapes on the adaxial-abaxial midrib sides. The palisade parenchyma was continuous on the adaxial midrib side of *S. fasciculatus*, and substituted by 1-2 colenchyma layers on the abaxial side. In the case of *S. gracilipes*, strong subepidermal multilayered collenchyma reinforcements were observed on both epidermal sides. The vascular system in *S. fasciculatus* was constituted by 1 to 3 poorly defined collateral vascular bundles surrounded by a parenchymal sheath with thickened walls (Figure 3A). *S. gracilipes* presented 4 to 6 poorly defined collateral vascular bundles and 4-7 minor inverted accessory bundles that may or may not be present (Figure 3B). Schizogenous ducts with secretory epithelium were observed in the phloem, typically 3 in *S. fasciculatus* and 2-4 in *S. gracilipes*. A characteristic endodermoid band was observed surrounding the secretory epithelium in *S. gracilipes* (Figure 3F). Several druses and prismatic crystals were evident in the ground and phloem parenchyma. Partial views in cross-section of the leaf lamina are also observed in Figure 3. Both *Schinus* species exhibited thick cuticles and one layered epidermis formed by polyhedral-rectangular cells with thickened periclinal walls. The mesophyll was isobilateral in *S. fasciculatus* with an upper palisade parenchyma (2-3 cell layers), compact spongy parenchyma (2-4 cell layers) and an inferior palisade parenchyma (2-3 cell layers) with cells shorter than those observed in the upper palisade (Figure 3C). These tissues layers had average thicknesses of 166 ± 37 µm, 68 ± 19 µm and 66 ± 21 µm, respectively. *S. gracilipes* exhibited a dorsiventral mesophyll of a palisade parenchyma with 2-3 layered palisade parenchyma (104 ± 14 µm thickness), 4-6 layers of compact spongy parenchyma (93 ± 18 µm thickness) (Figure 3E). Both *Schinus* species revealed minor collateral vascular bundles, surrounded by a single-layered parenchymatous sheath, immersed in the mesophyll (Figure 3C, E), vascular bundles of higher order veins exhibited secretory ducts similar to those described in the mid vein. Prismatic crystals and druses were also observed in the mesophyll (Figure 3D). In terms of the leaf petioles, transverse sections at their middle lengths showed round shape in *S. fasciculatus* (Figure 3G) and a slightly sub-circular winged form in *S. gracilipes* (Figure 3H). Their anatomical features were very similar to those observed in the leaf blades.

Table 1. Quantitative features recorded for stomata and trichomes found in leaves of *Schinus fasciculatus* and *S. gracilipes*.

Leaf structures	***S. fasciculatus***		***S. gracilipes***	
	Adaxial surface	Abaxial surface	Adaxial surface	Abaxial surface
Stomata				
Average density (stomata/mm^2)	58 ± 2	159 ± 5	Few	333 ± 10
Average size (length x width, µm)	28±3 x 25±1	28±2 x 24±2	26±2 x 22±2	25±2 x 18±2
Trichomes (number/ mm^2)	.			
Non-glandular	3 ± 1	2 ± 1	16 ± 4	2 ± 1
Glandular	3 ± 1	4 ± 2	6 ± 1	47 ± 2

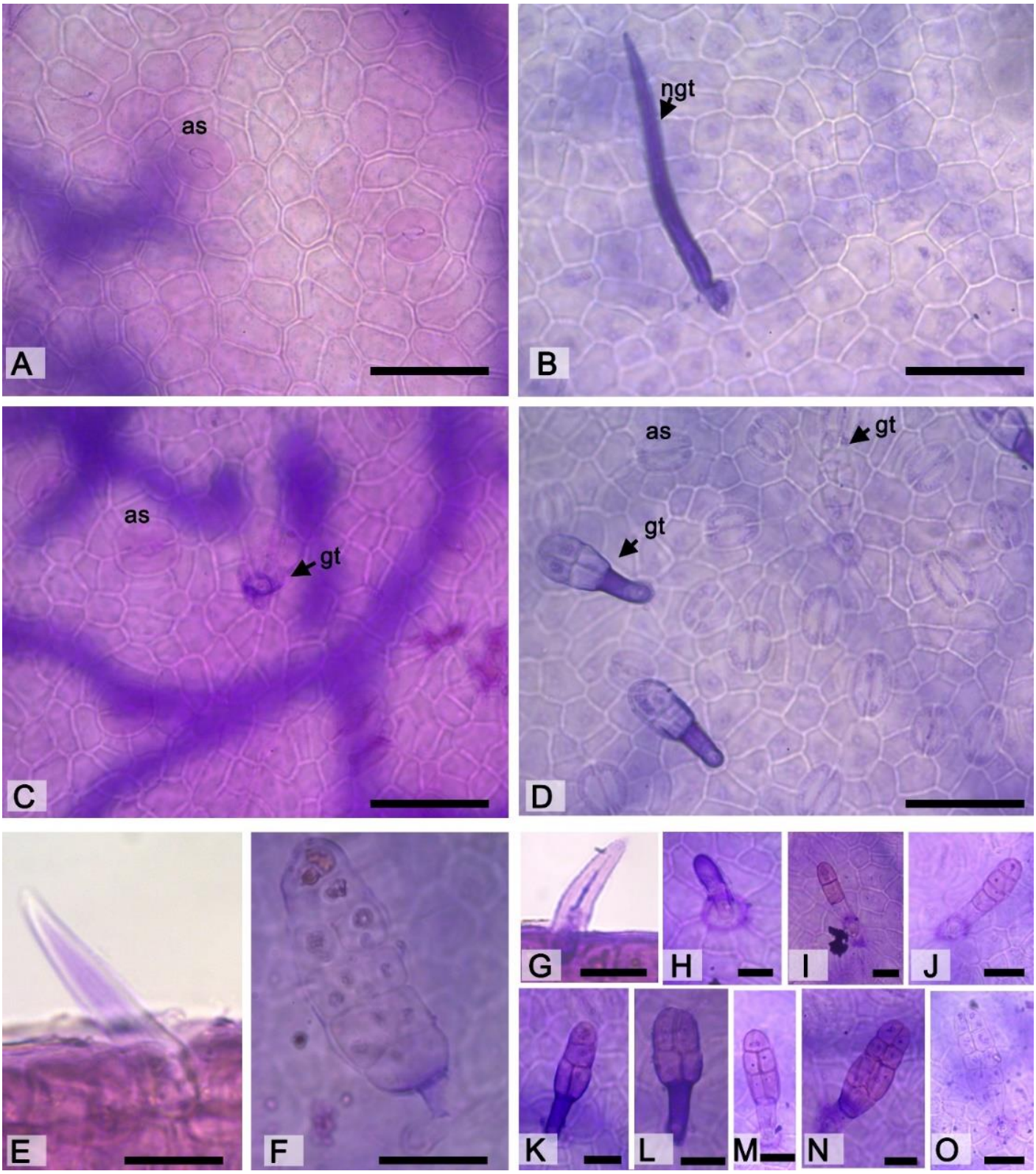

Figure 2. Superficial view of epidermis and trichomes features observed on leaf samples of *Schinus fasciculatus* and *S. gracilipes*. (A,C,E,F) *S. fasciculatus* and (B,D,G-O) *S. gracilipes* stained with cresyl violet. The epidermis is visualized from its (A,B) adaxial and (C,D) abaxial sides. (E,G) Unicellular non-glandular trichomes and (F,H-O) glandular trichomes are observed at different maturity stages. Scales: A-D, 50 µm; E-O, 20 µm. References: as, anomocytic stomata; gt, glandular trichome; ngt, non-glandular trichome.

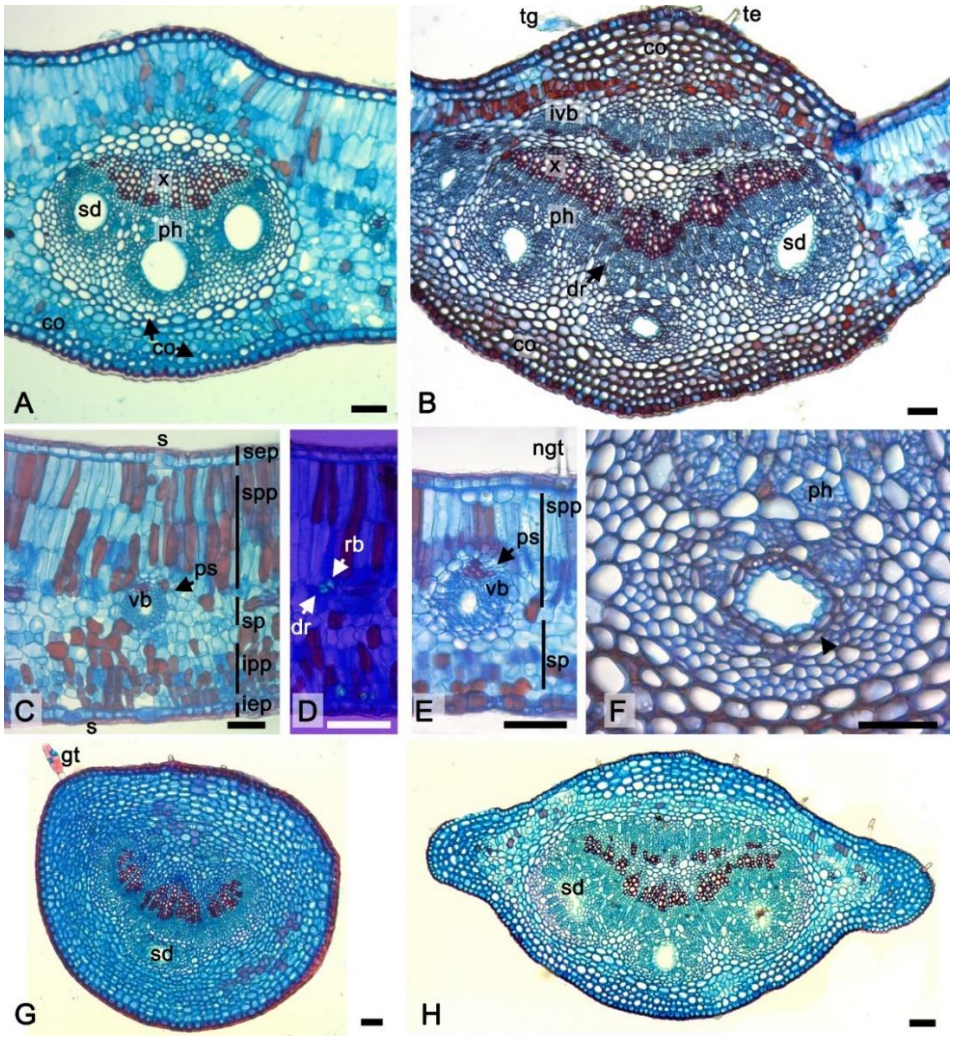

Figure 3. Leaf and petiole sections of *S. fasciculatus* and *S. gracilipes*. (A,C-D,G) Leaf and petiole sections stained with astra blue-safranin of *Schinus fasciculatus* and (B,E-F,H) *S. gracilipes*. (A-B) Leaf section at the mid vein. (C,E) Lamina section with bright and (D) polarized light. Detail of schizogen duct with endodermoid band in *S. gracilipes* (F). (G-H) Petiole section. Scales: 50 µm. References: arrow head, endodermoid band; co, collenchyma; dr, druse; gt, glandular trichome; iep, inferior epidermis; ipp, inferior palisade parenchyma; ivb, inverted vascular bundle; ngt, non-glandular trichome; ph, phloem; ps, parenchyma sheath; rb, rhomboidal crystal; s, stomata; sd, schizogen duct; sep, superior epidermis; sp, spongy parenchyma; spp, superior palisade parenchyma; vb, vascular bundle; x, xylem.

Histochemistry

The main findings obtained by the histochemical analysis are briefly summarized in Table 2. Unstained leaf sections (Figure 4A-B) revealed the mesophyll containing green chlorophyll, translucent refringent content in the secretory ducts, and amber content in the glandular trichomes. Sudan IV staining showed that the cuticles and the lumen of the secretory ducts had a red color, indicating the presence of lipids (Figure 4E-H). NADI reagent indicated the presence of terpenoids through a purple dye in the palisade and spongy chlorenchyma mesophyll cells, the contents of the secretory ducts and the head cells of glandular trichomes (Figure 4I-M). Phenolic compounds reacted with ferric chloride (Figure 4N-R) and Fast Blue B (Figure 4S-V), resulting in intense dark grey and brownish red colours, respectively. They were observed in the mesophyll palisade and spongy parenchyma (Figure 4N,P,S and U), the head and stalk cells of glandular trichomes (Figure 4O,R,T and V) and the epithelial cells of the phloem ducts (Figure 4S, Q and U). The lumen of the ducts in *S. fasciculatus* was stained dark red by Dragendorff reagent, which detects alkaloids (Figure 5A-D). Tannins were visualized as red in the mesophyll cells of both *Schinus* species with vanillin-HCl staining (Figure 5E-H). PAS reaction stained the mesophyll palisade and spongy parenchyma, as well as the contents of the head-cell of the glandular trichomes in a magenta color, indicating the presence of reducing polysaccharides (Figure 5I-L), while starch was not detected by lugol test. Steroids were detected in some cells of the palisade parenchyma of *S. fasciculatus* by the Liebermann Burchard test. However, they were not found in *S. gracilipes*.

Table 2. Summary of the histochemical analysis performed on leaf sections of *Schinus fasciculatus* and *S. gracilipes*.

Reagent	Identified compounds	Cuticle[1]		Epidermal Cells		Mesophyll		Secretory duct		Epithelial Cells		Glandular trichomes	
		Sf	Sg	Sf	Sg	Sf	Sg	Sf	Sg	Sf	Sg	Sf	Sg
Sudan IV	Terpenes and non-terpene lipids	++ red	++ Red	-	-	-	-	++,L red	++,L red	-	-	-	-
NADI	Essential oil/ terpenes	-	-	-	-	+, P-Sg Purple	+, P-Sg purple	+,L purple	+,L purple	-	-	++, H purple	++, H purple
$FeCl_3$	Phenolic compounds	-	-	-	-	+, P-Sg dark blue	+, P-Sg dark blue	++,E	++,E	+ dark blue	+ dark blue	+, H-S dark blue	+, H-S dark blue
Fast blue B	Phenolic compounds	-	-	-	-	++,P-Sg Orange	++,P-Sg orange	++,E	++,E	+ orange	+ orange	-	-
$AlCl_3$	Flavonoids	+ yellow cyan fl	+ yellow cyan fl	++ yellow cyan fl	+ yellow cyan fl	-	-	-	-	-	-	+, H orange fl	+, H yellow fl
Dragendorff	Alkaloids	-	-	-	-	-	-	+,L dark red	-	-	-	-	-
Vainillin HCl	Tannins	-	-	-	-	++,P-Sg red	++,P-Sg red	-	-	-	-	-	-
PAS	Polysaccharides (no starch)	-	-	-	-	++,P-Sg magenta	++,P-Sg magenta	-	-	-	-	+, H magenta	+, H magenta
Lugol	Starch	-	-	-	-	-	-	-	-	-	-	-	-
Liebermann-burchard	Triterpenes, steroids	-	-	-	-	+,P green-blue	-	-	-	-	-	-	-
Neu´s reagent	Phenolic compounds/flavonoids	++ yellow orange fl	++ yellow orange fl	-	-	+,P-Sg orange fl	+,P-Sg orange fl	-	-	+ orange fl	+ orange fl	++, H orange fl	++, H yellow-orange fl
KOH 5%	Phenyl-Propanoids	-	-	-	-	-	-	-	-	-	-	+, H bright blue fl	+, H bright blue fl

[1]References: Sf, *Schinus fasciculatus*; Sg, *Schinus gracilipes*; +, positive reaction or coloration; ++; strong positive reaction or coloration; -, negative reaction or coloration; fl, fluorescence. H, head cells; S, stalk cells; P, palisade; Sg, spongy; L, lumen, E, epithelial cells.

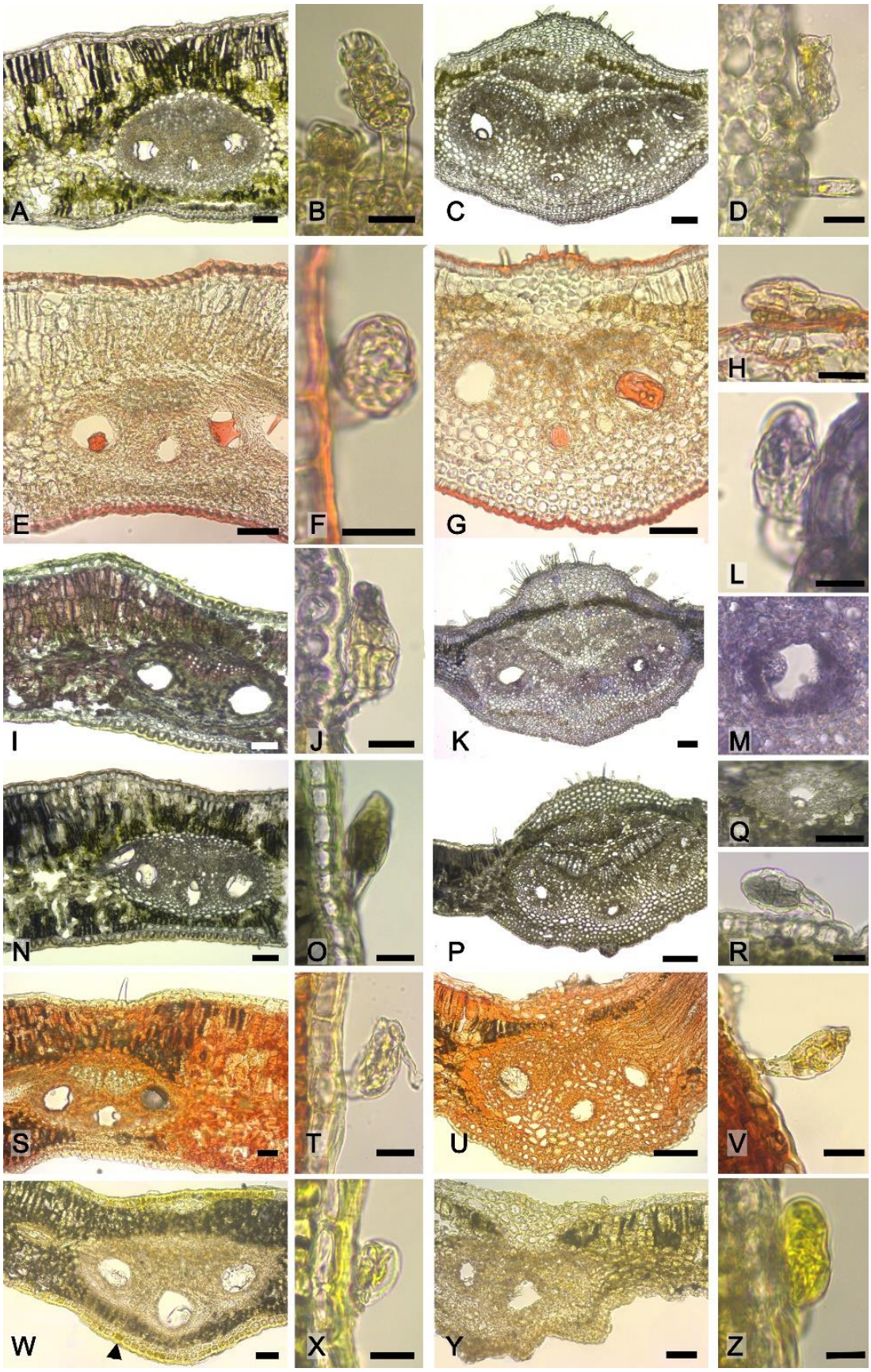

Figure 4. Cross sections of leaf samples showing ducts, and glandular trichomes of *Schinus fasciculatus* (A-B, E-F,I-J, N-O, S-T, W,X) and *S. gracilipes* (C-D, G-H, K-M, P-R, U-V, Y-Z). Sections are observed fresh (A-D) and after staining with reagents used for visualization of secondary metabolites: Sudan IV (red) for terpenes and non-terpene lipids (E-H), NADI reagent (purple) for terpenoids (I-M), FeCl3 (gray-black) for phenolic compounds (N-R), Fast Blue B (orange) for phenolic compounds (S-V), AlCl3 for flavonoids (orange-yelow) marked with arrow heads (W-Z). Scales: A, C, E, G, I, K, M, N, P, S, U, W, Y, 50 µm; B, D, F, J, L, O, Q, R, T, V, X, Z, 20 µm.

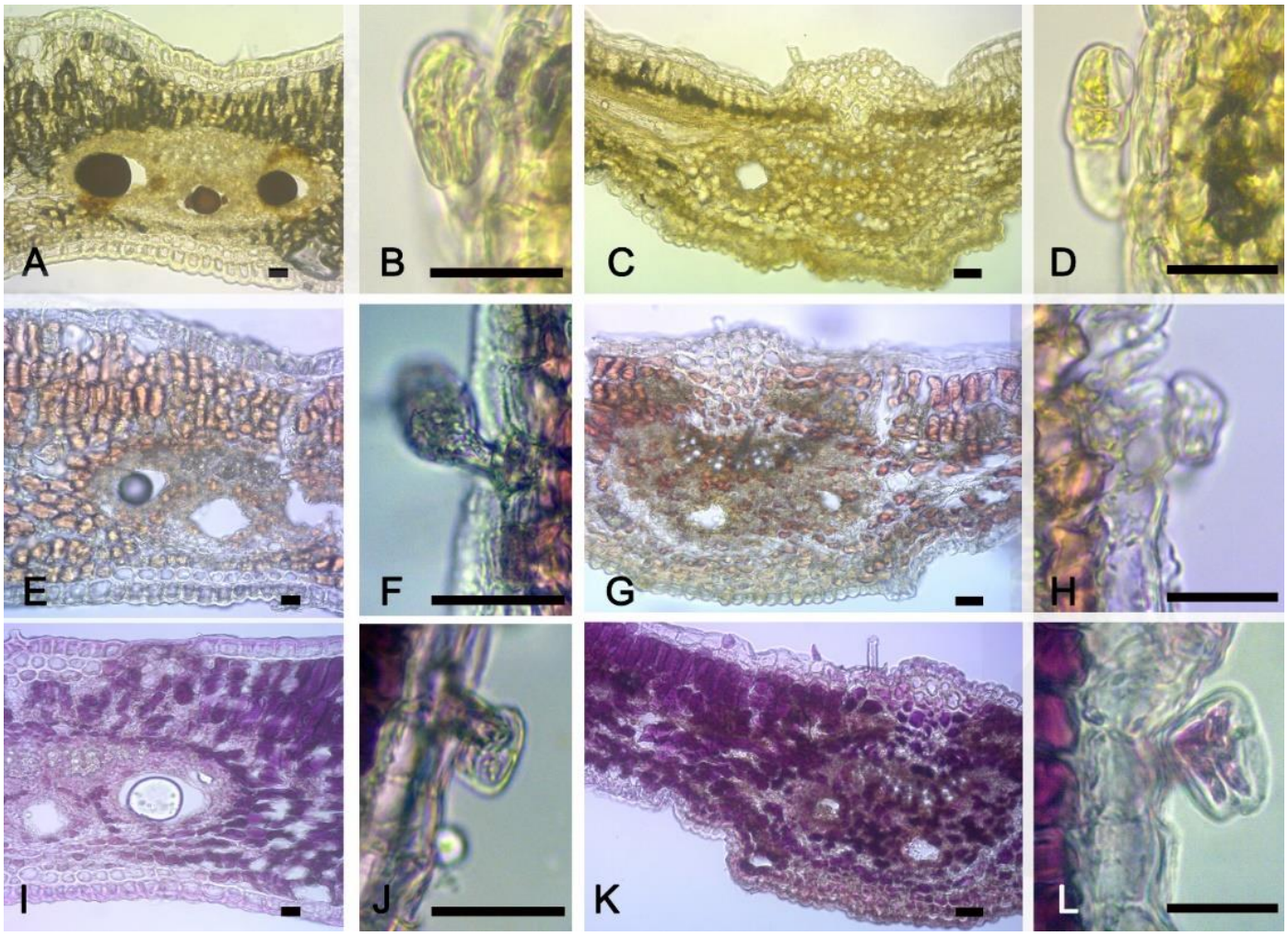

Figure 5. Cross sections of leaf samples showing ducts, and glandular trichomes of *Schinus fasciculatus* (A-B, E-F, I-J.) and *S. gracilipes* (C-D, G-H,K-L). Alkaloids stained dark brown with Dragendorff reagent (A-D), tannins visualized red with vanillin-HCl (E-H), and polysaccharides other than starch stained magenta with PAS reaction. Scales: A-D, 20 µm.

The unstained leaf sections exhibited red fluorescence emitted by chlorophyll, which was located in the mesophyll (Figure 6A and C). Blue fluorescence was observed in various parts of the mesophyll, the cuticles, glandular trichomes and secondary cell walls of the xylem vessels (Figure 6A-D). Leaves treated with the hydroalcoholic Neu´s reagent displayed orange fluorescence, indicating the presence of flavonols and flavones accumulated in the xylem vessels, the palisade parenchyma and the cuticle at the upper and lower leaf sides of *S. fasciculatus*. In *S. gracilipes*, orange fluorescence was observed in the xylem vessels and cuticle of the abaxial leaf surface (Figure 6E-H). Glandular trichomes of both species and the epithelial tissue of the phloem ducts in *S. fasciculatus* also exhibited orange fluorescence. Furthermore, the palisade mesophyll fluoresced mostly pink to red, and sometimes yellow-orange, with aluminum chloride. This reagent also revealed yellow to yellow-orange fluorescence in the content of the glandular trichomes and cyan-yellow fluorescence in the xylem vessels, epidermal cells and cuticles (Figure 6I-L). Cell wall-bound phenylpropanoids exhibited a bright blue fluorescence in the epidermal cuticles, glandular trichomes, and xylem vessels while it displayed a deep blue fluorescence in the mesophyll and ground parenchyma cells (Figure 6M-P).

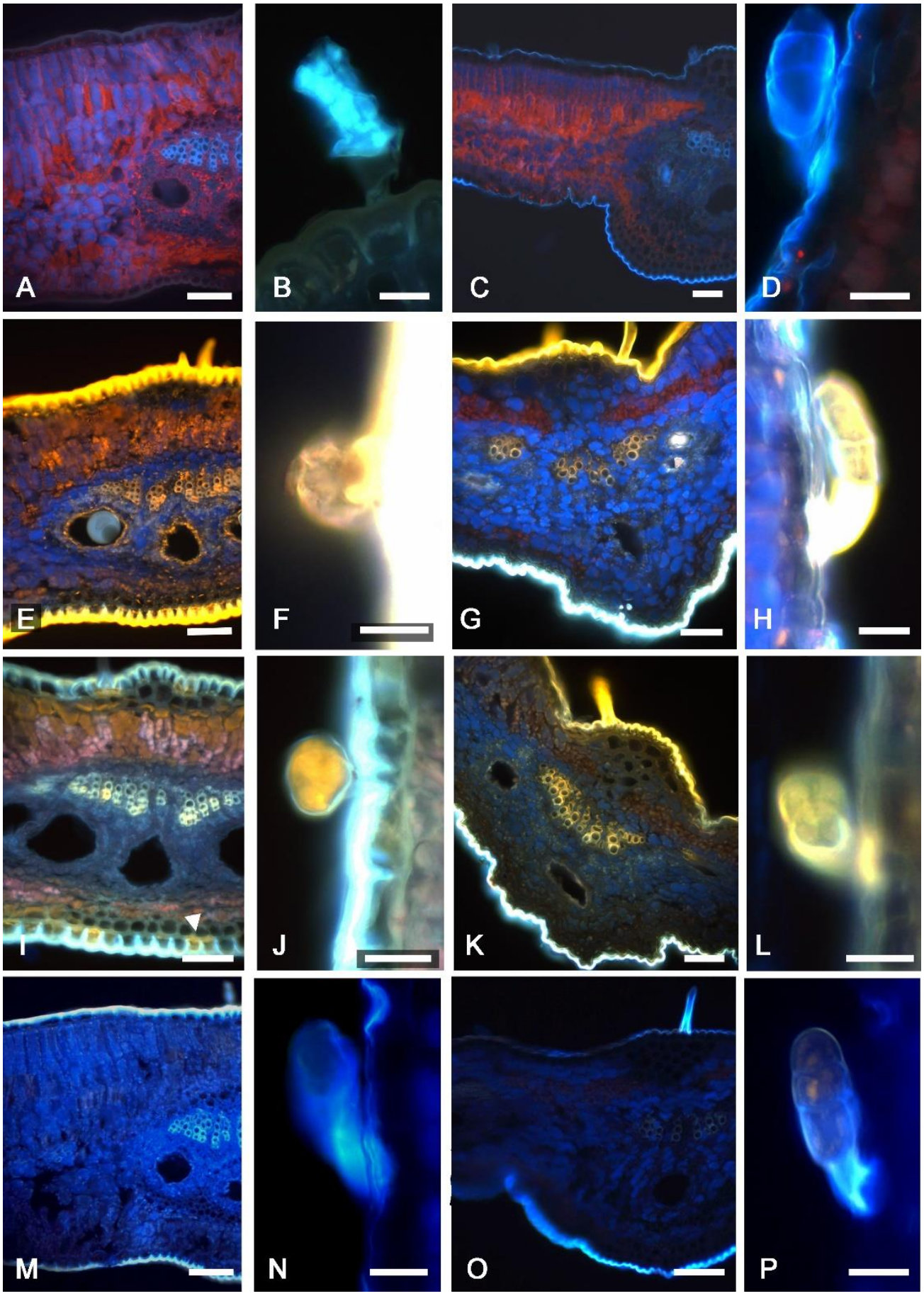

Figure 6. Cross sections of leaf samples under fluorescence microscropy of *Schinus fasciculatus* (A-B, E-F, I-J, M-N) and *Schinus gracilipes* (C-D, G-H, K-L, O-P). Fresh sections (A-D), sections treated with hydroalcoholic 1% Neu´s reagent (E-H) and 5% AlCl3 for flavonoids visualization (I-L), and 5% KOH for phenylpropanoids (M-P). Scales: A, C, E, G, I, K, M, O; 50 µm; B, D, F, H, J, L, N, P, 20 µm.

DISCUSSION

The plant and leaf architectures observed in both *Schinus* species were generally consistent with earlier reports [1], although some features such as a semicraspedromous venation in *S. gracilipes*, and fourth to sixth order veins mentioned for both species were absent in the analyzed material [22]. These shrubs showed a leaf anatomy well adapted for growth in xerophytic habitats exposed to strong solar radiation, high heliophany and prolonged periods of drought stress [23]. The presence of amphistomacy and anomocytic stomata in *S. fasciculatus* and *S. gracilipes* is consistent with findings in most *Schinus* species [24]. Amphistomacy maximizes CO_2 conductance, enabling high photosynthetic rates in leaves exposed to environments with elevated solar irradiance and low humidity [25]. Regarding the leaf trichomes, they were sparse in *S. fasciculatus* as observed in most *Schinus* species [26,27]. The abundance of non-glandular trichomes on the adaxial leaf surfaces of *S. gracilipes* might help to reflect sunlight and/or reduce water loss [28]. Additionally, they may serve as mechanical protection against phytophagous invertebrates and/or generate unfavourable conditions for the progress of phytopathogenic microorganisms [7]. On the other hand, glandular trichomes may have an important defensive role in *S. gracilipes*, where they appeared at high densities. These multifunctional structures protect leaves from abiotic stress factors and secrete defense metabolites [29]. Other leaf features described in this work, such as the mesophyll structure and leaf thickness, suggest that *S. fasciculatus* is better adapted to extreme environments than *S. gracilipes*. Both species contained druses and prismatic cristals, which are also observed in other *Schinus* species [27]. These structures reflect the sunlight into the photosynthetic tissues, serve as calcium storage, and likely provide protection against phytophagous organisms [30].

The histochemical analysis indicated that phenolic compounds and terpenoids were widely distributed in the mesophyll tissues, whereas alkaloids were strictly compartmentalized in secretory structures. Glandular trichomes contained a complex mixture of polysaccharides, terpenoids and phenolic compounds including flavonoids. Their contents could be secreted or released upon breakage, where the polysaccharide matrix likely acts as a sticky coating, coming into contact with the surfaces of other organisms and ensuring exposure to the defense metabolites [31]. The chemical constituents of glandular trichomes in other Anacardiaceae species resemble the composition reported here. For example, glandular trichomes of *Anacardium humile*, *Lithraea molleoides*, *Spondias dulcis* and *Tapirira guianensis* were rich in mucilage, lipids, and phenolic compounds [32], while tannin compounds were found in *Mangifera indica* [33].

The leaves of *S. fasciculatus* and *S. gracilipes* shared the presence of a high number of schizogenous phloem ducts, which is an ubiquitous trait among *Schinus* members [26]. The chemical composition of the ducts is reported here for the first time in *S. fasciculatus* and *S. gracilipes,* and it essentially consisted of lipids, with confirmed presence of terpenes. These constituents were also detected in the phloem ducts of *A. humile*, *L. molleoides* and *S. dulcis* along with mucilages that were absent in our analyses, and phenolic compounds that were only found in the epithelial cells of both *S. fasciculatus* and *S. gracilipes* [34]. Phloem ducts of other Anacardiaceae species (i. e. *T. guianensis*) only contained non-terpene lipids. Alkaloids were only found in phloem ducts of *S. fasciculatus.* Although unusual in this family, alkaloids have been reported in leaf extracts of *Schinus* species (*S. terebinthifolius*, *S. molle*, *S. montanus* and *S. polygamous*), *Lithraea caustica*, and mesophyll idioblasts of *A. humile* [35, 36, 37]. Phloem ducts of both *Schinus* species were devoid of phenolic lipids of the alkylresorcinol type (e. g. alkenylresorcinols and alkenylphenols). These compounds have been identified in *S. terebintifolius* and other *Schinus* species [38, 39] and are often found in phloem ducts of Anacardiaceae species where likely have a defensive role [6,7].

CONCLUSION

The strong presence and wide distribution of terpenoids and phenolic compounds suggest that they exert several simultaneous functions in the leaves of both *Schinus* species. They may act as antifeedants against insects and grazing animals and also participate as regulators of the water balance [40]. In the case of tannins and flavonoids, they possess UV-absorbing and antioxidant properties that not only protect plants from excessive radiation but also counteract the release of oxidating free radicals occurring under extreme environmental conditions [41]. The histochemical analysis confirm ed the previously reported high presence of phenolic compounds and terpenoids in the leaves of *S. gracilipes* and *S. fasciculatus* reported by our group [6,8,42]. Furthermore, it may contribute to optimize leaf extraction of specific bioactive constituents.

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