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Structural features of species of Asteraceae that arouse discussions about adaptation to seasonally dry environments of the Neotropics[1]

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ABSTRACT

Seasonally-dry environments of the Neotropics, such as the South American Chaco, exert selective pressures on vegetation through a pronounced water deficit. We describe the underground system and leaf anatomy of three species of Asteraceae from the Brazilian portion of the Chaco (*Pterocaulon purpurascens* Malme, *Wedelia trichostephia* DC., and *Pectis gardneri* Baker), aiming to describe their structural and adaptive features using standard plant anatomy techniques. *Pterocaulon purpurascens* and *W. trichostephia* exhibited slightly thickened xylopodia, with gemmiferous character and self-grafted stem shoots; *Pectis gardneri* displayed a slightly-thickened tuberous root with storage substances. Longitudinal sequences of cells with highly thickened walls forming globular protrusions were found throughout the extension of the periderm of *Pectis gardneri*, while senescent trichomes were found in the periderm of *W. trichostephia*. Schyzogenous aerenchyma was found in *P. purpurascens*. Aquifer cells, composing vascular rays of secondary phloem and xylem, are reported for this species. Leaves of the three species are perennial and amphistomatic, with aquifer cells in a variety of tissues. *Pectis gardneri* exhibits a "Kranz-type" anatomy with lignified bristles with stomata. The features described for the species play important role in water uptake and/or storage, which prevent excessive water loss during environmental or physiological stress periods.

Keywords: Asteroideae, drought, steppic savanna, underground systems, water stress, xylopodia

Introduction

Seasonal water stress, which is common to Neotropical arid and semi-arid environments, determines ecological and evolutionary patterns resulting in water transport safety and efficiency. Such patterns include variation in vessel length, diameter and frequency; type of perforation plate; and axial and radial parenchyma with storage substances, among other features, which are mainly apparent in arboreal species (Kraus *et al*. 2007; Carlquist 2009; Yang *et al*. 2010), However, such tissues patterns in species of the herbaceous-subshrub stratum of most biomes is poorly known. The main challenge faced by anatomists is determining which anatomical changes are environmentally induced and which are a result of genetic alterations, which provides crucial anatomical responses and the interpretation of the adaptations of species to their environment (Metcalfe & Chalk 1979; Kraus *et al*. 2010; Scremin-Dias *et al*. 2011).

Several botanical families have phenotypically similar species that inhabit arid and semi-arid environments and share adaptive convergences to seasonal water stress (Fahn & Cutler 1992). Species of the family Asteraceae, especially herbaceous and subshrub species, possess features related to water stress. Such features include

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the development of bud-bearing underground storage systems, which are related to the occurrence of secretory tissues that are efficient in maintaining individuals during seasonal water stress, and vegetative propagation, which provides resistance to extreme climatic variation (Anderberg *et al*. 2007; Appezzato-da-Glória 2015). Such features help in understanding adaptive radiations of recent groups on a global scale and their successful establishment in habitats with adverse climatic and edaphic conditions (Bremer 1994; Panero & Funk 2008; Funk *et al*. 2009).

When compared to rainforests, seasonally dry tropical areas have received little attention from ecologists and conservationists, which has resulted in a lack of knowledge about the vegetation of these regions and has hindered inferences regarding Neotropical biogeography (Pennington *et al*. 2000). South American seasonally dry forests possess assemblages of vegetation types that are related to climatic fluctuations with a well-defined dry season of variable duration. A well-defined dry season is a determining factor for physiognomies and needs to be studied separately in biogeographic analyses due to species and environmental diversity (Pennington *et al*. 2000; Prado 2000).

The South American sedimentary plain of the Chaco (or Gran-Chaco), is one of the most extensive seasonally dry Neotropical environments and the largest continuously semi-arid dry forest (Adámoli *et al*. 1990; Lewis *et al*. 1990; Prado 1993). Distinctly Chaquenian phytophysiognomies vary according to climatic and edaphic gradients, and possess a great diversity of environmental and species heterogeneity compared to other arid and semi-arid environments (Adámoli 1987; Spichiger *et al*. 1991). In Brazil, Chaquenian plains are located in the wetlands of the Pantanal and vary according to mild climatic seasonality. The protection of the biodiversity of this ecosystem in Brazil is vitally important due to its restricted distribution in the country and its floral specificity (Prado *et al*. 1992; Silva & Caputo 2010; Silva *et al*. 2010). Unfortunately there remain few botanical studies on this ecosystem.

Chaco vegetation exhibits a variety of strategies for existence in this ecosystem characterized by stress from marked water deficit for periods of months. The loss of leaves during the dry season with resprouting in the rainy season, combined with the development of underground storage systems with buds, are two important strategies that have evolved in several herbaceous species of the Cerrado (Moraes *et al*. 2016), and which are also possessed by some Chaquenian species. Such characteristics can be similar or vary among taxa. Thus, we investigated the anatomy and histochemistry of underground systems and leaves of three species of Asteraceae (*Pterocaulon purpurascens*, *Wedelia trichostephia* and *Pectis gardneri*) that compose the herbaceous-subshrub stratum of the Brazilian Chaco in order to evaluate the adaptive character of these organs.

Materials and methods

Environmental data and sampling

The Chaco encompasses about 800.000 km^2 throughout the north and central regions of Argentina, eastern Paraguay, southeastern Bolivia and extreme western Brazil (Adámoli *et al*. 1990; Lewis *et al*. 1990; Prado 1993). The Brazilian portion of the Chaco is restricted to southwest region of the Pantanal and occupies approximately 7 % of the Nabileque sub-region (Prado *et al*. 1992) (Fig. 1)

The Chaco is classified by phytophysionomic and floristic elements, and is divided into four subtypes: I) “Forested Steppic Savanna”, also called Chaco, dry forest or Chaquenian forest; II) “Steppic Scrub Savanna”; III) “Steppic Park Savanna” or “Carandazal”; and, IV) “Steppic Woody-Grassy Savanna” (see also Pott *et al*. 2011; Sartori 2012). The climate of the Chaco is hot and dry. It possesses a dry season from April to September, with mean rainfall below 100 mm for the entire season that leads to physiological drought, and a rainy season from November to February (≈ 970 mm), with March and October being considered transitional months (Carvalho & Sartori 2015). The soil is classified as compacted and saline, with slow drainage that can lead to temporary flooding during the rainy season (Pennington *et al*. 2000).

The species *Pterocaulon purpurascens* Malme, *Wedelia trichostephia* DC., and *Pectis gardneri* Baker possess perennial leaves during dry the season and are components of the herbaceous-subshrub stratum of their respective Chaco subtypes. Five individuals of each species were collected in different Chaco remnants in the municipality of Porto Murtinho, state of Mato Grosso do Sul, Brazil (see Tab. 1). Individuals of *Pterocaulon purpurascens* were collected in a “Steppic Park Savanna” site; individuals of *Wedelia trichostephia* were collected in a “Steppic Scrub Savanna” site, with elements of “Steppic Park Savanna”; and individuals of *Pectis gardneri* were collected in a “Steppic Scrub Savanna” site (see Fig. 1).

Anatomical analysis

Underground systems and leaves of three individuals per species were studied. Underground systems were analyzed from thickened and non-thickened portions, whereas leaves, which were selected from the fourth node, were analyzed at the midrib, and the internervural and edge regions. Morphological classification of the underground systems followed Appezzato-da-Glória (2015), while the classification of xeromorphic anatomical structures followed Fahn & Cutler (1992).

Samples were fixed in formalin-acetic acid-50 % ethanol solution (FAA 50, 1:1:18), dehydrated in a graded ethanol series and stored in 70 % ethanol (Johansen 1940). The underground systems were embedded in

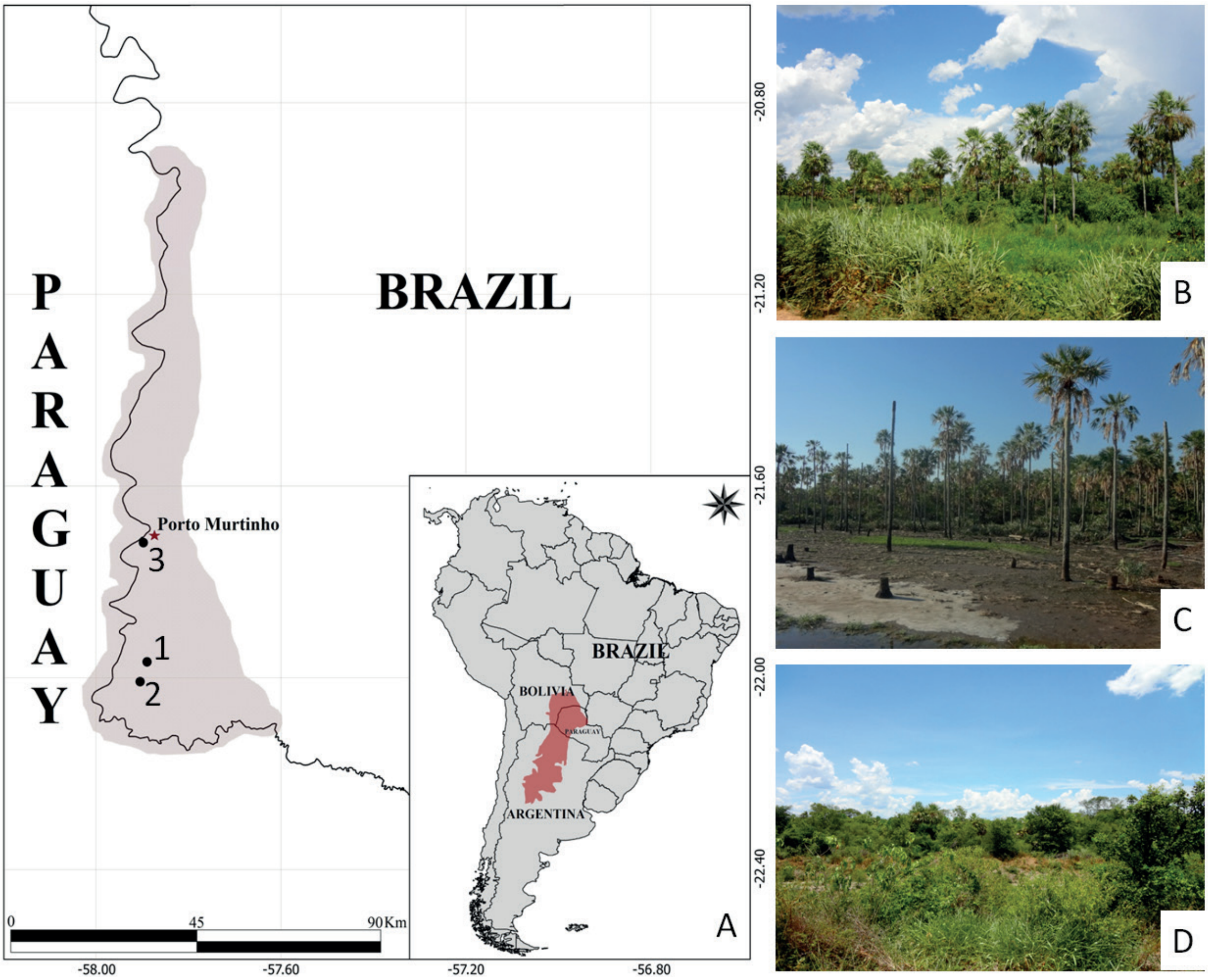

Figure 1. Brazilian portion of the Chaco in the municipality of Porto Murtinho, state of Mato Grosso do Sul, highlighting its distribution in South America (**A**). Black dots (●) represent sampling areas, where *Wedelia trichostephia* (1) were sampled in a "Steppic Scrub Savanna" site, with elements of "Steppic Park Savanna" (**B**), *Pterocaulon purpurascens* (2) were sampled in a "Steppic Park Savanna" site (**C**), and *Pectis gardneri* (3) were sampled in a "Steppic Scrub Savanna" site (**D**). Map by Renan S. Olivier.

paraffin blocks, cross- and longitudinally sectioned (20-30 μm thick) using a slide microtome (Model Leica SM 2000 R, Leica Microsystems, Germany) and stained with 1 % ethanol alcian blue and 1 % hydroalcoholic safranin (Bukatsch 1972 *apud*. Kraus & Arduin 1997). The leaves were dehydrated in a graded ethanol series, embedded in plastic resin (Leica Historesin®), cross-sectioned (7 μm thick) using a rotary microtome (Model Leica RM 2145, Leica Microsystems, Germany) and stained with 0.05 % Toluidine Blue O in a pH 4.5 citrate-phosphate buffer (Sakai 1973). Both underground system and leaf sections were mounted in Entellan® synthetic resin (Merck, Darmstadt, Germany). When required, free-hand paradermic (only on leaves) and cross-sections were made using disposable steel blades, which were then were stained with 0.1 % alcian blue and 0.1 % safranin, and mounted in glycerinated gelatin (Bukatsch 1972 *apud*. Kraus & Arduin 1997).

We performed the Franklin (1946) technique for dissociation of secondary xylem, followed by 1 % ethanolic safranine staining and mounting in glycerinated gelatin. The diameter, length and number of vessel elements per mm^2 were measured for 30 cells of three individuals per species, and the mean and standard deviation calculated.

Histochemical analysis was performed on sections of both fresh and fixed underground systems and leaves. Zinc-chloride iodide was used for starch grains (Johansen 1940), ferric chloride for phenolic compounds (Johansen 1940), Ruthenium red for pectin and mucilage (Gregory & Baas 1989), Sudan IV for lipophilic substances (Pearse 1985), and acidified phloroglucinol for lignin (only on leaves; Johansen 1940).

Photo documentation was performed using an optical microscope and a stereomicroscope (Leica DMBL) coupled to a Leica DC 300F image capture system with projection of the corresponding micrometric scales.

Table 1. Analyzed species and respectively geographic data and vouchers

Species	Locality, State and coordinates	Phytoecological Subtype	CGMS Voucher
Pterocaulon purpurascens Malme	Porto Murtinho/MS (21°42’59,31”S 57°53’57,51”W)	Steppic Park Savanna	40805
Wedelia trichostephia DC.	Porto Murtinho/MS (22°00’22,67”S 57°54’13,23”W)	Steppic Scrub Savanna	40807
Pectis gardneri Baker	Porto Murtinho/MS (21°58’02,58’’S 57°53’24,94’’W)	Steppic Scrub Savanna	40809

For scanning electron microscopy (SEM), $1\ cm^2$ portions of leaf blade were dehydrated in a graded ethanol series, critical-point dried using CO_2 (Horridge & Tamm 1969), mounted on aluminum stubs and coated with gold (Denton Vacuum Desk IV, LLC). The samples were analyzed and photomicrographed using a JEOL JSM-6380LV SEM operating at 20 kV.

Results

The evaluated adaptive and structural features are provided in Tables 2 and 3, respectively, as were structural characteristics (Tab. 2) and storage substances (Tab. 3).

Underground system anatomy

The underground systems of *Pterocaulon purpurascens* and *Wedelia trichostephia* were classified as slightly thickened xylopodia (Fig. 2A-B), while for *Pectis gardneri*, the underground system was classified as a slightly thickened tuberous root (Fig. 2C). Both xylopodia exhibit non-thickened lateral roots and self-grafted stem shoots (Fig. 2D-E), while those features are absent on the tuberous root (Fig. 2F). Buds are located at the base of stem branches and previously originated on vascular cambium (Fig. 2G-I). *Pectis gardneri* exhibit the presence of interspersed storage parenchyma cells with highly lignified parenchyma cells in secondary xylem (Fig. 2J).

The periderm of the three species consists of 4-7 layers of cells ranging in shape from cubic to flattened rectangles (Fig. 3A-C), with phenolic and lipid storage in *Pectis gardneri* (Fig. 3D-E) and lipid storage in *W. trichostephia* (Fig. 3F). Persistent trichomes with lipid storage occur in the epidermis-periderm transitional stage in *W. trichostephia* (Fig. 3G). Longitudinal sequences of cells with highly thickened walls forming globular protrusions occur throughout the extension of the periderm of *Pectis gardneri* (Fig. 3H-I). Cortical parenchyma occupies a short extension, and comprises mainly regularly-shaped cells with periclinal divisions in the layers closest to the periderm in all species (Fig. 3A-C). The cortical parenchyma possesses schyzogenous aerenchyma in *P. purpurascens* (Fig. 3A); clusters of fibers and sclereids in the inner layers in *P. purpurascens* (Fig. 3A); and randomly occurring isolated and/or clustered sclereids with fibers in *W. trichostephia* (Fig. 3B); this feature was not seen in *P. gardneri*. The underground systems of *P. purpurascens* and *W. trichostephia* have secretory canals comprised of one layer of epithelial cells surrounding the lumen (Fig. 3J-K). Cells containing starch grains occur in the outer layers of the cortical parenchyma in *P. gardneri*, and the endodermis exhibits a Casparian strip (Fig. 3L).

The secondary phloem exhibits conducting cells with reduced lumen flanked by bulky radial parenchyma cells with less thickened walls, some of which are evidently aquifers functioning as water reservoirs (Fig. 4A-C). The secondary xylem is well developed, with a radial arrangement of vessels *in P. purpurascens* and *W. trichostephia* (see Fig. 2A-B), and a diffuse arrangement in *P. gardneri* (see Fig. 2C). The vessel elements are long (± 142.45 μm), narrow (± 22.24 μm) and dense (± 324.4/mm^2) (see Tab. 2), with single perforation plates and alternate intervessel pits. Bulky cells that store water are present mainly in longitudinal sections of vascular rays, which are multisseriate in *P. purpurascens* and *W. trichostephia*, and unisseriate in *P. gardneri* (Fig. 4D-F). Histochemical analysis indicated storage of mucilage and phenolic compounds in vascular rays and cells of the vascular cambium in *P. purpurascens* (Fig. 4G-H). Greater lignification of the cell walls of the axial parenchyma forming seasonal rings is visible in *W. trichostephia* only during later stages of development (Fig. 4I).

Leaf anatomy

The analyzed species have perennial leaves with variable morphology in the same individual (Fig. 5A-C). Cross sections of all three species show a unisseriate epidermis with juxtaposed irregularly-sized cells that are usually elliptic, and with little variation in size and shape. Anticlinal cell walls are sinuous, while the external periclinal walls vary from being smooth to papillose for all species (Fig. 6A-G). Leaves are dorsiventral, with heterogeneous mesophyll and slightly curved edges in *P. purpurascens* (Fig. 6B-C) and *W. trichostephia*(Fig. 6E-F), and homogenous mesophyll in *P. gardneri* (Fig. 6G-I). All species are amphistomatic, with anomocytic stomata (Fig. 6J-L) and stomatal ridges with epicuticular waxes impregnating the cells (Fig. 6M-R). In *P. purpurascens*, stomatal guard cells are positioned above the other epidermal cells on the abaxial surface (Fig. 6B-C).

The midrib of *P. purpurascens* possess angular collenchyma underlying the adaxial epidermis, with collateral vascular bundles surrounded by a lignified sheath (Fig. 6A), while

Table 2. Adaptive and structural features of underground systems and leaves of *Pterocaulon purpurascens* Malme, *Wedelia trichostephia* DC., and *Pectis gardneri* Baker

Structural Features		Species		
		P. purpurascens Malme	*W. trichostephia* DC.	*Pectis gardneri* Baker
Underground System	Classification	Xylopodia	Xylopodia	Tuberous Root
	Bud development	+	+	-
	Periderm with trichomes	-	+	-
	Small cortical parenchyma	+	+	+
	Aerenchyma	+	-	-
	Secretory ducts	+	+	-
	Globular protrusions on the periderm	-	-	+
	Aquifer Cells	+	+	+
	VE diameter (**mean**) (µm)	(12.06)-**23.09**-(37.88) ± 5.56	(11.29)-**24.85**-(49.88) ± 6.12	(2.93)-**18.78**-(42.7) ± 6.6
	VE length (**mean**) (µm)	(96.71)-**142.05**-(251.45) ± 36.4	(86.63)-**142.93**-(361.29) ± 53.43	(88.66)-**142.39**-(294.02) ± 24.43
	VE/mm^2 (**mean**)	(233)-**247.6**-(266) ± 12.16	(316)-**333.4**-(352) ± 15.74	(308)-**392.2**-(443) ± 52.2
Leaves	Perennial	+	+	+
	Amphistomatic	+	+	+
	Stomata type	Anomocytic	Anomocytic	Anomocytic
	Stomata above epidermic cells	+	-	-
	Large substomatic chambers	-	+	-
	Stomatal ridges	+	+	+
	Epicuticular waxes	+	+	+
	Epicuticular striae	-	+	+
	Stomata in bristles	-	-	+
	Trichomes in one/both epidermis	+	+	-
	Glandular trichomes on furrows	+	-	-
	Ornamented trichomes	-	+	-
	Curved edges	+	+	-
	Kranz-type anatomy	-	-	+
	Aquifer Cells	+	+	+

Note: + for presence; - for absence.

Table 3. Histochemistry of underground systems and leaves of *Pterocaulon purpurascens* Malme, *Wedelia trichostephia* DC., and *Pectis gardneri* Baker

Storage	Reagent	Species					
		P. purpurascens Malme		*W. trichostephia* DC.		*Pectis gardneri* Baker	
		Xylopodia	Leaves	Xylopodia	Leaves	T. Roots	Leaves
Fenolics	Ferric trichloride	VR	CC	Absent	CC	PE	CC
Starch	Zinc-chloride iodide	Absent	CC	Absent	CC	PE, CP	CC
Lipids	Sudan IV	PE,VR	CC, GT	PE, GT, CP	GT	PE, CP	CC
Mucilage	Ruthenium red	VR	Absent	Absent	GT	Absent	Absent

Note: PE, Periderm; CP, Cortical Parenchyma; VR, Vascular Rays; CC, Chlorenchyma Cells; GT, Glandular Trichomes.

chlorenchyma possess bulky cells with thin anticlinal walls and a regular shape, which probably function in water storage, similar to those found in the underground system (Fig. 6B-C). Similarly to *P. purpurascens*, the midrib of *W. trichostephia* possess angular collenchyma with collateral vascular bundles (Fig. 6D), which in the chlorenchyma possess sheath extension, and also, bulky cells with thin anticlinal walls and a regular shape, with similar function in water storage, and the presence of large substomatic chambers (Fig. 6E-F). The mesophyll of *P. gardneri* has a hypodermis composed of a single layer of

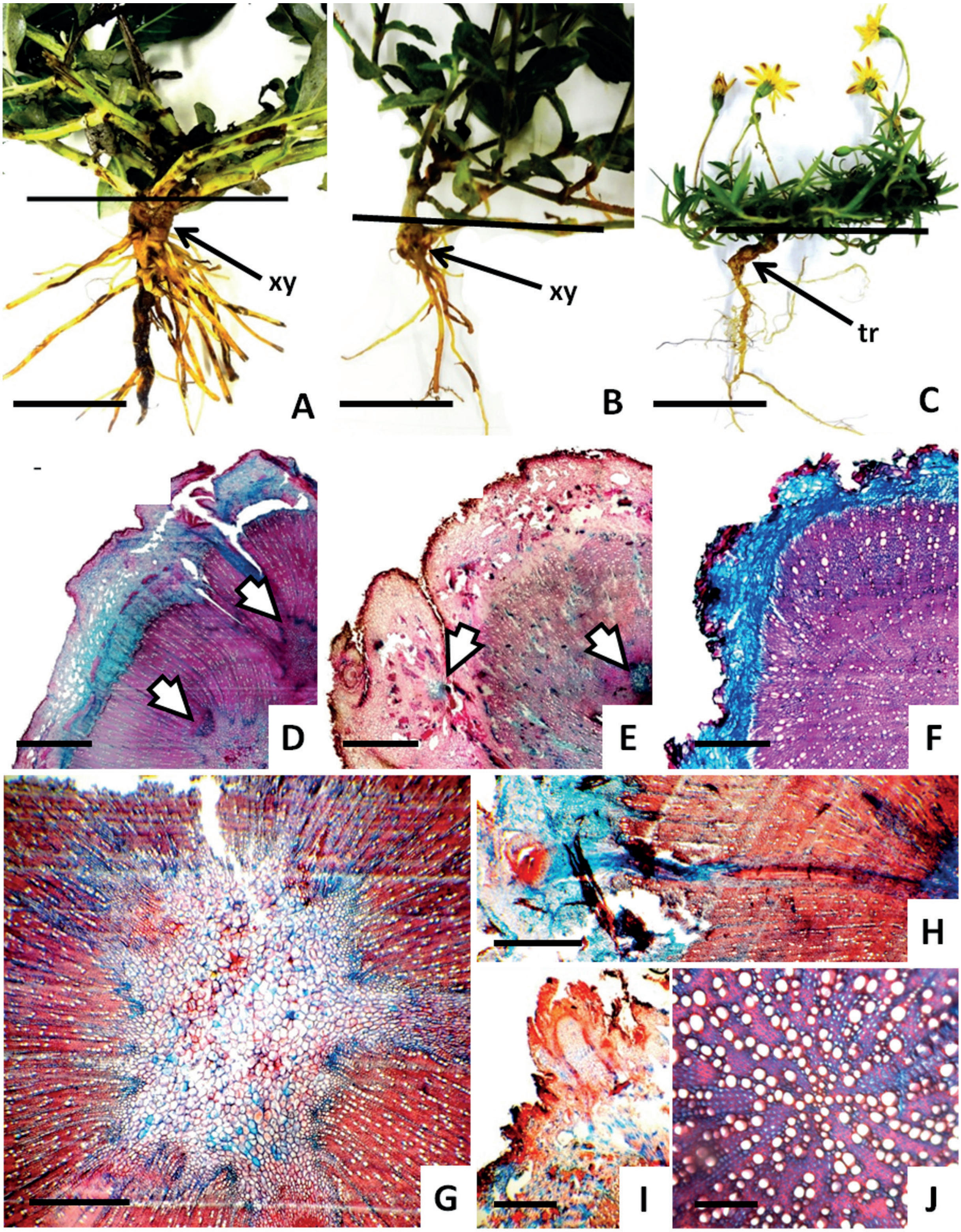

Figure 2. The morphological and anatomical structure of underground systems. Xylopodia (xy) of *Pterocaulon purpuracens* Malme (**A**) and *Wedelia trichostephia* DC. (**B**), and tuberous root (tr) of *Pectis gardneri* Baker (**C**), with lines indicating soil level and thin arrows indicating the sectioned region of each species. Cross-sections from *P. purpurascens* (**D**) and *W. trichostephia* (**E**), where thick arrows show self-grafting. Cross-section of *Pectis gardneri* exhibiting lignified parenchyma (**F**). Central portion of the xylopodium of *P. purpurascens* (**G**) exhibiting the direction of primary xylem. Both *P. purpurascens* (**H**) and *W. trichostephia* (**I**) exhibit buds originating from the vascular cambium. Storage parenchyma cells interspersed with highly lignified parenchyma cells in secondary xylem on *Pectis gardneri* (**J**). Scale bars: A-C=1 cm; D-E = 1mm; F=500 µm; G -J=100 µm.

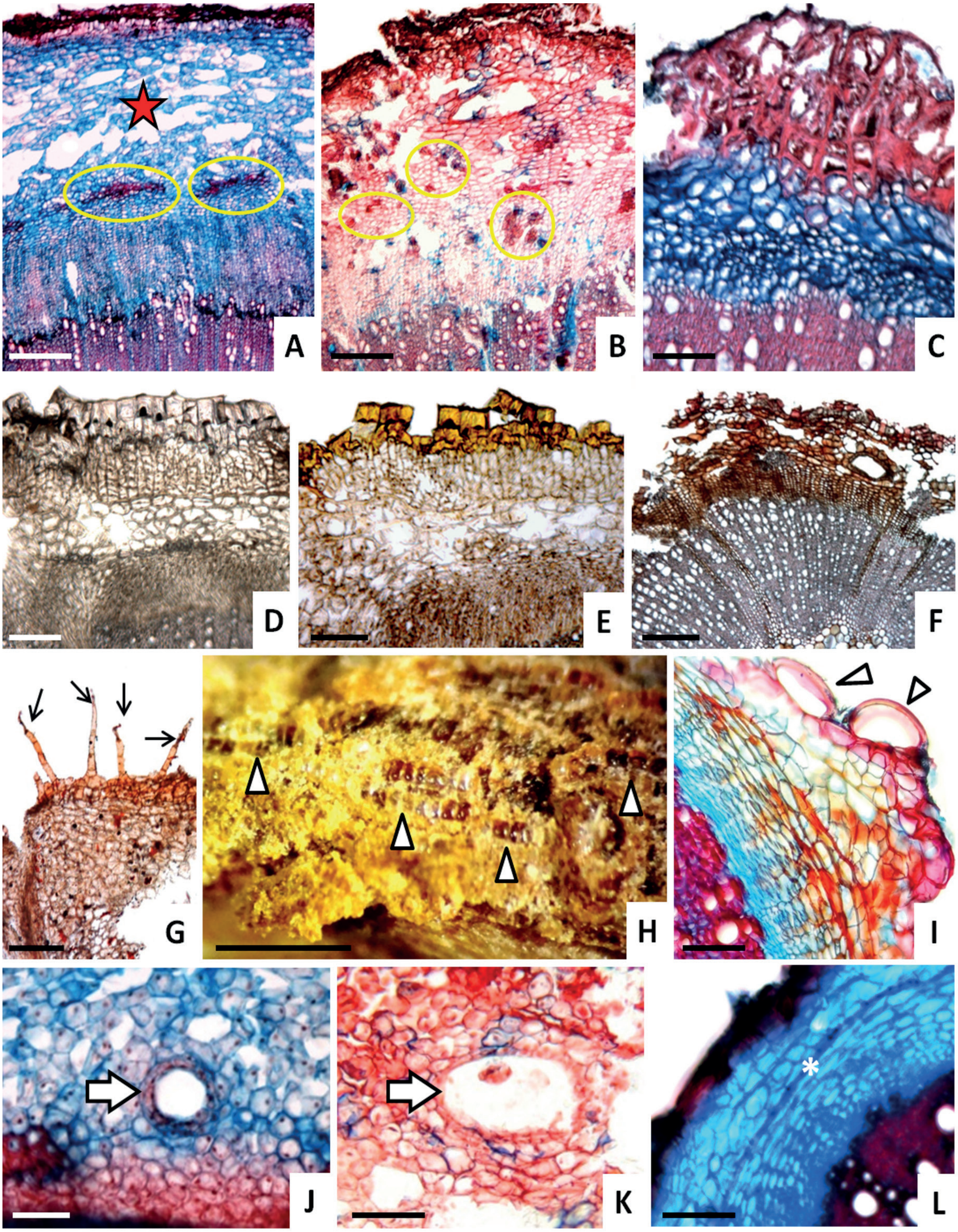

Figure 3. Main anatomical differences of the underground systems of the analyzed species. (**A**) *Pterocaulon purpurascens* with schyzogenous aerenchyma (red star), (**B**) *W. trichostephia* and (**C**) *Pectis gardneri*. Fiber clusters and sclereids (yellow ellipses) surrounding cortical parenchyma in (A) *P. purpurascens*, and isolated sclereids and/or fiber clusters (yellow circles) randomly distributed in (B) *W. trichostephia*. (**D-E**) Phenolic compounds in the periderm of *P. gardneri*, as evidenced by histochemical analysis, and the presence of starch grains in the outer layers of the cortical parenchyma. (**F**) Lipid storage in different tissues of *W. trichostephia*. (**G**) Persistent trichomes (thin arrows) in the periderm of *W. trichostephia*, with lipid storage as evidenced by histochemical analysis. (**H-I**) Longitudinal sequences of cells with highly thickened walls forming globular protrusions throughout the periderm of *P. gardneri* (arrow heads). Secretory ducts (thick arrows) in (**J**) *P. purpurascens* and (**K**) *W. trichostephia*. (**L**) Casparian strip (asterisk) in *P.gardneri*. Scale bars: A-G=100 µm, H=1mm, I=100 µm; JK=50 µm; L=100 µm.

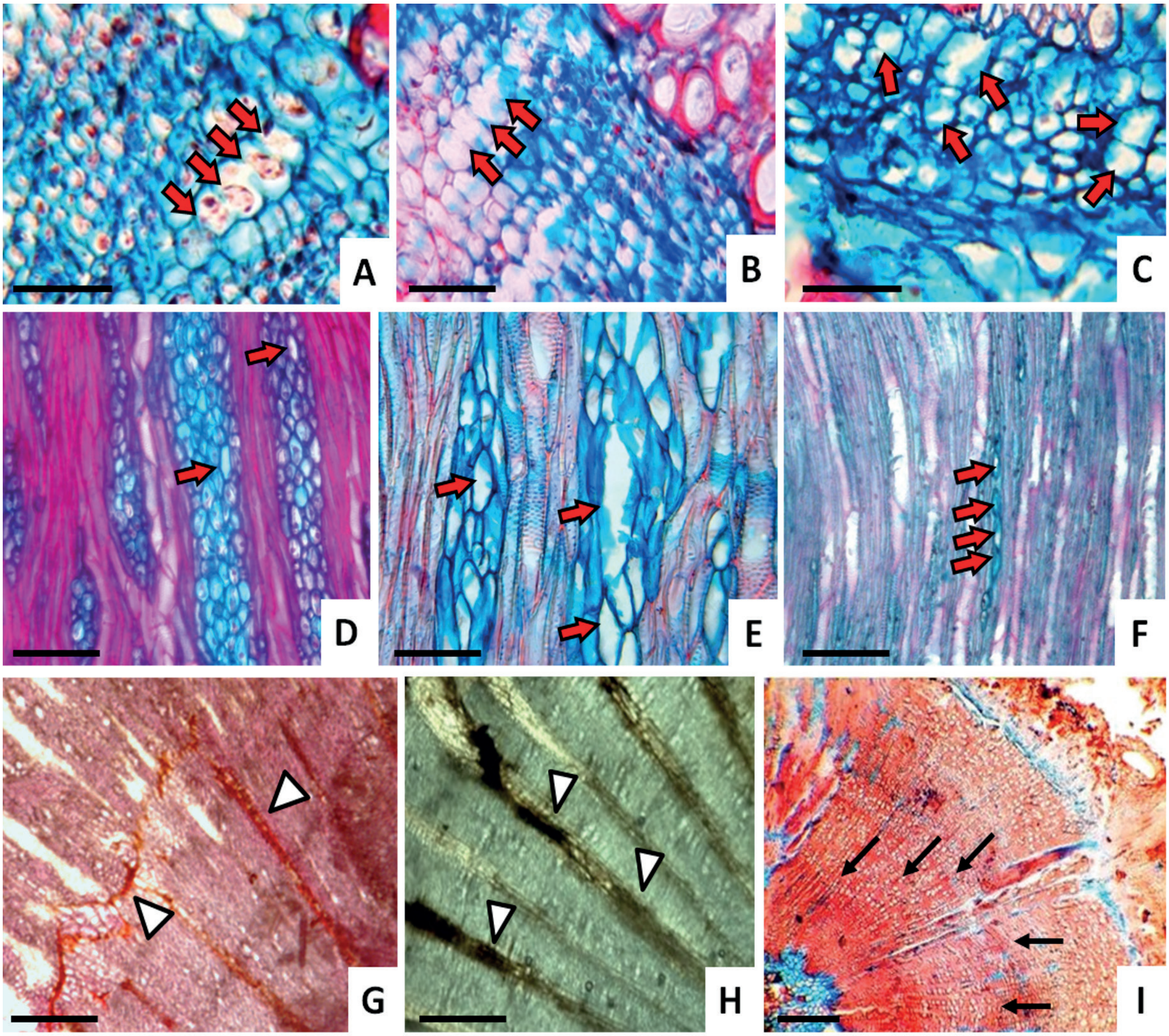

Figure 4. Structural differences in secondary phloem and xylem. Aquifer cells (red arrows) occurring on secondary phloem of (**A**) *P. purpurascens*, (**B**) *W. trichostephia*, and (**C**) *P. gardneri*. Longitudinal sections of vascular rays of (**D**) *P. purpurascens*, (**E**) *W. trichostephia*, and (**F**) *P. gardneri*, showing the occurrence of aquifer cells (red arrows). Histochemical analysis indicated storage of (**G**) mucilage and (**H**) phenolic compounds in vascular rays and cells of the vascular cambium of *P. purpurascens*. (**I**) Thin arrows indicate seasonal rings in *W. trichostephia*. Scale bars: A-C=50 µm; D-F=50 µm; G-I=100 µm.

bulky cells, which are probably aquifers, underneath which are secretory canals (Fig. 6G). This species also exhibits palisade parenchyma with a concentric arrangement around the vascular bundles, referred to as "*Kranz* type" anatomy, with a slightly irregular distribution, an interrupted pericycle internal to the mestome sheath (Fig. 6H) and schyzolysigenous secretory cavities (Fig. 6I).

Non-glandular trichomes of *P. purpurascens* are uni- to multicellular, non-branched, composed of 1-4 cells, and occur only on abaxial surface epidermis (see Fig. 6A-C), whereas the glandular trichomes are globular, restricted to furrows of the epidermis of both surfaces (but more common abaxially), predominantly composed of 2-3 pairs of secretory cells (Fig. 7A-B), and store lipids (Fig. 7C). The non-glandular trichomes of *W. trichostephia* are multiseriate, similar to emergences, and composed of a column of 3-5 stalk cells and 5-12 basal cells. They possess a thin apex and an oblong base with ornamented external periclinal walls (Fig. 7D), with mucilage and lipids storage (Fig. 7E-F; see Tab. 3), and seen mainly by SEM analysis (Fig. 7G). At the bases of the leaves of *P. gardneri*, 2-3 pairs of bristles are present, formed basically of lignified epidermal cells with a peculiar occurrence of stomata, as shown by SEM analysis (Fig. 7H-J). Epicuticular striae are visible in both *W. trichostephia* (Fig. 7G) and *P. gardneri* (Fig. 7I) by light microscopy but better by SEM analysis.

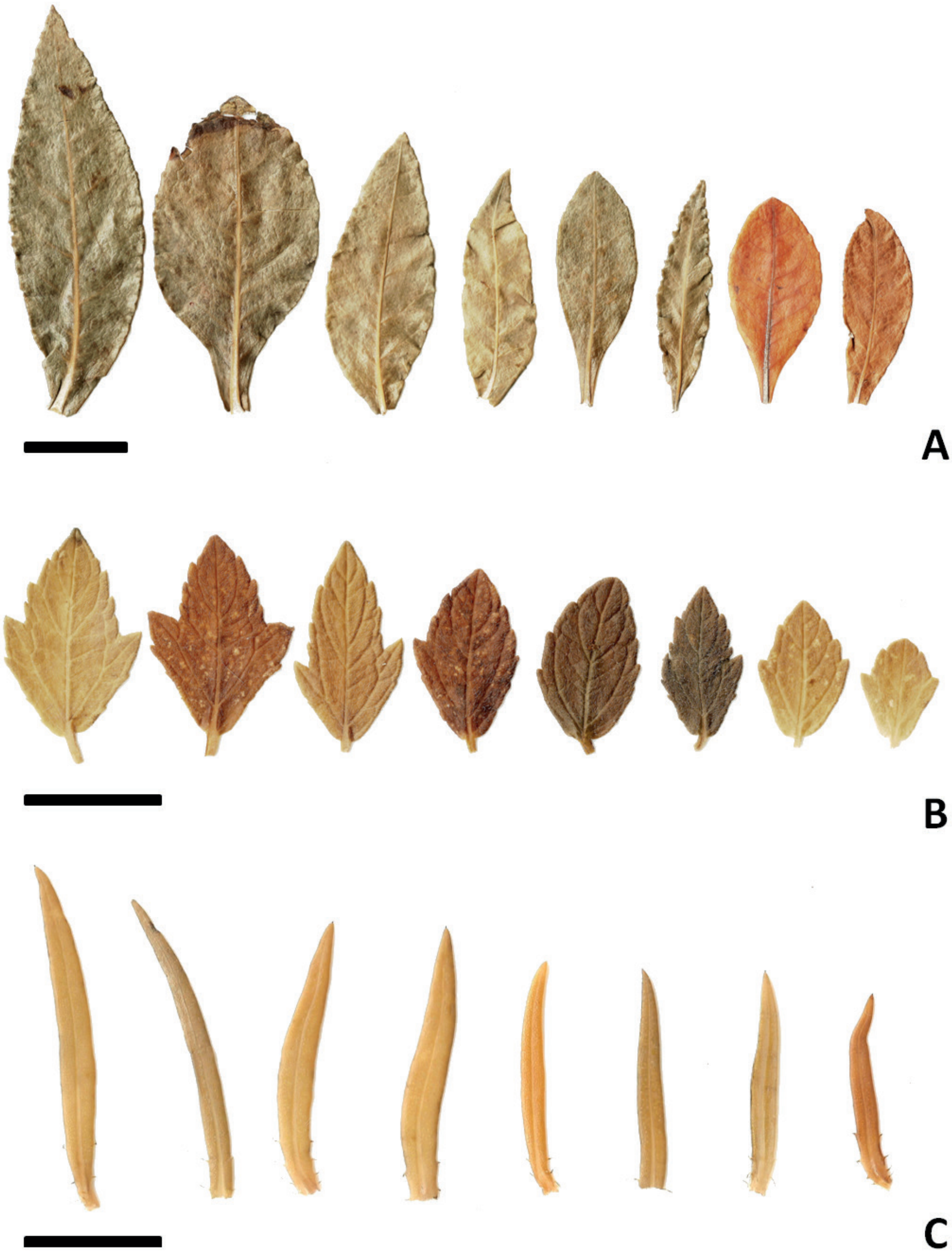

Figure 5. Morphological differences among perennial leaves of (**A**) *P. purpurascens*, (**B**) *W. trichostephia*, and (**C**) *Pectis gardneri*. Scale bars: A=0.5 cm; B, C=1 cm.

Discussion

Underground system anatomy

The classification of the underground systems of *P. purpurascens* and *W. trichostephia* as xylopodia was based mainly on the occurrence of self-grafting stem branches, their gemmiferous character and the absence of parenchyma storage cells on xylem components. On the other hand, the underground system of *P. gardneri* was classified as a tuberous root based on the presence of parenchyma storage cells distributed throughout secondary xylem, and the absence of self-grafting and a gemmiferous character (Appezzato-da-Glória 2015). Our results showed that

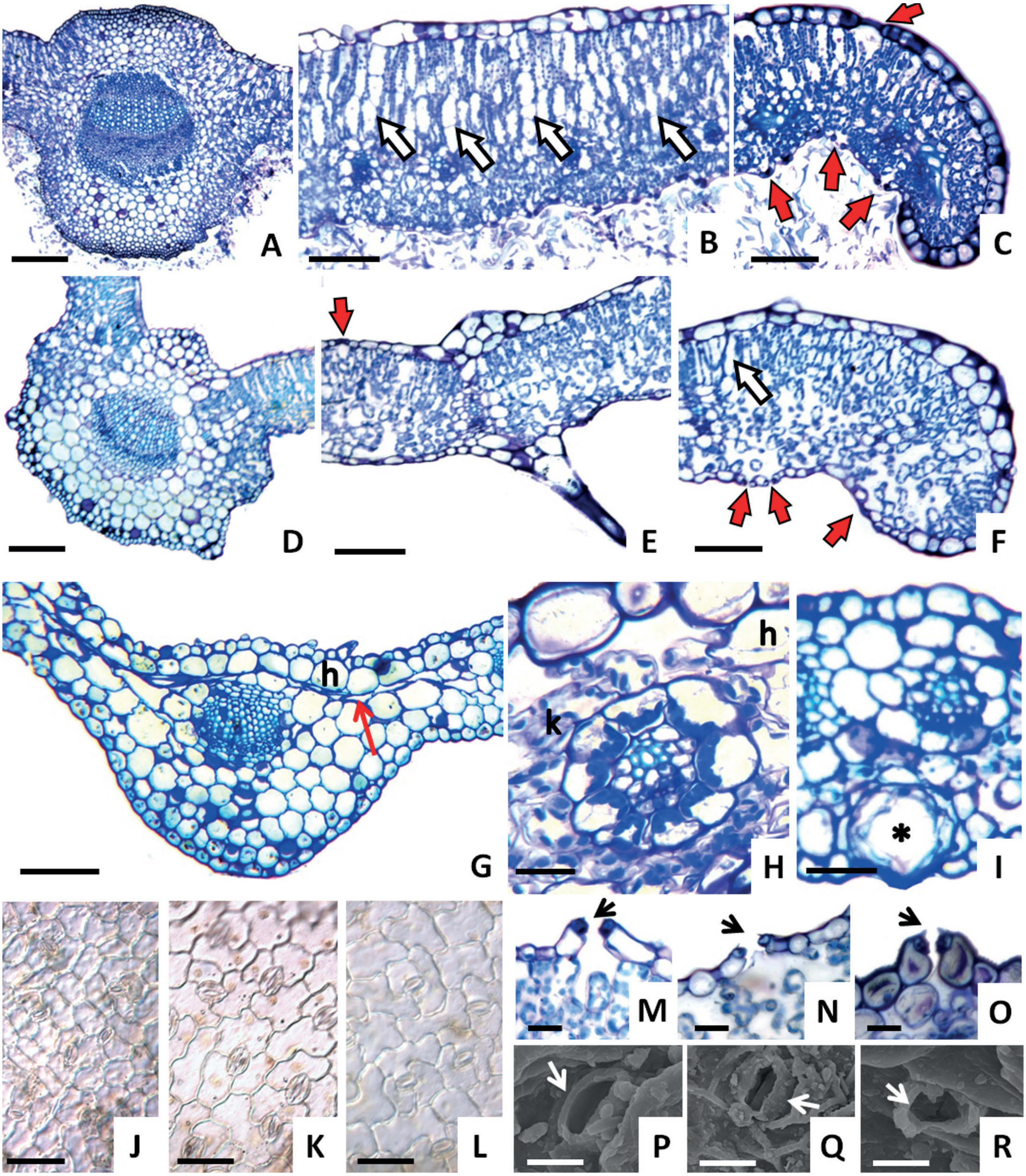

Figure 6. Anatomical differences among leaves. Cross-sections of the leaf blade of (**A-C**) *P. purpurascens*, (**D-F**) *W. trichostephia*, and (**G-I**) *P. gardneri*. (**A, D, G**) Midrib of (**A**) *P. purpurascens*, (**D**) *W. trichostephia*, and (**G**) *P. gardneri*. (**C, E-F**) Stomata occur on both surfaces (thick red arrows). (**G-I**) Mesophyll of *P. gardneri* showing a hypodermis (h) with a role in water storage, (**H**) "Kranz type" anatomy (k), and (**I**) schyzolysigenous secretory cavities (asterisk). Aquifer cells occurring in different tissues are indicated by thick white arrows. (**J-L**) Paradermic section of adaxial surface exhibiting anomocytic stomata in (J) *P. purpurascens*, (**K**) *W.trichostephia*, and (**L**) *Pectis gardneri*. Cross-sections (**M-O**) and SEM images (**P-R**) showing stomatal ridges impregnated with epicuticular waxes in (M, P) *P. purpurascens*, (**N, Q**) *W. trichostephia*, and (**O, R**) *P. gardneri* (black and white arrows). Scale bars: A-G=100 µm; H=50 µm; I-L=100 µm; M-O= 50 µm; P=5 µm; Q=10 µm; R=5 µm.

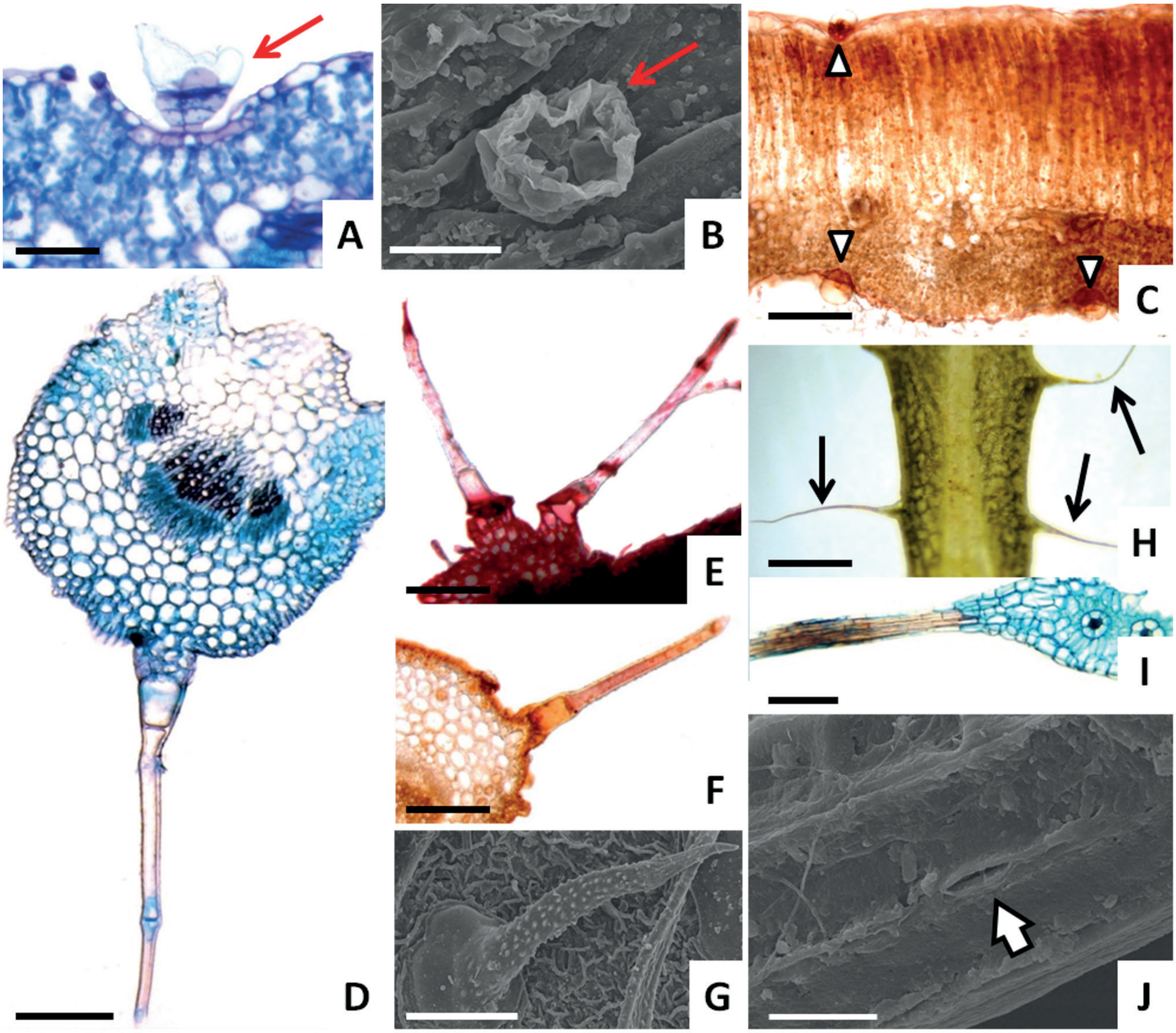

Figure 7. Epidermal appendages. (**A-B**) Glandular trichome (red arrow) in *P. purpurascens* restricted to epidermal furrows; (**A**) cross-section, and (**B**) SEM image. (**C**) Lipid storage (**D-G**) non-glandular trichome in *W. trichostephia*, similar to emergences, with ornamented external periclinal walls; (**D**) free-hand cross-section, (**E**) mucilage (**F**) lipid storage, and (**G**) SEM image. (**H-J**) Lignified bristles (black arrows) at the base of *P. gardneri* leaves; (**H**) morphology, (**I**) free-hand cross-section and (**J**) SEM image. Stoma (white arrow). Epicuticular striae occur in **(G)** *W. trichostephia* and **(J)** *P. gardneri*, visible by light microscopy but better shown by SEM. Scale bars: A=20 µm; B=10 µm; C-F=100 µm; G=50 µm; H=1mm; I=100 µm; J=10 µm.

underground systems of several types and origins are common in species of the Chaco, as previously described by Silva (2011) and Yule (2012) for species of Fabaceae. Thickened underground organs, such as xylopodia and tuberous roots, confer adaptive advantages for species that are capable of developing them. Xylopodia facilitate resprouting and restablishment after disturbance through budding activity, while tuberous roots mainly provide efficient water and fructan storage, which are involved in the distribution of energy to aerial organs between periods of dormancy and flowering (Rizzini & Heringer 1961; Appezzato-da-Glória *et al*. 2008a; Silva *et al*. 2014). We also infer that *P. gardneri* prioritizes the development of drought resistance features due to the occurrence of storage cells interspersed, along with highly lignified parenchyma cells, in the secondary xylem of its underground system.

Bud development, as seen in *P. purpurascens* and *W. trichostephia*, and their viability guarantee population persistence after seasonal disturbances, in addition to providing vegetative propagation by forming a persistent or seasonal bud bank, similar to what has been reported for several Brazilian Cerrado species (Klimešová & Klimeš 2007; Clarke *et al*. 2013; Moraes *et al*. 2016). Several functional traits, such as bud location and type, resprout type, resource location, and bud protection, among others described by Clarke *et al*. (2013), deserve further investigation in plants of different vegetative strata subjected to the restrictive climatic and edaphic characteristics of the Chaco.

In addition to regulating and mobilizing the organic and inorganic compounds, some strategies function directly in plant defense, (Levin 1973; Johnson 1975), such as trichomes with an accumulation of lipids, which are persistent even during the development of the periderm of *W. trichostephia*, and are probably senescing. Such structures may also be related to other strategies that aim to resist degradation and desiccation of subsequent tissues (Evert 2013), such as the phenolic compounds of the periderm of *P. gardneri*, as also reported for species of Asteraceae with tuberous roots that occur on rocky outcrops of the Brazilian Cerrado (Joaquim *et al*. 2014). The occurrence of longitudinal sequences of cells with highly thickened walls forming globular protrusions throughout extent of the periderm of *P. gardneri* seems to be a novelty for Asteraceae. We suggest that these are hypertrophied periderm cells resulting from extensive cellular differentiation in response to heat stress, which increases their production and volume, and accumulates them in layers. This response promotes thermal insulation because these hypertrophied cells are filled with air, and prevents invasion by pathogens and desiccation of subsequent tissues due to the thickened suberized wall, as proposed by Ginzberg *et al*. (2009), to Solanaceae species.

The Brazilian portion of the Chaco is located in domains of the Pantanal that, in addition to having saline soils, experience flooding on a lesser scale than the other domains of the wetland, but which are frequent during the rainy season (Prado 1993; Sartori 2012). In addition to stress caused by water deficit, strategies are also required to avoid and/or tolerate stress from flooding. Thus, the uncommon development of aerenchyma in the xylopodia of *P. purpurascens* constitutes an adaptive feature for environments with periodic flooding and salinity, since this structure can be associated with both saturated and/or saline soil (Fahn & Cutler 1992; Marschner 1995). This feature, discussed by Yule (2012) as occurring in the periderm of the underground system of *Neptunia pubescens* (Fabaceae), reflects the adaptive success of this species in inhabiting areas with periodical flooding.

The secondary xylem of the three species has similar features of vessel elements and perforation plates, which indicates similar strategies acting on water conduction efficiency, protection of conducting cells and resistance to water flow reduction (Carlquist & Hoekman 1985; Metcalfe & Chalk 1979; Alves & Angyalossy-Alfonso 2000). The occurrence of bulky aquifer cells in the uni- and multiseriate vascular rays of the three studied species, and the substance storage cells that also occur in this tissue in *P. purpurascens* and *W. trichostephia*, are evidence of the priority given to developing storage tissues rather than support tissues. This prioritization promotes resistance to high temperatures and consequent drought by maintaining efficient transport and water flow over short distances, thereby altering the osmotic equilibrium and resulting in water retention during seasons of water stress (Metcalfe & Chalk 1979; Fahn & Cutler 1992).

Studies carried out over the last two decades with species in different Brazilian phytophysiognomies have shown that several species with different types of underground systems develop features related to morphogenesis, proportion of storage tissue, storage location, and chemical structure of stored compounds, which provides a variety of different data useful in the identification and differentiation of species and families, and helps to elucidate their adaptive roles in leading to successful establishment in their respective environments (Appezzato-da-Glória & Estelita 1997; Milanez & Moraes-Dallaqua 2003; Appezzato-da-Glória *et al*. 2008b; Cury & Appezzato-da-Glória 2009; Appezzato-da-Glória *et al*. 2012; Oliveira *et al*. 2013; Bombo *et al*. 2014; Moraes *et al*. 2016). Such compounds, usually water-soluble carbohydrates, particularly fructans, and secondary metabolites, provide protection against desiccation and extreme temperatures, in addition to being an extremely useful energy source for underground systems bearing buds leading to resprouting and vegetative propagation (Klimešová & Klimeš 2007; Joaquim *et al*. 2014; Moraes *et al*. 2016).

The remarkable rainy and drought seasons of the Chaco interfere directly with growth and tissue arrangement of species subjected to this environment, with different cells undergoing substantial changes in volume with minimal variation in turgor, which reflects another strategy for water deficit (Holbrook 1995; Scholz *et al*. 2007). For example, the reduction of cell expansion and growth in *W. trihostephia* during periods of low water availability, combined with the stable volume of radial parenchyma cells and the consequent cambium inactivity, forms seasonal growth rings. Marcati *et al*. (2006) discussed the occurrence of seasonal growth rings in a variety of different Cerrado species, and concluded that it may be related to a distinct annual dry season; however, this feature is poorly understood for species of the Chaco and deserves further investigation into its adaptive value.

Leaf anatomy

Considering how severe the dry seasons of the Chaco are, the presence of perennial leaves stands out as contradictory to the widely reported pattern for plant species of the Cerrado and the Mediterranean, two environments that also have dry seasons but where non-succulent plants lose their leaves during stressful events of water deficit (Fisher & Turner 1978; Moraes *et al*. 2016). This apparent contradiction may reflect the ability of the evaluated tissues of the studied species to accumulate water. Similarly, the curved margins and trichomes on the abaxial surface of the leaves of *P. purpurascens* and *W. trichostephia*, serve as adaptations to dry environments by forming a wet barrier that interferes with evapotranspiration and influences water diffusion, thereby reducing the loss of water vapor from the mesophyll to the atmosphere, and increases reflection of solar radiation, thereby decreasing leaf temperature (Payne 1978; Fahn 1986; Fahn & Cutler 1992; Werker 2000; Evert 2013).

Some variant epidermal cell patterns, which are juxtaposed, prevent water loss by evaporation and aid cell resistance to physical rupture when subjected to constant drought, thus avoiding compression of internal tissues, which could reduce or cease photosynthesis (Fahn & Cutler 1992; Turner 1994; Larcher 2006). Stomatal ridges and epicuticular striae and waxes are important to the successful distribution of these species in seasonally dry environments because they increase water flow to the epidermal surface and hinder water and solute diffusion through the cuticle (Struwig *et al*. 2011; Evert 2013). The amphistomatic character of leaves of *P. purpurascens* differs from that described for other species occurring in seasonally dry environments and, allied with the aerenchyma in xylopodia, may reflect the main structural adaptations of this species to the seasonally dry Chaco environment, which is subjected to both stress from water deficit and excess in the dry and rainy seasons of the Pantanal, respectively.

The presence of a lignified sheath in *P. purpurascens* is an important adaptive characteristic because it provides efficient protection from high solar incidence and physiological drought (Fahn & Cutler 1992). In addition to this feature, the aquifer cells in the palisade parenchyma of *P. purpurascens*, which also occur in *W. trichostephia*, and the aquifer potential of the hypodermis of *P. gardneri*, have been poorly studied in plants of different Neotropical arid and semi-arid environments. These structures, being associated with water storage and transport, promote anatomical differences resulting from physiological adaptations at different levels, in addition to maintaining water potential in leaves and acting on water balance and photosynthesis (Fahn & Cutler 1992; Dettke & Milaneze-Gutierre 2008; Rossatto & Kolb 2010).

The Chaco is subjected to short periods of adequate water supply followed by long periods of water deficit and high temperature and solar incidence, which favors the establishment of xeromorphic plants with two main strategies: one aimed at decreasing photosynthesis and transpiration rates, and the other aimed at intensifying these two processes (Shields 1950; Fahn & Cutler 1992). The first strategy can be inferred from the presence of large substomatic chambers, such as in *W. trichostephia*, which establishes a superficial diffusion gradient between chlorenchyma and the environment, thus helping to reduce transpiration rates and promoting greater absorption of CO$_2$ into these intercellular spaces (Pickard 1981; Fahn & Cutler 1992; Roth-Nebelsick 2007). The second strategy is represented by plants with "*Kranz*-type" structure, such as *P. gardneri*, which are efficient in maximizing photosynthesis under intense light. Whereas this anatomical mechanism is quite diffuse throughout Asteraceae, mainly present in the genus *Pectis*, it also occurrs in *Isostigma*, *Chrysanthellum*, and *Flaveria* (Solereder 1908; Smith & Turner 1975; Peter & Katinas 2003; Muhaidat *et al*. 2007; Sage *et al*. 2011). As widely reported, C$_4$ plants have an adaptive advantage over C$_3$ plants, since their water usage is twice as efficient, and they possess specific genetic differences that affect leaf anatomy, ultrastructure, and metabolic and energetic pathways (Metcalfe & Chalk 1979; Fahn & Cutler 1992; Hibberd & Covshoff 2010).

Various trichomes and cells involved with the storage of mucilaginous and lipophilic substances, such as in *W. trichostephia*, are also associated with water regulation, mobility and retention in different tissues, conferring an adaptive value to water deficit, in addition to serving as a defense mechanism against herbivores (Levin 1973; Johnson 1975; Fahn 1979; Metcalfe & Chalk 1979; Werker 2000). The occurrence of glandular trichomes in epidermal furrows on both surfaces of the leaves of *P. purpurascens*, seems to have adaptive value in seasonally dry environments, similar to that described for *Mikania glomerata*, a species of Asteraceae from the Brazilian Cerrado (Milan *et al*. 2006). However, this feature is in need of further investigation among plants of the Chaco in order to confirm this adaptive value. The ornamented trichomes of *W. trichostephia* may aid in the reduction of water loss during stress from water deficit, as described by Gil *et al*. (2012) for *Wedelia buphthalmiflora*, a species from the Chaco.

Pectis gardneri exhibits bristles at the base of leaves, which are rigid due to impregnation with lignin, a taxonomic feature of the subtribe *Pectidinae* (Anderberg *et al*. 2007). The quite uncommon occurrence of stomata on this structure, along with the "Kranz-type" anatomy and reduced leaf area, favor transpiration and water balance and mobility. This feature represents the most effective adaptation this species exhibits, in addition to features of the underground storage system described herein, and is fundamental to successful establishment in the Chaco.

Conclusion

Information about the anatomical features of plants of the herbaceous-subshrub stratum in seasonally dry environments is lacking, particularly for those of the Chaco. The structural adaptive mechanisms (both morphological and anatomical) possessed by underground systems and leaves of *P. purpurascens*, *W. trichostephia* and *P. gardneri* are considered important for water regulation and maintenance, which was expected for species occurring in environments with water deficit. The underground systems and leaf cells and tissues described here corroborate previous descriptions of xeromorphic species, which, through various mechanisms, improve water uptake and storage and/or prevent excessive water loss during environmental and/or physiologically stressful periods. Thus, the occurrence of perennial leaves allied with the features of underground systems provide evidence of the presence of an efficient ecophysiological apparatus associated with resistance to water deficit, and is deserving of further investigation. These features could be useful on discussions about adaptive issues of plants native to arid and semi-arid environments that are influenced by marked seasonality, generally scarce water availability and variable extreme temperatures.

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