ANATOMY OF VEGETATIVE ORGANS IN *ALLIONIA* (NYCTAGINACEAE), WITH EMPHASIS ON THE VASCULAR SYSTEM

Israel L. Cunha Neto*, Juliana P. Silva, Veronica Angyalossy

Department of Botany, Institute of Bioscience
University of São Paulo, São Paulo, SP, 05508-090, SP, BRAZIL
israellopescn@gmail.com, israelneto@usp.br
Orcid: 0000-0002-0914-9974

ABSTRACT

Allionia is a small genus within the tribe Nyctagineae (Nyctaginaceae) which has a controversial, infrageneric delimitation. Here, we investigated the two known species of *Allionia* in order to characterize the anatomy of leaves, stems and roots, with further notes on vascular system development. Additionally, the present study aimed to broaden our knowledge of stem vascular diversity and to survey for anatomical features with diagnostic value in distinguishing *A. choisyi* from *A. incarnata*. Leaf anatomy of other Nyctagineae taxa was also analysed. Anatomical and ontogenetic observations from the vegetative organs in *Allionia* revealed no diagnostic features to distinguish the two species. We illustrated the occurrence of Kranz anatomy, which in Nyctaginaceae is only known in *Allionia, Boerhavia,* and *Okenia*. The stem primary vascular system was unusual in showing a polycyclic eustele (medullary bundles + continuous concentric procambium). Likewise, mature stems and roots show vascular cambial variants (successive cambia) that arise from the pericycle. The anatomy and histochemistry of multicellular glandular trichomes observed in aerial organs were presented. Raphids were seen in all organs. Although no strong xerophytic features were observed in *Allionia,* several characteristics can be associated with their arid habitats. Our findings on the vascular system of *Allionia* showed the two species to be much the same and reinforced earlier findings that the stem anatomy of Nyctaginaceae is complex and intriguing.

RESUMEN

Allionia es un género pequeño dentro de la tribu Nyctagineae (Nyctaginaceae) con delimitación infragenérica controversial. Analizamos las características anatómicas de hojas, tallos y raíces de las dos especies conocidas de *Allionia* e incluimos comentarios sobre el desarrollo del sistema vascular. El presente estudio pretende, examinar características diagnósticas entre *A. choisyi* y *A. incarnata* y de esta forma ampliar el conocimiento sobre la diversidad vascular del tallo. Adicionalmente, analizamos la anatomía foliar de otros taxa de Nyctagineae. Las observaciones anatómicas y ontogenéticas de los órganos vegetativos en *Allionia* no mostraron características diagnósticas que permitieran diferenciaran entre las dos especies. La anatomía Kranz para Nyctaginaceae, restringida únicamente a *Allionia, Boerhavia* y *Okenia* fue ilustrada. Presentamos la anatomía e histoquímica de tricomas glandulares multicelulares observados en órganos aéreos. El sistema vascular primario del tallo era incomum al mostrar un eustele policíclico (haces medulares + procambio concéntrico continuo). Así mismo, tallos y raíces maduras mostraron variantes cambiales vasculares (cambios sucesivos) que surgen en el periciclo. Todos los órganos presentaron rafidios. No fueron observadas características xerofíticas en *Allionia*, sin embargo, varias características pueden estar relacionadas con ambientes áridos. Estos hallazgos esclarecen y corroboran la complejidad anatómica de las especies de Nyctaginaceae, y muestran la intrigante diversidad de patrones anatómicos caulinares.

KEY WORDS: *Allionia choisyi, Allionia incarnata,* Caryophyllales, cambial variants, Nyctagineae, ontogeny

INTRODUCTION

Nyctaginaceae have about 30 genera and 400 species which include trees, shrubs, subshrubs, lianas and herbs (Douglas & Manos 2007; Douglas & Spellenberg 2010; Hernández-Ledesma et al. 2015). The species are distributed mostly in the tropics and subtropics of the New World, except for some genera that occur in the Old World (e.g., *Boerhavia, Commicarpus, Pisonia*, *Phaeoptilum,* and *Mirabilis)* (Hernández-Ledesma et al. 2015). In the most recent classification, the family has been divided into 7 tribes: Nyctagineae, Boldoeae, Leucastereae, Bougainvilleeae, Pisonieae, Colignonieae, and Caribeeae (Douglas & Spellenberg 2010).

Allionia L. belongs to tribe Nyctagineae and comprises species of annual or perennial herbs with procumbent, decumbent or prostrate stems (Fig. 1). Two species are recognized *A. choisyi* Standl. and *A. incarnata* L. which are very similar morphologically, differing only in some fruit characteristics (e.g., number of lateral expansions, length of glands) (Spellenberg 2003), which makes the delimitation of infrageneric categories

J. Bot. Res. Inst. Texas 14(2): 373 – 394. 2020
https://doi.org/10.17348/jbrit.v14.i2.1016

Fig. 1. Specimens of *Allionia incarnata* in their natural habitat. **1a–c.** Chihuahuan Desert, New Mexico, USA. **1a.** Overview of the environment with plants growing on the ground (notice the pinkish dots from the inflorescences). **1b.** Prostrate habit, leaves and inflorescence. **1c.** Details of the inflorescence; **1d–f.** Parque Nacional Amboró, Bolivia. **1d.** Environment and habit. **1e.** Details of leaves and inflorescence buds. **1f.** Detail of primary root. Photographs: Israel L. Cunha Neto.

controversial. In general, most authors have followed Standley's work (1931) and accepted *Allionia* as having two species (Phillips 1976; Bittrich & Kühn 1993; Turner 1994; Hernandez-Ledesma & Olvera 2003; Spellenberg 2003; López & Anton 2006; Hernandez-Ledesma et al. 2015; Sandoval-Ortega et al. 2020), whereas other studies have treated the genus consisting of one variable species (Heimerl 1932; Fay 1980; Rzedowski & Rzedowski 2001; Spellenberg 2012). The two species grow in a variety of habitats, mostly in warmer, xeric regions of North America (southwestern United States of America and northern Mexico)—

including on gypsum soils (Waterfall 1946; Alexander et al. 2014)—and South America (Argentina, Bolivia, Venezuela) (Phillips 1976; Bittrich & Kühn 1993; Turner 1994). The species have been found also in the Antilles (Turner 1994; Hernandez-Ledesma & Olvera 2003; Douglas & Manos 2007).

Anatomical information on *Allionia* is almost entirely restricted to the dissertation of Phillips (1976) entitled "Anatomy and developmental morphology of *Allionia* L. (Nyctaginaceae)." This work sought to understand the structural aspects of reproductive and vegetative organs, among other biological aspects of the genus. Although Phillips (1976) had described several anatomical features in *Allionia,* the taxon sampling was restricted to specimens from North America. Over the years, additional ontogenetic data obtained for the family has promoted interest in investigating the stem vascular system within Nyctaginaceae due to its remarkable anatomical diversity. Whether the various vascular architectures are associated with their different habits and habitats are still uncertain. We here present further information on the anatomy and development of *Allionia,* a poorly studied group within the herbaceous representatives of the Nyctaginaceae, enhancing our understanding on the developmental aspects of vascular characteristics in the family.

Many herbs produce secondary tissues making possible the study of the "wood" (secondary xylem) and "bark" (secondary phloem + periderm) (Schweingruber 2011; Carlquist 2012; Dória et al. 2018). Interestingly, several of these plants have anatomies known as vascular cambial variants (i.e., alternative patterns of secondary growth) that are found in adult stems and/or roots of species within different families across eudicots (Schweingruber et al. 2011). This is the case of *Allionia* and other species within Nyctaginaceae, such as *Boerhavia* and *Mirabilis* (Phillips 1976; Rajput & Rao 1998; Rajput et al. 2009; Hernández-Ledesma et al. 2011). In addition, most Nyctaginaceae species (including *Allionia*) present medullary bundles as a major component of their stem, resulting in an increase in the complexity and diversity of primary vascular system within the family (Cunha Neto et al. 2020).

Here, we revisit the anatomy of vegetative organs in *Allionia* emphasizing the aspects of the stem vascular system. We studied the two currently recognized species *A. incarnata* and *A. choisyi* aiming to assess the uncertainty on the infrageneric status of the genus. In addition, the leaves of other representatives of Nyctagineae were studied and compared with the anatomical features in *Allionia.*

MATERIAL AND METHODS

Taxon sampling and material collection

In this study, the taxon sampling covered the two main distribution regions of the genus, emphasizing specimens from South America that were not investigated by Phillips (1976). Samples from *Allionia incarnata* L. with procumbent habit were collected in their natural habitats both in North and South America. For *Allionia choisyi* Standl., we obtained only one stem sample from an herbarium voucher (Table 1). Additional information for the species was obtained from Phillips (1976). See Appendix 1 for other species studied.

Each specimen collected from natural population consisted of whole plants, including root and shoots with stems, leaves, and inflorescences. Samples from these plants were fixed in FAA 70 (formaldehyde–acetic acid–ethanol) for 24 h (Johansen 1940) or in 70% isopropanol, and then transferred to 70% ethyl alcohol.

Anatomical procedures for light microscopic

Samples from different ontogenetic stages of each organ were selected. We used fully expanded leaves which were trimmed to obtain the middle portion of the petiole, midrib, and leaf margin. From stems we selected samples near the apex, middle, and base in order to ensure that all developmental stages of vascular system ontogeny would be sampled. Similarly, primary and secondary roots were selected for the study.

Samples of leaves and young stems and roots were dehydrated either in an ethanol–t-butanol series and embedded in paraplast (Fisher Healthcare, Houston, Texas, USA) or dehydrated in ethanol series and embedded in Historesin (Leica; LeicaMicrosystems, Heidelberg, Germany) (Johansen 1940; Ruzin 1999). These samples were sectioned in a rotary microtome (Leica RM2145, Nussloch, Eisfeld, Germany), typically 3–10 µm thick, and stained with 1.5% alcoholic safranin O and 1% aqueous Astra blue (Gerlach 1969) or stained with toluidine blue (O'Brien et al. 1964), respectively.

Table 1. List of species analysed with information on environment, locality, collector, collector numbers, and herbaria where vouchers were deposited.

Taxon	Environment	Locality	Collector (herbarium)
Allionia choisyi Standl.	Chihuahuan Desert	Mesilla Valley, New Mexico, USA	US 498327
Allionia incarnata L.	Valles secos	Parque Nacional Amboró, Santa Cruz, Bolívia	Nee, MH 64124–64126 (USZ)
	Chihuahuan Desert	Luna County, New Mexico, USA	Douglas, NA 2292 (FLAS)
	Chihuahuan Desert	Malone Mountains, Sierra Blanca, Texas, USA	Douglas, NA 2276 (FLAS)

Stems of *Allionia choisyi* from dried herbarium vouchers were rehydrated in distilled water until submersion, dehydrated in ethylic series and then included in Historesin (Leica; LeicaMicrosystems, Heidelberg, Germany) and stained with toluidine blue (O'Brien et al. 1964).

Adult stem and roots were embedded in polyethylene glycol 1500 (Rupp 1964) and sectioned in transverse, longitudinal radial, and longitudinal tangential planes with a sliding microtome (Leica SM2010R, Nussloch, Eisfeld, Germany) with the aid of a styrofoam resin (Barbosa et al. 2010). These anatomical sections were double stained with safrablau (Kraus & Arduin 1997) or safranin and alcian blue (Johansen 1940).

All the sections were mounted on permanent slides with synthetic resin (Permount; Fisher Scientific, Fair Lawn, NJ).

In order to characterize the morphology of each cell type we performed macerations using Jeffrey's solution (10% aqueous nitric acid + 10% aqueous chromic acid; Johansen 1940) and mounted slides in 50% glycerine. Macerations were performed independently for the secondary xylem and secondary phloem from samples near the cambium and for the pith. The samples were obtained from adult stems.

Histochemical analyses and diaphanization

Histochemical tests were performed on hand-free sectioned samples of the stem of *Allionia incarnata* (*Douglas 22* and *Nee 61124*), as follow: Sudan IV and Sudan Black B (Johansen 1940; Pearse 1985) to detect total lipids; Nile Blue (Cain 1947) to detect neutral and acidic lipids; ruthenium red (Gregory & Baas 1989) to detect pectates and acidic mucilage; ferric chloride to identify phenolic compounds (Johansen 1940); NADI reagent for terpenoids (David & Carde 1964), Aniline Blue Black for proteins (Fisher 1968) and lugol to detect starch (Johansen 1940). Standard control procedures were carried out as required for each test, and the sections were mounted between slides and cover slips with Kaiser's jelly glycerin (Kraus & Arduin 1997).

Fully expanded and intact leaves were treated in 5% sodium hydroxide, washed in distilled water, then cleared in sodium hypoclorite and stained with 1% aqueous safranin (Kraus & Arduin 1997). After clearing, the leaves were cut into small pieces and mounted with glycerinated gelatin.

All slides were analysed using a Leica DMBL light microscope coupled with a digital camera (Leica DFC310, Leica Microsystems, Wetzlar, Germany).

Anatomical descriptions

Anatomical descriptions for secondary xylem and secondary phloem followed the International Association of Wood Anatomists's guides, including the "IAWA List of Microscopic Features for Hardwood Identification" (IAWA Committee 1989) and the "IAWA List of Microscopic Bark Features" (Angyalossy et al. 2016), respectively. All measurements were performed for both regular secondary xylem and phloem and for the medullary bundles. We measured the diameter, length, frequency, and occupied area for vessels, sieve-tube elements, and fibers. The wall thickness of fibers was also calculated. The cell frequencies were calculated within a grid of 0.1 mm^2 for the xylem and 0.01 for the phloem. The occupied areas were calculated for mature xylem and phloem using 50 points per analysis (adapted from Ziemińska et al. 2015). All measurements were performed using the free software ImageJ (ver. 1.45s; Rasband 2012), with a minimum of 30 repetitions.

RESULTS

Here we describe the anatomical aspects leaves, stems, and roots, with emphasis on the structure and ontogeny of the vascular system in the stem. The anatomical description below refers to both *A. incarnata* and *A. choisyi*.

Leaf anatomy

The epidermis shows polygonal cells with straight anticlinal walls on both surfaces (Fig. 2a–b). The leaves are amphistomatic (Fig. 2c, e) with anomocytic stomata that are scattered on the leaf blade (Fig. 2a–b). In cross section, the stomata are disposed at the same level of the ordinary epidermal cells and are underlined by a notorious substomatal chamber (Fig. 2c, e). Multicellular and uniseriate glandular trichomes occur on both surfaces of the leaves (Fig. 2g).

The epidermis is uniseriate and composed by cells with roundish or polygonal contour and variable sizes in cross view (Fig. 2c, e, 3a–d). The mesophyll is dorsiventral (bifacial), usually with two layers of palisade parenchyma, and lacunose parenchyma characterized by roundish cells of various sizes and wide intercellular spaces (Fig. 2c–d, 3a, c, d). Generally, one layer of longer palisade cells occurs toward the adaxial epidermis, while another layer of shorter cells occurs below the vascular system, which occurs in the middle of the mesophyll (Fig. 2c–e, 3a–d). The same arrangement is observed in the mesophyll near the leaf blade margin (Fig. 3d). Leaf blade margins with smaller and round cells throughout the mesophyll were also observed in one specimen collected in Bolivia (Fig. 3b). The leaf blade margins are straight (right edges) in all specimens (Fig. 3b, d).

The vascular system is composed of collateral bundles with xylem toward the adaxial surface and phloem toward the abaxial surface (Fig. 2e). The bundles are surrounded by large sheath cells containing several chloroplasts characteristic of Kranz anatomy (Fig. 2c–e, 3a–d). Within the tribe, similar anatomy is observed only in other two genera, *Okenia* and *Boerhavia* (Fig. 2c–e, 4a–h). Cells containing crystals (raphids) are found throughout the mesophyll (Fig. 2f, 3a–b).

The midrib is prominent with larger portion toward the abaxial surface (Fig. 5a, c). The epidermal cells are similar to the ones in the leaf blade (Fig. 5a, c). A few collenchyma cells occur in subepidermal layers immediately beneath the adaxial epidermis (Fig. 5c). The mesophyll is composed of round parenchymatic cells of various sizes (Fig. 5a, c). The vascular system is formed by a single unit which eventually may divide into two or more collateral bundles (Fig. 5a, c, 6a). There are one to three accessory bundles on the adaxial side of the main vascular system (Fig. 6a).

The petiole is roundish or slightly plane-convex in cross section (Fig. 5b, d). The epidermis is uniseriate and contains multicellular uniseriate glandular trichomes (Fig. 5b, d). The cortex is composed of round parenchymatic cells (Fig. 5b, d). The vascular system is formed by four or five collateral vascular bundles (Fig. 5b, d, 6b).

Stem anatomy

Early stages of development.—In early developmental stages, the epidermis of the young stem is uniseriate with both multicellular, unbranched non-glandular trichomes and multicellular, uniseriate glandular trichomes (Fig. 7a). Initially, the cortex is composed of large and round parenchyma cells (Fig. 7a) which later become flattened (Fig. 7b). The primary vascular system consists of six to eight medullary bundles—collateral vascular bundles located in the pith—organized in a ring and encircled by a continuous concentric procambium (CCP) (Fig. 7a, b). The CCP produces a ring of collateral vascular bundles externally to the medullary bundles and internally to the starch sheath (endodermis) (Fig. 7a, c-d). This way the CCP can be divided into a fascicular and an interfascicular portion. The pith is parenchymatous with large round cells that can store starch (Fig. 7a, b).

Secondary vascular system and cambial variants.—The beginning of secondary growth is marked by the establishment of the cambium, and lignification of peripheral pith cells (Fig 7b, c). The cambium is formed by the fascicular and interfascicular cambium, which are derived from the fascicular and interfascicular regions

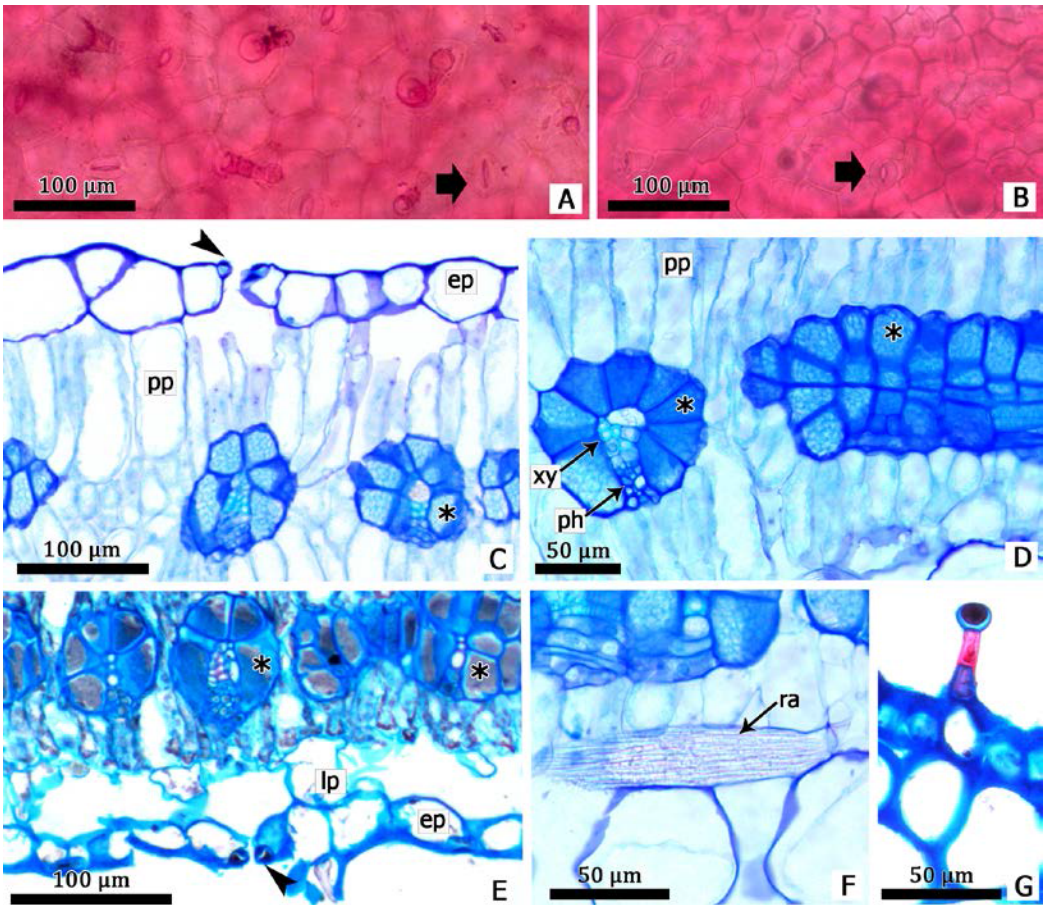

Fig. 2. Anatomy of leaf blade in *Allionia incarnata*. **2a–b.** Leaf surface showing epidermal cells and anomocytic stomata. **2c.** Adaxial surface with stomata (arrowhead). **2d.** Bundle sheath cells (asterisks) forming Kranz anatomy. **2e.** Abaxial surface with stomata (arrowhead). **2f.** Crystals (raphides). **2g.** Uniseriate glandular trichome. *Asterisks,* bundle sheath cells; *ep,* epidermis; *lp,* lacunose parenchyma; *pp,* palisade parenchyma; *ra*, raphides.

of the CCP. In addition to the residual cells of the CCP, pericyclic cells may also contribute to the formation of the interfascicular cambium. Initially, fascicular cambium forms secondary xylem centripetally and secondary phloem centrifugally containing the vessels and sieve-tube elements, respectively (Fig. 7b). On the other hand, the interfascicular cambium produces mainly xylem fibers internally, while the external cells remain parenchymatic (Fig. 7b). Later, conducting cells (i.e., vessels and sieve-tube elements) are also formed by the interfascicular cambium (Fig. 7b, c).

After a period of secondary growth, the pericyclic parenchyma located between the starch sheath and the primary phloem, undergoes periclinal divisions giving rise to a new meristematic zone. From this meristem, several tangential segments of new cambia differentiate along the stem circumference (Fig. 7d, e). Initially, the new cambia produce fibers and few vessels internally, whereas parenchyma and sieve-tube elements are formed externally (Fig. 7e, 8a–c). Between the new vascular increments and the regular cylinder some cells remain parenchymatic forming a conjunctive tissue (Fig. 8c). This pattern of secondary growth characterizes the stems of *Allionia* as having successive cambia (Fig. 9a) of which, however, from all analysed samples only one complete ring of new cambium was observed (Fig. 9a).

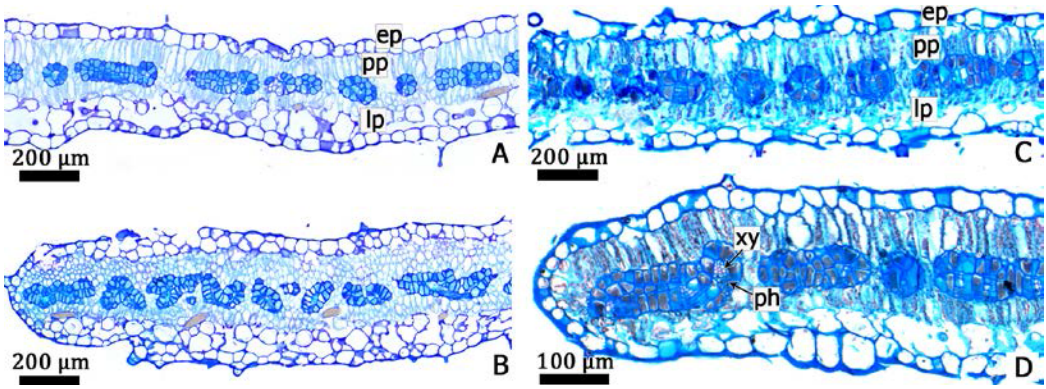

Fig. 3. **Cross section of the leaf blade in *Allionia incarnata*. 3a–b.** Specimens of *A. incarnata* collected in Bolivia, Parque Nacional Amboró. **3c–d.** Specimens of *A. incarnata* collected in the Chihuahuan Desert, New Mexico, USA. **3a, 3c.** Leaf blade showing palisade parenchyma (one layer above and other below the vascular system), and one to two layers of lacunose parenchyma. **3b, 3d.** Straight leaf margins showing round cells in **3b,** and elongated palisade-like cells in **3d**. *ep,* epidermis; *lp, lacunose* parenchyma; *ph,* phloem; *pp,* palisade parenchyma; *xy,* xylem.

Qualitative and quantitative characteristics of wood and bark.—In adult stems, a periderm (the secondary protective tissues formed by the rise of the phellogen and its derivatives, which can replace the epidermis) was not observed (Fig. 9a). The cortex undergoes a dilatation process due to numerous cell divisions in periclinal and anticlinal planes (Fig. 9a).

In the vascular system, the conducting elements are usually confined to small areas of the vascular cambium, with sieve-tube elements opposing the vessels (Fig. 7b, e, 9a). The sieve-tube elements are diffuse, each one associated with a single companion cell (Fig. 7b). Sclerenchyma was not observed in the phloem. In the secondary xylem, vessels are diffuse or radially arranged (Fig. 7b–e). Vessels are solitary or in multiples, predominantly 2–4 (Fig. 7b–d). Fibers are non-septate, fusiform or with slightly straight ends, and with large lumen (Fig. 8d-e). Axial parenchyma is diffuse and represented by two to four cells per parenchyma strand (Fig. 8d). Regular rays were not observed although some regions had some radially elongated cells forming a ray-like structure—radially elongated cells in cross section (Fig. 7e). The quantitative anatomical features of both xylem and phloem are described in Table 2.

Root anatomy

In early developmental stages, the epidermis is replaced by the periderm that arises at subepidermal layers (Fig. 10a). The cortical region possesses several cells containing raphides (Fig. 10a–c). The root is diarch—with two protoxylem poles (Fig. 10a). The secondary growth is initiated by the formation of a regular vascular cambium (Fig. 10b) that produces secondary xylem and secondary phloem in the usual manner (Fig. 10b–d). Soon, a new cambium arises from the pericyclic parenchyma, forming a new ring of vascular tissue (Fig. 10c). This new cambium produces variant xylem centripetally and variant phloem centrifugally (Fig. 10c, e). Between the central cylinder and the first ring of successive cambia, some cells remain parenchymatic forming the conjunctive tissue (Fig. 10c). Subsequently, new increments are formed from remaining pericyclic parenchyma cells outside the first ring (Fig. 11a–c). This process repeats a few times producing a thick root with several increments of new cambia at maturity (Fig. 11a–c).

Anatomy and histochemistry of glandular trichomes.—Multicellular uniseriate glandular trichomes are found in both leaves and stems (Fig. 2g, 7a). There are small and large trichomes with thick walls (Fig. 12a). Trichome heads stain darkly with safranin (Fig. 2g). In fixed and non-stained sections, the secretion produced by the trichome head is greenish in colour and is on the exterior of the head (Fig. 12a). The presence of lipids was detected by the positive reactions to Sudan IV, Sudan Black and Nile Blue (Fig. 12b–d). Lipophilic

substances were also identified, such as mucilage detected by Ruthenium Red (Fig. 12e) and proteins detected by Aniline Blue Black (Fig. 12f). The Lugol reagent reacted positively only in the starch sheath (Fig. 12g).

DISCUSSION

Taxonomic and systematic notes.—Here we performed a comparative anatomical analysis of the two recognized species of *Allionia*. Our results showed that the vegetative anatomy of *Allionia* species is remarkably similar, and diagnostic features to distinguish the species are lacking. Although previous studies have shown the value of characteristics of the vascular system for the distinction of similar species in Nyctaginaceae (e.g., arrangement of medullary bundles in the genus *Pisoniella*—Cunha Neto et al. 2020), we found that most of the variation in the anatomy of *Allionia* species is seen in size, number of cells or amount of tissue rather than qualitative differences. Similar observations have been proposed for external vegetative and reproductive morphology (Spellenberg 2003). Moreover, preliminary molecular data do not support the distinction between the two species, but additional loci and populations will need to be sampled to confirm this (Emily Humphries and Michael J. Moore, pers. comm.).

Anatomical and developmental aspects.—Leaf characteristics observed in *Allionia* confirm previous results by Phillips (1976) and is consistent with that of other members of the family (Metcalfe & Chalk 1950; Bittrich & Kühn 1993; Struwig et al. 2011). A striking feature found in *Allionia* is the occurrence of Kranz anatomy. In Nyctaginaceae, Kranz anatomy has been reported also in *Okenia* and some species of *Boerhavia*, all belonging to Nyctagineae (Carolin et al. 1978; Bittrich & Kühn 1993; Struwig et al. 2011; this study). Although the sheath cells are easily recognized in these taxa, they do not have a second layer of radially elongated cells, as firstly described by Haberlandt (1882) (*Kranz* = crown, in German]. In *Allionia,* the sheath cells are larger and round without intercellular spaces, while the neighbouring cells resemble regular palisade or lacunose parenchyma. These characteristics observed in *Allionia, Boerhavia,* and *Okenia* are similar to descriptions for other Caryophyllales, but variation in Kranz anatomy is also observed in some taxa which may have important physiological, ecological and systematic significance (Carolin et al. 1978; Antonucci 2010; Voznesenskaya et al. 2010; Ocampo et al. 2013). According to Antonucci (2010), who investigated the leaf development and ultrastructure of leaves in *Gomphrena* (Amaranthaceae) species, the sheath cells were identified as the inner layer of the mesophyll, that is, the endodermis (or starch sheath).

Among the specimens analysed, the only notable difference was found in the shape of margin cells of the leaf mesophyll (round vs. palisade). However, the round pattern was restricted to a single specimen, and such leaf characteristics are known to be strongly influenced by growing conditions, especially light intensity, water availability, temperature, and nutrient supply (Dickinson 2000; Chen et al. 2010).

The leaves, stems, and anthocarps of *Allionia* are covered with trichomes that release a viscid exudate. According to Spellenberg (2003) these glands remain sticky in specimens of Nyctaginaceae for decades in the herbarium. In the shoot of *Allionia,* these structures are glandular multicellular trichomes that were shown to produce an exudate composed by both hydrophobic and hydrophilic substances. The occurrence of secretory structures producing sticky substances has been observed in Nyctaginaceae by different authors (Willson & Spellenberg 1977; Spellenberg 2003; Struwig et al. 2011; Cunha Neto et al. 2019; Sukhorukov et al., in press). In the genus *Anulocaulis*, these structures are unicellular trichomes constituting secretory rings found in each stem internode, which secrete a complex exudate (Cunha Neto et al. 2019). In *Boerhavia* and *Commicarpus,* they are multicellular and uniseriate (Struwig et al. 2011), as observed in *Allionia.* Such trichomes or similar structures producing sticky substances are also present on the fruits of some species (Struwig et al. 2011; pers. obs.; Sukhorukov et al., in press). In these cases, there is evidence that the sticky substances might facilitate the anchoring of the diaspore on substrates or facilitate epizoochory, but additional studies are still needed to confirm these hypotheses (Sukhorukov et al., in press).

The structure of the stem vascular system in *Allionia* presents some unusual characteristics. The primary system is remarkable for the occurrence of medullary bundles (named "central collateral bundles" by Phillips 1976), and vascular bundles derived from a continuous concentric procambium that delimits the pith.

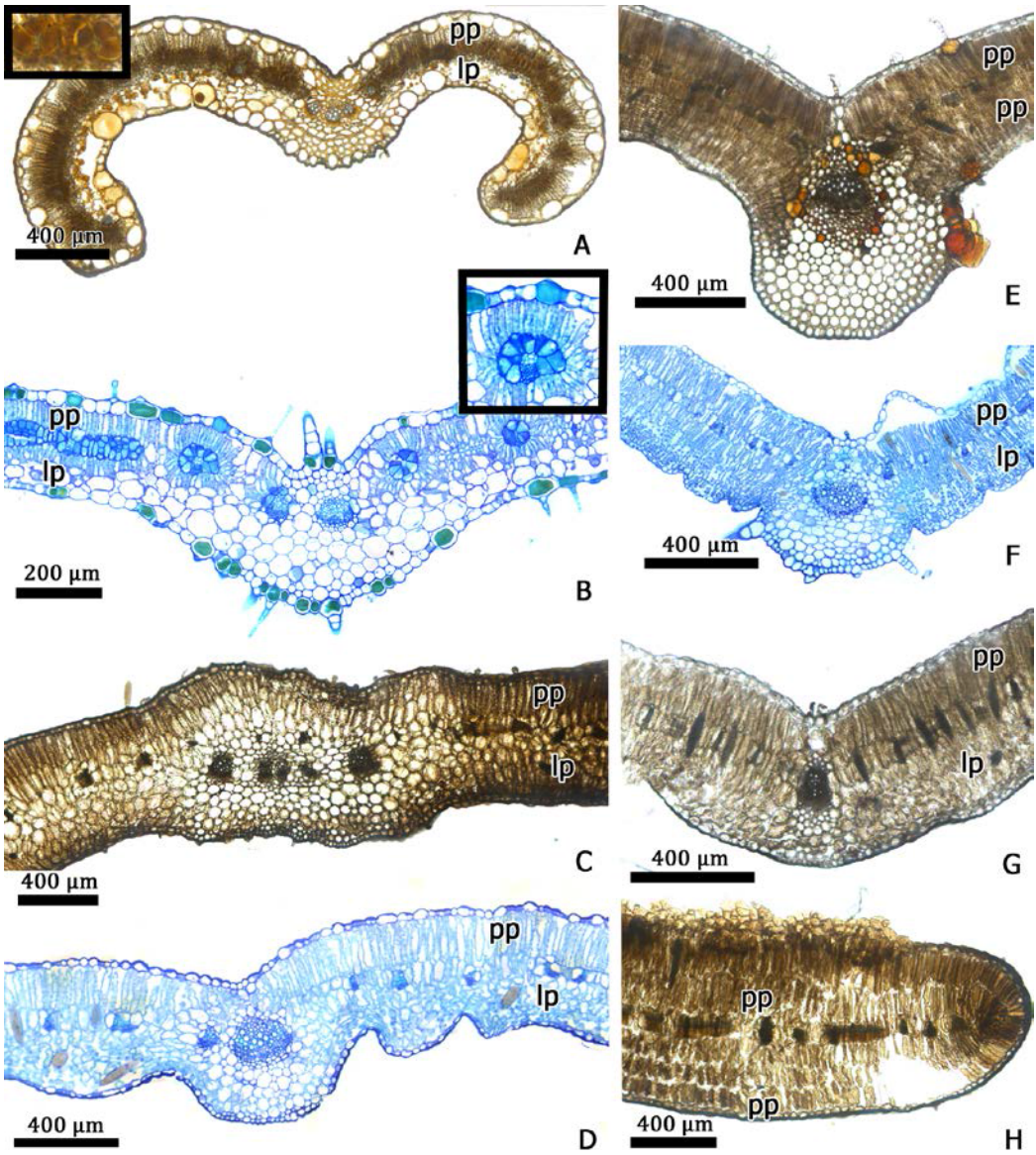

Fig. 4. Leaf anatomy in related species of Nyctagineae. **4a–b.** Species with Kranz anatomy. **4a.** *Boerhavia linearifolia.* **4b.** *Okenia hypogaea.* **4c–h.** Species without Kranz anatomy. **4c.** *Acleisanthes lanceolata*. **4d.** *Commicarpus scandens.* **4e.** *Anulocaulis leiosolenus* var. *gypsogenus.* **4f.** *Nyctaginia capitata*. **4g.** *Cyphomeris gypsophilioides*. **4h.** *Abronia nealleyi.*

Together, these vascular units constitute the primary vascular system (the stele). This stele organization, named polycyclic eustele, was recently investigated in a broader scale by Cunha Neto et al. (2020). In this study, authors performed a thorough developmental and evolutionary analysis of the presence of medullary bundles for Nyctaginaceae. The polycyclic eustele was found to be ancestrally present in Nyctaginaceae and sister families (phytolaccoid clade), with a reversion to the regular eustele in one lineage of the family (tribe Leucastereae) (Cunha Neto et al. 2020).

In *Allionia* stems, the transition from primary to secondary growth follows the typical establishment of a

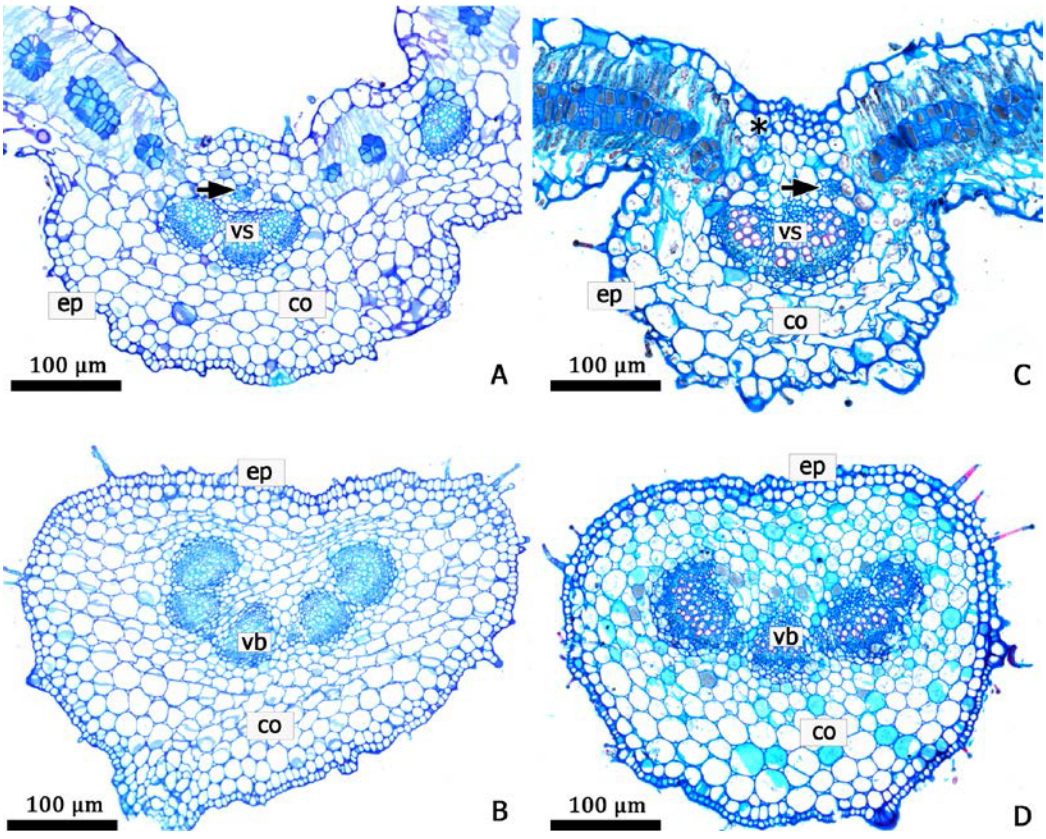

Fig. 5. Cross section of midrib and petiole in *Allionia incarnata*. 5a–b. Specimens collected in Bolivia, Parque Nacional Amboró. 5c–d. Specimens collected in the Chihuahuan Desert, New Mexico, USA. 5a, 5c. Prominent midrib showing collenchyma cells (asterisk) and the vascular system (vs) including an additional accessory bundle (arrow). 5c, 5d, Petiole showing four or five vascular bundles (vb) arranged in an arc toward the adaxial surface. *co,* cortex: *ep,* epidermis.

regular cambium resulting from fascicular and interfascicular cambium, except for the fact that it arises in a continuum with the continuous concentric procambium (CCP). Due to the rapid transition from the CCP to the vascular cambium, previous authors had interpreted the CCP as a "secondary meristem" (De Bary 1884; Balfour 1965). However, we have found that in early developmental stages this meristem shows characteristics of a typical procambium which form primary vascular tissue (vascular bundles) evidenced by wall thickenings commonly found in primary xylem (Cunha Neto et al. 2020). Concomitantly with regular cambium development, several layers of peripheral pith cells became lignified during this vascular transition. This unusual but consistent characteristic was found in *Allionia* and other genera within Nyctaginaceae (pers. obs.). The regular stem secondary growth in *Allionia* produce relatively little secondary phloem and xylem until the establishment of the successive cambia system. As reported for other taxa within the family (Carlquist 2004; Hernández-Ledesma et al. 2011), there are no typical vascular rays in *Allionia*, although some cambial derivatives peripheral to interfascicular regions can be sometimes organized in radially oriented rows. The ray-like cells in *Allionia* and other Nyctaginaceae should be better explained as resulting from the continuity on divisions of the meristem that originates the additional cambia (Carlquist 2004, 2007) and/or from proliferation of the conjunctive tissue.

The occurrence of cambial variants is a well-known feature for stems of Nyctaginaceae (De Bary 1884;

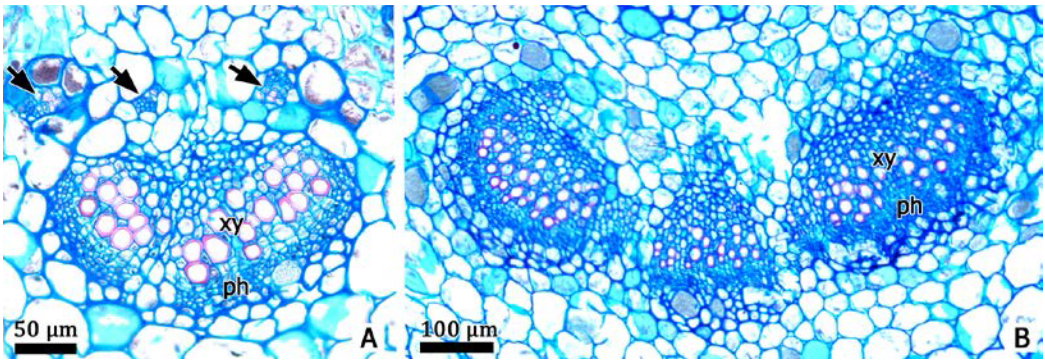

Fig. 6. Details of the vascular system in the midrib and petiole in *Allionia incarnata*. 6a. Collateral vascular bundles in the midrib. 6b. Three collateral vascular bundles in the petiole. *Arrow,* accessory bundles; *ph,* phloem, *xy,* xylem.

Schenck 1893; Metcalfe & Chalk 1950; Carlquist 2001, 2007, 2010). However, the interpretation of the origin and development of these systems have always been a matter of debate. In the case of *Allionia,* two aspects should be emphasized. First, different from Phillips' (1976) interpretation, we noticed that there is some period of regular growth in the stems of *Allionia* and only later the cambial variant arises. Second, our observation of a pericyclic origin for successive cambia (both in stems and roots) is in accordance with Phillips (1976) but differs from Carlquist's (2004) interpretation for other Nyctaginaceae—which are also described as having successive cambia. Here, we showed that the outermost layer of the stele differentiates into perivascular fibers and the innermost layer of the cortex is characterized as the starch sheath. Since we have identified these limits of the stele and the cortical region, and given that all events related to the formation of the successive cambia occur internally to these two cell layers, the additional cambia are not established in the cortex, as has been stated by Carlquist (2004, 2007, 2010) and others following him (Rajput et al. 2009; Hernández-Ledesma et al. 2011). The ontogenetic approach carried out in this study was fundamental in developing our current understanding of the cambial variant present in *Allionia*. Similarly, there have been various studies aimed at better understanding the origin and ontogeny of cambial variants in different groups (e.g., Sapindaceae—Cunha Neto et al. 2018; Vitaceae—Pace et al. 2018; Fabaceae—Leme et al. 2020).

The formation of successive cambia can be observed also in lateral and primary roots of *Allionia*, as well as in roots of other herbs and subshrubs within Nyctagineae, such as *Anulocaulis leiosolenus, Cyphomeris gypsophiloides,* and *Mirabilis albida* (pers. obser). Successive cambia in roots of Nyctaginaceae have been previously reported for *Abronia latifolia* (Carlquist 2004), *Bougainvillea spectabilis* (Esau & Cheadle 1969; Stevenson & Popham 1973; Carlquist 2004) and *Mirabilis jalapa* (Mikesell & Popham 1976). The origin and evolution of such variant anatomies is remarkable within Nyctaginaceae and other Caryophyllales (e.g., Aizoaceae, Amaranthaceae [including the well-known beet root of *Beta vulgaris,* Chenopodiaceae], Basellaceae, Phytolaccaceae, Polygonaceae, Nepenthaceae) since it is a feature with multiple independent origins (Gibson 1994; Carlquist 2010; Schwallier et al. 2017).

Ecological and functional interpretations.—*Allionia* consists of annual and perennial plants. Although these plants produce stems and leaves in the summer (Phillips 1976; Mulroy & Rundel 1977), the foliage of *Allionia* is not strikingly xerophytic and only rarely is it heavily pubescent or cutinized (Mulroy & Rundel 1977). Nevertheless, according to Phillips (1976), there are some morphological traits in *Allionia* that might be associated as adaptations for harsh desert environments; they are the large taproot, anisoclady, and seeds with abundant storage. Anatomically, the presence of trichomes and Kranz anatomy in relation to C_4 photosynthesis (a common adaptations for summer annual species in the Sonoran Desert, Syvertsen et al. 1976; Mulroy & Rundel 1977) would represent the main leaf traits that provide the ability to survive in hot, dry conditions. In

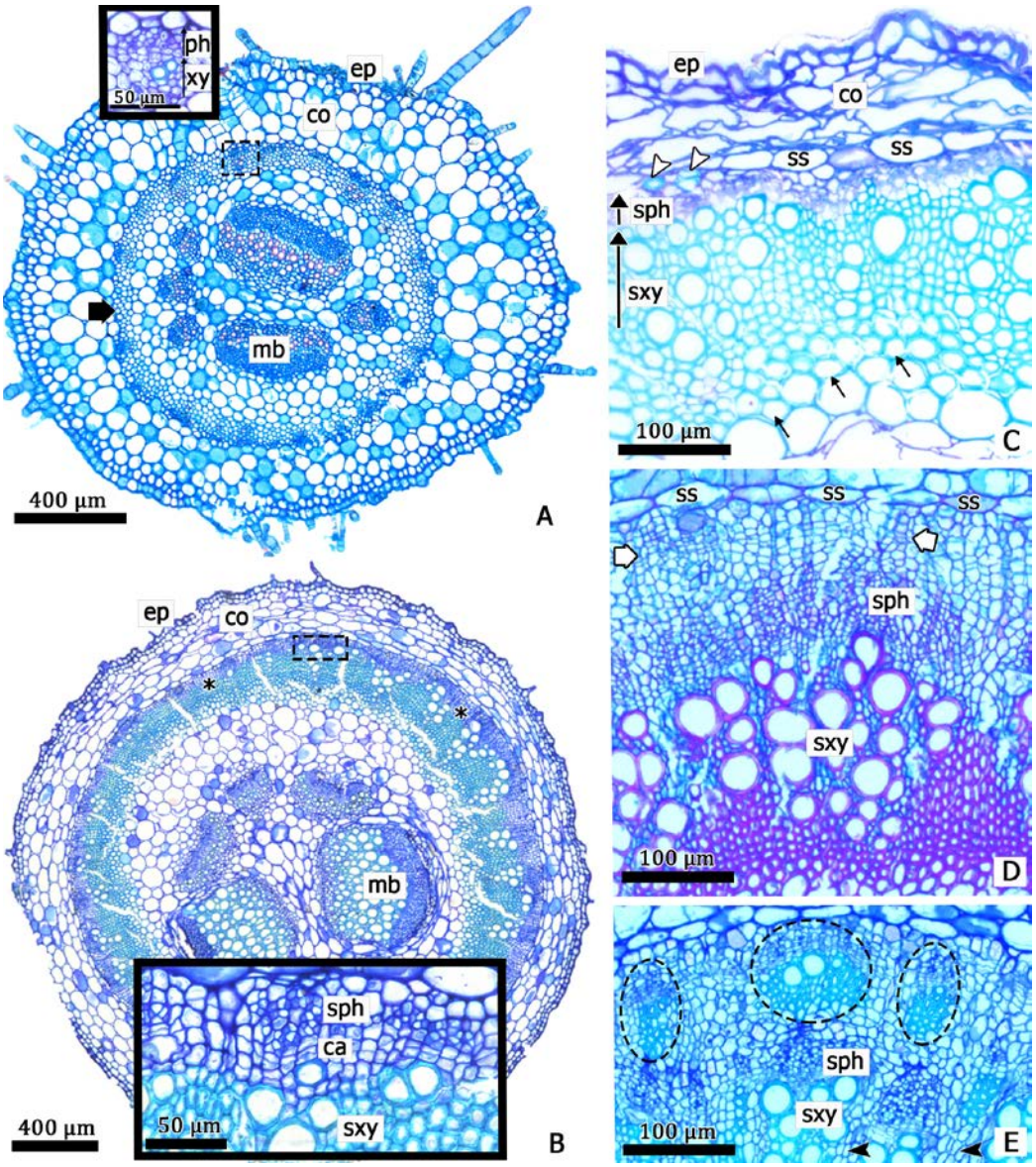

Fig. 7. Anatomy and development of the stem in *Allionia* in cross section. **7a–b, d–f.** *A. incarnata;* **7c.** *A. choisyi.* **7a.** Primary structure showing polycyclic eustele (medullary bundles + vascular bundles [box and inset] derived from the continuous concentric procambium [thick arrow]). **7b.** Early secondary growth formed by regular activity of the vascular cambium forming xylem centripetally and phloem centrifugally (box). Asterisks indicate interfascicular region formed mainly by fibers in the xylem and parenchyma in the phloem side. **7c.** Detail of secondary vascular tissues, lignified pericyclic cells (white arrowheads), starch sheath (ss) and lignified pith cells (arrows). **7d.** Divisions on pericyclic parenchyma giving rise to the meristematic zone that will form the first ring of successive cambia (white arrows). **7e.** Developing arcs of successive cambia which form xylem centripetally and phloem centrifugally. Arrowheads indicate ray-like cells. *Arrow* (yellow), vessels formed by interfascicular cambium; *ep,* epidermis; *co,* cortex; *ph,* primary phloem*; mb,* medullary bundles; *sph,* secondary phloem; *ss,* starch sheath; *sxy,* secondary xylem; *xy*, primary xylem.

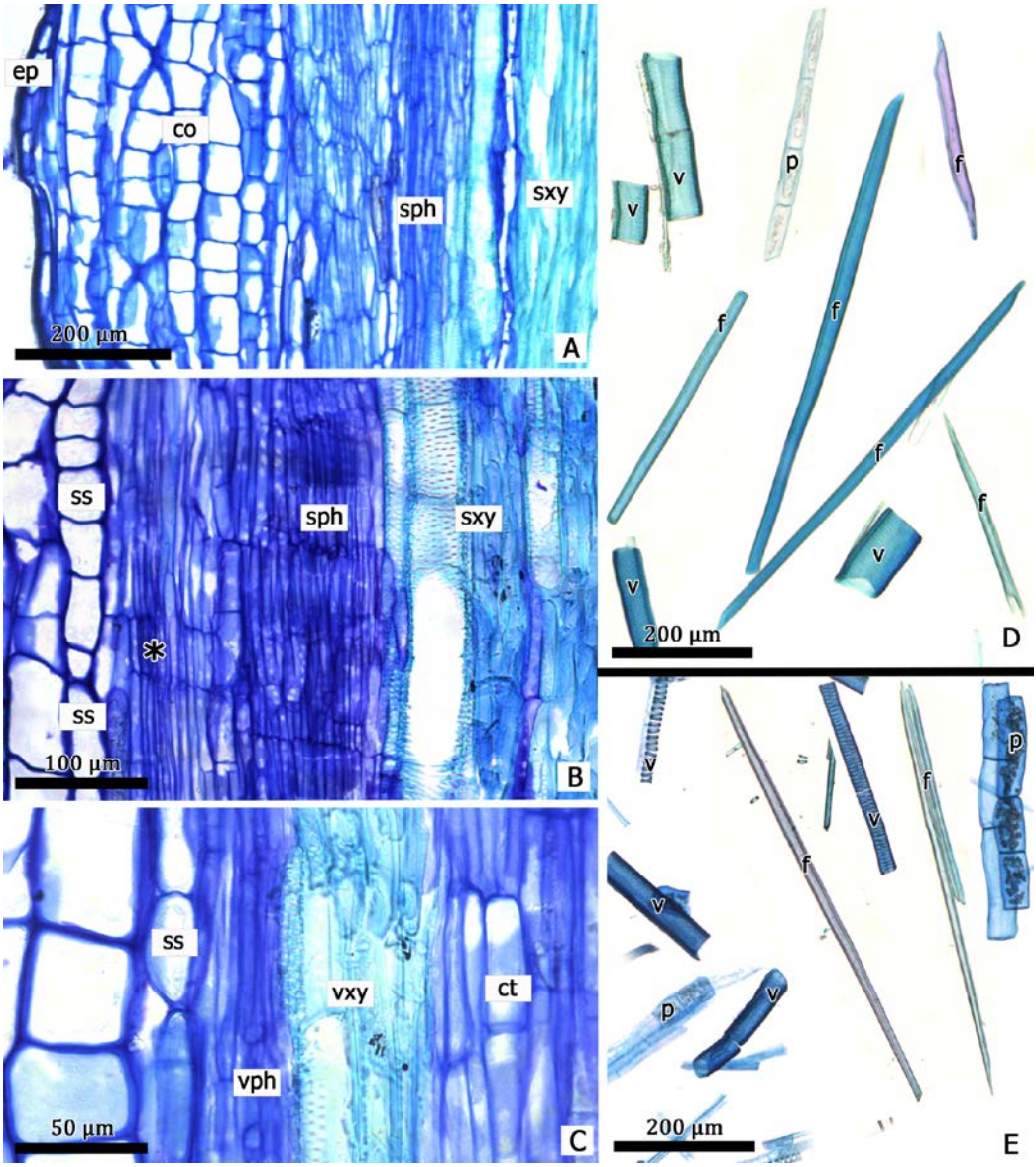

Fig. 8. Details of secondary growth, establishment of successive cambia and wood cell types in *Allionia incarnata*. **8a–c.** Longitudinal radial sections. **8a.** Regular secondary xylem and secondary phloem. **8b.** Divisions on pericyclic parenchymatic cells (asterisk) that give rise to the first successive cambia. **8c.** Differentiation of variant xylem (vxy) and variant phloem (vph) from the first arc of successive cambia. **8d–f.** Maceration. **8d.** Cells from the regular xylem showing vessels, axial parenchyma and fibers of different sizes and tip shapes (fusiform, "u"-shaped). **8e.** Cells from xylem of medullary bundles showing vessels with different wall thickenings, parenchyma with starch and fibers. *ep,* epidermis; *f,* fiber; *co,* cortex; *ct,* conjunctive tissue; *p,* parenchyma; *ss,* starch sheath; *sxy,* secondary xylem; *v,* vessel.

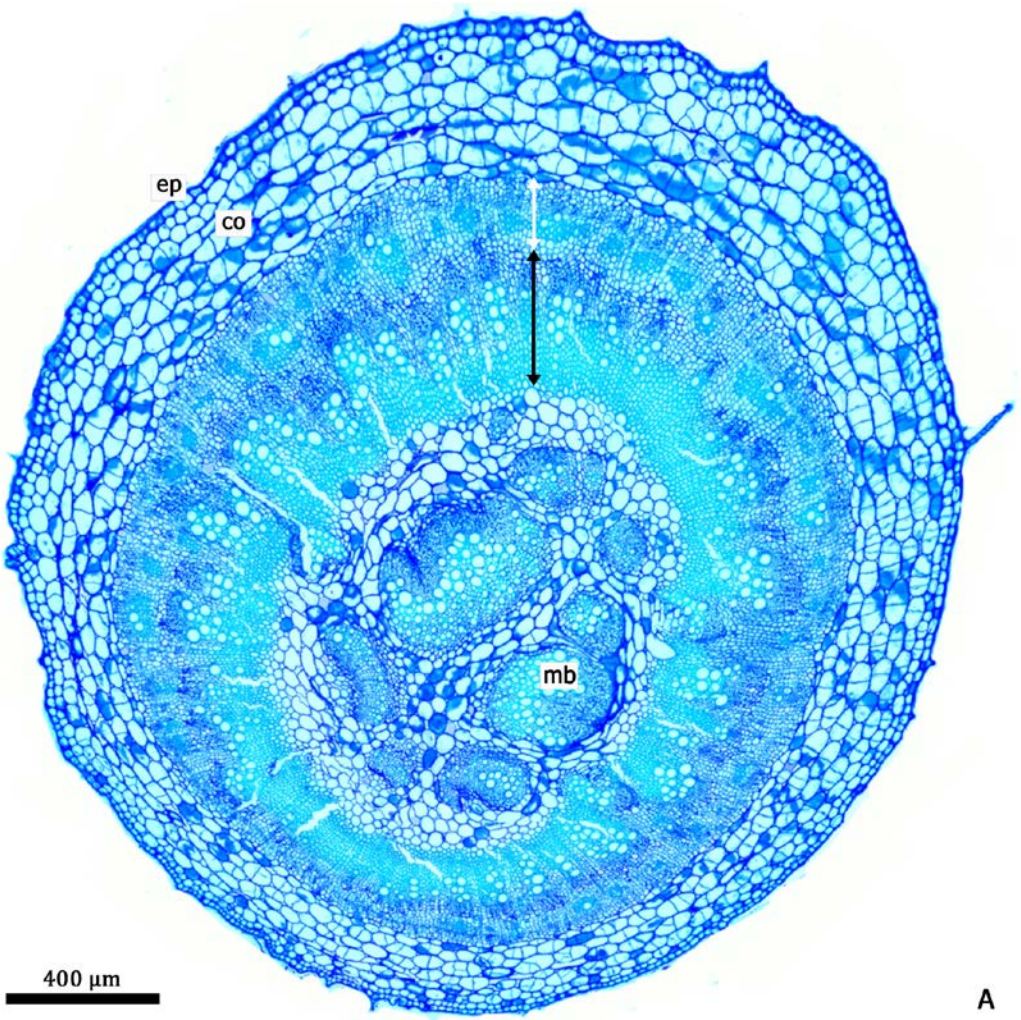

Fig. 9. General view of adult stem in *Allionia incarnata*. 9a. Note the regular cylinder of vascular tissues (black line) and the ring of vascular tissues produced by the successive cambia (white line) developed outwards; *ep*, epidermis; *co*, cortex; *mb*, medullary bundles.

addition, the formation of successive cambia, which is associated with an increase in water and starch parenchyma, can be also suggested as an adaptive feature for plants thriving in harsh environments (Carlquist 2001, 2012). In this sense, it is interesting to note that the usual thick perennial roots showed several rings of successive cambia, while the slender stems had only one or two additional rings. This condition seems to be true for several other species from tribe Nyctagineae that grows in similar conditions (*e.g., Acleisanthes chenopodioides, Anulocaulis gypsogenus* var. *gypsogenus, Boerhavia torreyana,* pers. obs.). The adaptation to arid environments and drought stress in *Allionia* has been linked also with the association with endophytes—arbuscular mycorrhizal—an adaptation that has been demonstrated for more than 40 species, including *Allionia* (Lugo et al. 2015). Other species of Nyctaginaceae growing in deserts (e.g., *Boerhavia* and *Commicarpus*) have acquired the ability to survive in dry conditions by avoiding desiccation also through the production of tannins, thickened stomata walls and additional collenchyma cells as supporting tissue (Struwig et al. 2011).

Table 2. Quantitative anatomical features of xylem and phloem in stems of *Allionia* species.

	Allionia incarnata					
	Xylem				Phloem	
	Vessel elements		Fibers		Sieve-tube elements	
Features/cell type	Medullary bundles	Regular cylinder	Medullary bundles	Regular cylinder	Medullary bundles	Regular cylinder
Diameter (µm)	39.9 ± 1.0 (16.2–66.4)	31.0 ± 8.2 (14–54.5)	13.4 ± 6.3 (5.7–23.1)	16.2 ± 5.8 (5.6–27.5)	14.5 ± 1.2 (12.6–18.4)	9.8 ± 2.6 (8.6–16.1)
(Length (µm)	257.1 ± 1.1 91.4–461)	127.2 ± 2.9 (36.5–201.6)	490.4 ± 192.7 (233.7–989.7)	422.4 ± 171.5 (215.3–755.0)	217.7 ± 24.3 (186.6–220.4)	111.7 ± 25 (54.7–140.0)
Wall thickness (µm)	–	–	3.7 ± 0.8 (1.8–5.5)	3.6 ± 0.7 (2.4–5.4)	–	–
Frequency (mm-2)	54.6 ± 5.8 (42.0–66.0)	35.0 ± 3.2 (22.0–38.0)	–	–	23.4 ± 4.6 (14–27)	11.7 ± 1.7 (9–15)
Area (%)	26.0 ± 5.9 (18.0–34.0)	27.0 ± 4.0 (20.0–30.0)	11.3 ± 2.5 (8.0–14.0)	22.0 ± 8. (14.0–31.0)	17.4 ± 5.2 (10.0–22.0)	15.0 ± 1.0 (14.0–19.0)

	Allionia choisyi					
	Xylem				Phloem	
	Vessel elements		Fibers		Sieve-tube elements	
	Medullary bundles	Regular cylinder	Medullary bundles	Regular cylinder	Medullary bundles	Regular cylinder
Diameter (µm)	25.8 ± 1.3 (8.6–54.3)	24.6 ± 6.8 (11.5–42.9)	6.1 ± 1.1 (4.2–7.7)	7.2 ± 1.4 (5.3–8.7)	6.4 ± 1.1 (4.9–11.2)	8.6 ± 1.5 (7.6–9.8)
Length (µm)	–	–	–	–	–	–
Wall thickness (µm)	–	–	2.5 ± 1.0 (1.6–4.0)	3.9 ± 1.0 (2.0–4.6)	–	–
Frequency (mm-2)	28.8 ± 3.4 (25.2–36.4)	35.0 ± 3.2 (22–38)	–	–	–	–
Area (%)	16.5 ± 3.7 (18.0–34.0)	27.0 ± 4.0 (20.0–30.0)	14.6 ± 3.5 (12.0–18.0)	16.5 ± 19.0 (14.0–21.0)	–	–

A common characteristic of roots, stems, and leaves in *Allionia* is the abundance of calcium oxalate crystals in the form of raphides, as also observed in other Nyctaginaceae (Metcalfe & Chalk 1950; Struwig et al. 2011). Calcium oxalate crystals play different roles in plants such as accumulation of excesses, ionic/osmotic calcium regulation, detoxification/heavy metal tolerance and defence against herbivores (Mauseth 1988; Franceschi & Nakata 2005; Molano-Flores 2001; Struwig et al. 2011). However, this defence system is probably constitutive rather than inducible and not likely associated with soil calcium concentration (Ruiz et al. 2002). Nonetheless, the occurrence of calcium oxalate crystals as a mechanism to sequester calcium in a physiologically unavailable form has been recently investigated on gypsophytes—plants that grow on gypsum (CaSO4·H2O) soils—given that they can extract structural H_2O molecules from gypsum (Merlo et al. 2011; Borer et al. 2012; Palacio et al. 2014a,b; Mota et al. 2017). *Allionia* can grow on gypsum (gypsovag) but is not a gypsophyte (Moore et al. 2014). Curiously, *Anulocaulis gypsogenus* var. *gypsogenus* and *Acleisanthes lanceolata* are endemic to gypsum and comparatively show less crystals in their vegetative parts compared to *Allionia* (pers. obs.).

In *Allionia,* some stem anatomical features can be associated to their herbaceous habit, including the absence of rays (raylessness), wide fibers with thin walls, delayed or absence of periderm and the reduced amount of secondary growth despite the presence of successive cambia. Most of these characteristics can be

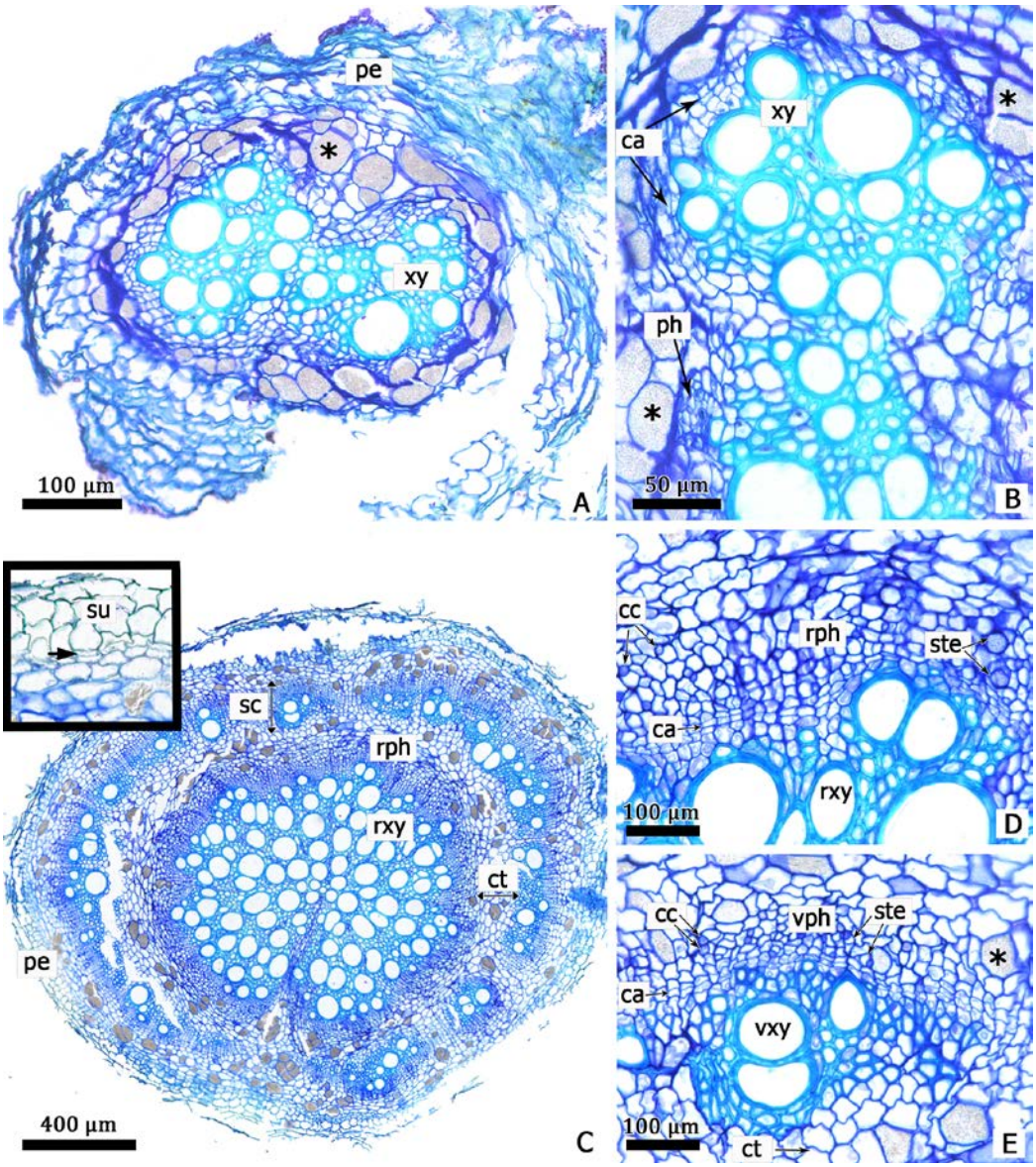

Fig. 10. Root anatomy and development of *Allionia incarnata* in cross section. **10a–b.** Lateral root. **10a.** Early secondary growth showing diarch vascular system. **10b.** Detail of previous image showing the establishment of regular cambium. **10c–e.** Primary root (taproot). **10c.** Regular vascular cylinder and first ring of successive cambia. **10d.** Detail of regular cambium, regular secondary xylem, and phloem. In the inset, note the phellogen (arrow) and suber. **10e.** Detail of variant cambium, variant xylem, and variant phloem. *Asterisks,* crystals (raphides); *ca*, cambium, *cc*, companion cell; *ct*, conjunctive tissue; *pe*, periderm; *ph*, phloem; *rph*, regular phloem; *rxy*, regular xylem; *sc*, successive cambia; *ste*, sieve-tube element; *su,* suber; *vph*, variant phloem; *vxy*, variant xylem; *xy*, xylem.

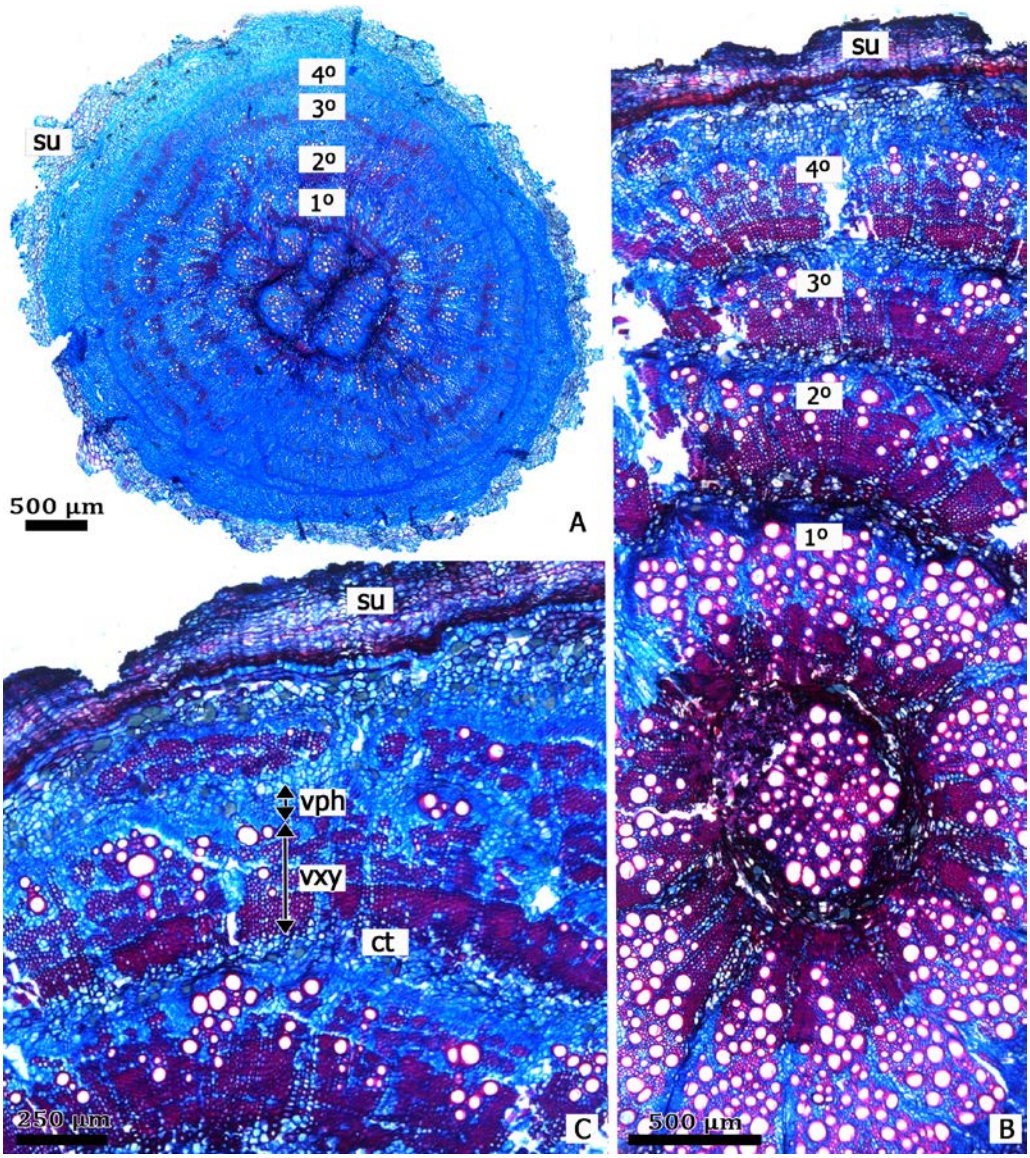

Fig. 11. **Adult primary root of *Allionia incarnata* in cross section. 11a.** General view showing four developed rings of successive cambia (ordinal numbers). **11b.** Detail of central cylinder and rings of successive cambia. **11c.** Close-up of successive cambia showing variant xylem, variant phloem, and conjunctive tissue. *ct,* conjunctive tissue; *su,* suber; *vph,* variant phloem; *vxy,* variant xylem.

linked with specific hydraulic and/or biomechanical demands of herbaceous plants. The absence of rays (raylessness), for instance, might be related to the fact that longitudinal conduction of photosynthates outweighs radial conduction (Carlquist 2012). In addition, given that medullary bundles seem to remain functional even in mature stems (Cunha Neto et al. 2020), their high proportion of vessels for a specific stem section can represent a crucial path for maintenance of its hydraulic function. However, the functional anatomy of herbaceous annuals and plants with medullary bundles remain underexplored, since this topic has been investigated predominantly in large woody plants.

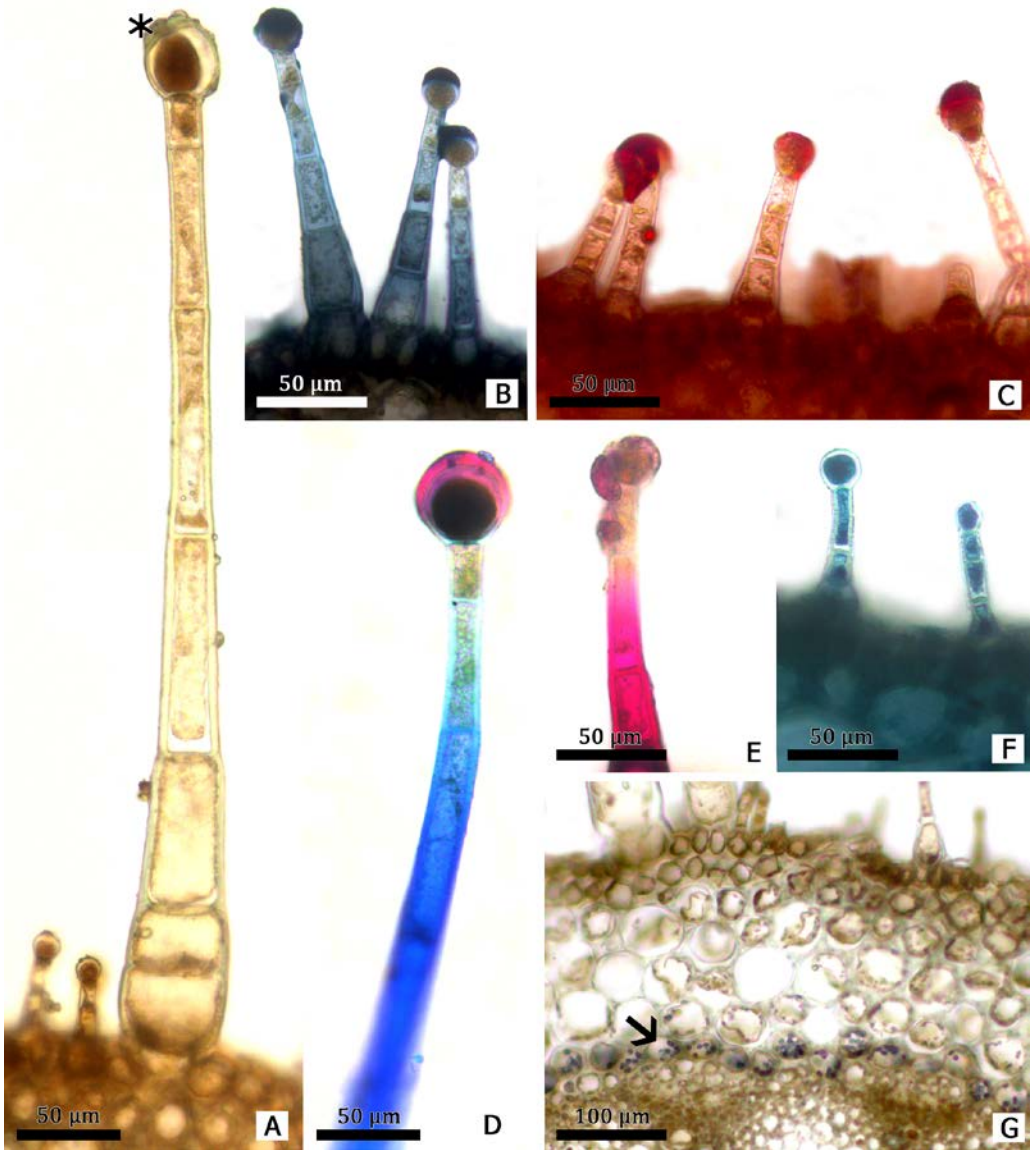

Fig. 12. **Anatomy and histochemistry of uniseriate glandular trichomes in *Allionia incarnata*. 12a.** Non-stained section showing greenish secretion (asterisk). **12b–d.** Positive reactions for lipids. **12b.** Sudan Black B. **12c.** Sudan IV. **12d.** Nile Blue. **12e.** Ruthenium red for mucilage. **12f.** Aniline Blue Black for proteins. **12g.** Starch grains detected in the starch sheath by Lugol reagent.

Overall, our results indicate that vegetative anatomy in *Allionia* species do not contribute to species delimitation. However, its general stem structure and development is unique. While we have revealed different interpretations for the phenomena accounting for the complexity of the stem vascular system in *Allionia* (e.g., polycyclic eustele, medullary bundles, continuous concentric procambium and successive cambia), a broader analysis of the diversity and evolution of the secondary structure within Nyctaginaceae is yet to be performed. Our future investigations will address this and other questions concerning the diversity and evolution of the vascular system for the family and sister lineages.

APPENDIX 1

Information on taxa, collectors, localities, and herbarium vouchers for the analyzed species.

Abronia fragrans Nutt. ex Hook., USA. New Mexico: Las Cruces, *Douglas 2290* (FLAS). ***Abronia neealleyi*** Standl., USA. New Mexico. Eddy Co.: Yeso Hills, *Douglas 2281*, (FLAS). ***Acleisanthes lanceolata*** (Wooton) R.A. Levin, USA. Texas: Malone Mountains, Sierra Blanca, *Douglas 2277* (FLAS). ***Acleisanthes chenopodioides*** (A. Gray) R.A. Levin, USA. New Mexico: Las Cruces, *Douglas 2289, 2293* (FLAS). ***Acleisanthes longiflora*** A. Gray, USA. Texas: Malone Mountains, Sierra Blanca, *Douglas 2279* (FLAS). ***Anulocaulis leiosolenus*** (Torr.) Standl. var. ***leiosolenus***, USA. *Texas:* Malone Mountains, Sierra Blanca, *Douglas 2278* (FLAS). ***Boerhavia linearifolia*** A. Gray, *USA. New Mexico: Douglas 2284* (FLAS). ***Boerhavia torreyana*** (S. Watson) Standl., USA. New Mexico: Las Cruces, *Douglas 2294* (FLAS). ***Boerhavia wrightii*** A. Gray, USA. New Mexico: Las Cruces, *Douglas 2288* (FLAS). ***Commicarpus scandens*** (L.) Standl., USA. New Mexico: *Douglas 2291* (FLAS). ***Cyphomeris gypsophiloides*** (M. Martens & Galeotti) Standl., USA. New Mexico: Las Cruces, Organ Mountains-Desert Peaks National Monument, *Douglas 2287* (FLAS). ***Mirabilis aggregata*** (Ortega) Cav., MEXICO. Hidalgo, Ixmiquilpan, *Pace 728* (MEXU). ***Mirabilis*** cf. ***albida*** (Walter) Heimerl., USA. New Mexico: *Douglas 2286* (FLAS). ***Mirabilis jalapa*** L., Mexico. Veracruz: *Acevedo-Rodríguez 16480* (US). ***Mirabilis viscosa*** Cav., Mexico. Hidalgo: Ixmiquilpan, *Pace 727* (MEXU). ***Nyctaginia capitata*** Choisy, USA, New Mexico: *Douglas 2282* (FLAS). ***Okenia hypogaea*** Schltdl. & Cham., Mexico. Veracruz: *Pace 749* (MEXU, SPF).

ACKNOWLEDGMENTS

The work was supported by the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP process 2017/17107-3) to I.L.C.N, Programa Unificado de Bolsas (PUB-USP) to J.P.S. and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior–Brasil (CAPES—Finance Code 001). We thank Norman A. Douglas, Patrick Alexander, Michael H. Nee, and Marcelo R. Pace for assistance during field collection. We are grateful to the National Museum of Natural History (NMNH) at the Smithsonian Institution for the Cuatrecasas Award granted to I.L.C.N. and for permission to sample at the herbarium collection. The authors thank Richard Spellenberg and an anonymous reviewer for their helpful suggestions and comments on early versions of the manuscript.

REFERENCES

Alexander, P.J., N.A. Douglas, H. Ochoterena, H. Flores-Olvera, & M.J. Moore. 2014. Recent finding on the gypsum flora of the rim of the Guadalupe Mountains, New Mexico, U.S.A.: A new Species of *Nerisyrenia* (Brassicaceae), a new state record, and an update checklist. J. Bot. Res. Inst. Texas 8:383–393.

Angyalossy, V., M.R. Pace, R.F. Evert, C.R. Marcati, A.A. Oskolski, T. Terrazas, E. Kotina, F. Lens, S.C. Mazzoni-viveiros, & G. Angeles. 2016. IAWA list of microscopic bark features. IAWA J. 37:517–615.

Antonucci, N.P. 2010. Estudos anatômicos, ultra-estruturais e bioquímicos da síndrome Kranz em folhas de duas espécies de *Gomphrena* L. (Amaranthaceae). (Doctoral dissertation). Universidade de São Paulo, São Paulo, Brazil.

Balfour, E. 1965. Anomalous secondary thickening in Chenopodiaceae, Nyctaginaceae and Amaranthaceae. Phytomorphology 15:111–122.

Barbosa, A.C.F., M.R. Pace, L. Witovisk, & V. Angyalossy. 2010. A new method to obtain good anatomical slides of heterogeneous plant parts. IAWA J. 31:373–383.

Bittrich, V. & U. Kühn. 1993. Nyctaginaceae. In: Kubitzki, K., J.G. Rohwer, V. Bittrich, eds. The families and genera of flowering plants, vol. 2. Springer, Berlin, Germany.

Borer C.H., M.N. Hamby, & L.H. Hutchinson. 2012. Plant tolerance of a high calcium environment via foliar partitioning and sequestration. J. Arid Environ. 85:128–131.

Cain, A. J. 1947. The use of Nile blue in the examination of lipids. J. Cell Sci. 3:383–392.

Carlquist, S. 2001. Comparative wood anatomy. Systematic, ecological and evolutionary aspects of dicotyledon wood. Springer, Berlin, Germany.

Carlquist, S. 2004. Lateral meristems, successive cambia and their products: A reinterpretation based on roots and stems of Nyctaginaceae. Bot. J. Linn. Soc. 146:129–143.

Carlquist, S. 2007. Successive cambia revisited: Ontogeny, histology, diversity, and functional significance. J. Torrey Bot. Soc. 134:301–332.

Carlquist, S. 2010. Caryophyllales: A key group for understanding wood anatomy character states and their evolution. Bot. J. Linn. Soc. 164:342–393.

Carlquist, S. 2012. How wood evolves: A new synthesis. Botany 90:901–940.

Carolin, R.C., S.W.L. Jacobs, & M. Vesk. 1978. Kranz cells and mesophyll in the Chenopodiales. Austral. J. Bot. 5:683–698.

Chen, F.S., D.H. Zeng, T.J. Fahey, C.Y. Yao, & Z.Y. Yu. 2010. Response of leaf anatomy of *Chenopodium acuminatum* to soil resource availability in a semiarid grassland. Pl. Ecol. 209:375–382.

Cunha neto, i.l., f.m. Martins, g.v. Somner, & n. Tamaio. 2018. Successive cambia in liana stems of Paullinieae and their evolutionary significance in Sapindaceae. Bot. J. Linn. Soc. 186: 66–88.

Cunha Neto, I.L., V. Angyalossy, & N.A. Douglas. 2019. What are the "sticky rings" on stems of *Anulocaulis* and related taxa (Nyctaginaceae) from arid regions? J. Bot. Res. Inst. Texas 2:477–485.

Cunha Neto, I.L., M.R. Pace, N.A. Douglas, M.H. Nee, C.F.C. Sá, M.J. Moore, & V. Angyalossy. 2020. Diversity, distribution, development and evolution of medullary bundles in Nyctaginaceae. Amer. J. Bot. 107: 1–19.

David R, & JP. Carde. 1964. Coloration différentielle des inclusions lipidiques et terpéniques des pseudophylles du *Pin maritime* au moyen du réactif Nadi. C. R. Acad. Sci. Paris 258: 1338–1340.

De bary, A. 1884. Comparative anatomy of the vegetative organs of the phanerogams and ferns. Clarendon Press, Oxford, UK.

Dickinson W.C. Integrative plant anatomy. 2000. Harcourt Academic Press, New York, U.S.A.

Dória L.C., D.S. Podadera, M. del Arco, T. Chauvin, E. Smets, S. Delzon, & F. Lens. 2018. Insular woody daisies (*Argyranthemum*, Asteraceae) are more resistant to drought-induced hydraulic failure than their herbaceous relatives. Funct. Ecol. 32:1467–1478.

Douglas, N.A. & P.S. Manos. 2007. Molecular phylogeny of Nyctaginaceae: Taxonomy, biogeography, and characters associated with a radiation of xerophytic genera in North America. Amer. J. Bot. 94:856–872.

Douglas, N.A. & R. Spellenberg. 2010. A new tribal classification of Nyctaginaceae. Taxon 59:905–910.

Esau, K. & V.I. Cheadle. 1969. Secondary growth in *Bougainvillea*. Ann. Bot. 33:807–819.

Fisher, D.B. 1968. Protein staining of ribboned epon sections for light microscopy. Histochemie 1:92–96.

Fay J.J. 1980. Nyctaginaceae. In: Gómez-Pompa A. ed. Flora de Veracruz 13. Instituto Nacional de Investigaciones sobre Recursos Bióticos, Xalapa, Mexico. Pp. 1–54.

Franceschi, V.R. & P.A Nakata. 2005. Calcium oxalate in plants: Formation and function. Ann. Rev. Pl. Biol. 56:41–71.

Gerlach, G. 1969. Botanische Mikrotechnik, eine Einführung. Thieme, Stuttgart, Germany.

Gibson, A.C. 1994. Vascular tissues. In: H.D. Behnke & T.J. Mabry, eds. Caryophyllales: Evolution and systematics. Springer, Berlin, Germany. Pp. 45–74.

Gregory, M. & Baas, P. 1989. A survey of mucilage cells in vegetative organs of the dicotyledons. Israel J. Bot. 2–3:125–174.

Haberlandt, G. 1882. Die physiologischen Leistungen der Pflanzengewebe. Trewendt.

Heimerl, A. 1932. Nyctaginaceen-Studien. Notizblatt des Botanischen Gartens und Museums zu Berlin-Dahlem, Germany. Pp. 450–470.

Hernández-Ledesma, P. & H. Flores-Olvera. 2003. Nyctaginaceae de Hidalgo, México. Anales Inst. Biol. Univ. Nac. Auton. México, Bot. 74:231–287.

Hernández-Ledesma, P., T. Terrazas, & H. Flores-Olvera. 2011. Comparative stem anatomy of *Mirabilis* (Nyctaginaceae). Pl. Syst. Evol. 292:117–132.

Hernández-Ledesma, P. et al. 2015. A taxonomic backbone for the global synthesis of species diversity in the angiosperm order Caryophyllales. Willdenowia 45:281–383.

IAWA Committee. 1989. IAWA list of microscopic features for hardwood identification. IAWA Bull. n.s. 10:219–332.

Johansen, D.A. 1940. Plant microtechnique. McGraw-Hill, New York, U.S.A.

Kraus, J.E. & M. Arduin. 1997. Manual básico de métodos em morfologia vegetal. EDUR, Rio de Janeiro, Brazil.

Leme, c.l.d., i.l. Cunha neto, & v. Angyalossy. 2020. How the neotropical liana *Machaerium multifoliolatum* (Fabaceae) develop their distinctive flattened stems? Flora 269: 151629.

López, H.A. & A.M. Anton. 2006. Nyctaginaceae. In: A.T. Hunziker ed., Flora fanerogámica Argentina, Programa Proflora, Córdoba, Argentina. Pp. 1–22.

Lugo, M.A., K.O. Reinhart, E. Menoyo, E.M. Crespo, & C. Urcelay. 2015. Plant functional traits and phylogenetic relatedness explain variation in associations with root fungal endophytes in an extreme arid environment. Mycorrhiza 2:85–95.

Mauseth, J.D. 1988. Plant anatomy. The Benjamin/Cummings Publishing Company, Menlo Park, California, U.S.A.

Mikesell, J.E. & R.A Popham. 1976. Ontogeny and correlative relationships of the primary thickening meristem in Four-O'clock plants (Nyctaginaceae) maintained under long and short photoperiods. Amer. J. Bot. 63:427–437.

Metcalfe, C.R. & L. Chalk. 1950. Anatomy of the dicotyledons: Leaves, stems, and wood in relation to taxonomy with notes on economic uses. Clarendon Press, Oxford, UK.

Merlo, M.E., J.F. Mota, & P. Sánchez Gómez. 2011. Ecofisiología y adaptaciones de las plantas vasculares a las características físicas y químicas de sustratos especiales. In: Mota, J.F., Sánchez- P. Gómez & J.S. Guirado Romero, eds. Diversidad vegetal de las yeseras ibéricas. ADIF-Mediterráneo Asesores Consultores. Almería, Spain. Pp. 51–74.

Molano-Flores, B. 2001. Herbivory and calcium concentrations affect calcium oxalate crystal formation in leaves of *Sida* (Malvaceae). Ann. Bot. 3:387–391.

Mota, J.F., J.A. Garrido-Becerra, M.E. Merlo, J.M. Medina-Cazorla, & P. Sánchez-Gómez. 2017. The edaphism: Gypsum, dolomite and serpentine flora and vegetation. In: Loidi, J. ed. The vegetation of the Iberian Peninsula. Springer, Cham., New York, U.S.A. Pp. 277–354.

Mulroy, T.W. & P.W. Rundel. 1977. Annual plants: Adaptations to desert environments. Bioscience 2:109–114.

Moore, M.J., J.F. Mota, N.A. Douglas, H. Flores-Olvera, & H. Ochoterena. 2014. The ecology, assembly, and evolution of gypsophile floras. In: N. Rajakaruna, R. Boyd & T. Harris, eds. Plant ecology and evolution in harsh environments. Nova Science Publishers, Hauppauge, New York, U.S.A. Pp. 97–128.

O'Brien, T.P., N. Feder, & M.W. Mac Cully. 1964. Polychromatic staining of plant cell walls by toluidine blue O. Protoplasma 59:368–373.

Ocampo, G., N.K. Koteyeva, E.V. Voznesenskaya, E.E. Edwards, T.L. Sale, R.F. Sage, & J.T. Columbus. 2013. Evolution of leaf anatomy and photosynthetic path-ways in Portulacaceae. Amer. J. Bot. 100:2388–2402.

Pace, M.R., V. Angyalossy, P. Acevedo-rodríguez, & J. Wen. 2018. Structure and ontogeny of successive cambia in *Tetrastigma* (Vitaceae), the host plants of Rafflesiaceae. J. Syst. Evol. 56:394–400.

Palacio S., M. Aitkenhead, A. Escudero, G. Montserrat-Martí, M. Maestro, & J. Robertson. 2014a. Gypsophile chemistry unveiled: Fourier Transform Infrared (FTIR) spectroscopy provides new insight into plant adaptations to gypsum soils. PLoS One 9:107–285.

Palacio S., J. Azorín, G. Montserrat-Martí, J.P. Ferrio. 2014b. The crystallization water of gypsum rocks is a relevant water source for plants. Nat. Commun. 5:46–60.

Pearse, A. 1985. Carbohydrates and mucosubstances. In: Histochemistry theoretical and applied Analytical technology. Churchill Livingstone, Edinburgh London Melbourne New York. 2:675–753.

Phillips, B. 1976. Anatomy and developmental morphology of *Allionia* L. (Nyctaginaceae). Ph.D. dissertation. The University of Arizona, Tucson, U.S.A.

Rajput, K.S. & K.S. Rao. 1998. Cambial anatomy and absence of rays in the stem of *Boerhaavia* species (Nyctaginaceae). Ann. Bot. Fennici 35:131–135.

Rajput, S., V.S. Patil, & K.K. Kapadne. 2009. Structure and development of secondary thickening meristem in *Mirabilis jalapa* (Nyctaginaceae). Polish Bot. J. 54:113–121.

Rasband, W.S. 2012. ImageJ: Image processing and analysis in Java. Astrophysics Source Code Library.

Ruiz N., D. Ward, & S. Saltz. 2002. Calcium oxalate crystals in leaves of *Pancratium sickenbergeri*: Constitutive or induced defense? Funct. Ecol. 16:99–105

Rupp, P. 1964. Polyglykol als Einbettungsmedium zum Schneiden botanischer Präparate. Mikrokosmos 53:123–128.

Ruzin, S.E. 1999. Plant microtechnique and microscopy. Oxford University Press, New York, U.S.A.

Rzedowski J. & Calderón, de R.G. eds. 2001. Flora fanerogámica del valle de México. Instituto de Ecología, A. C., Centro Regional del Bajío and Comisión Nacional para el Conocimiento y Uso de la Biodiversidad, México.

Sandoval-Ortega M.H., M.W. Siqueiros-Delgado, R. Cerros-Tlatilpa, & G. Ocampo. 2020. La familia Nyctaginaceae (Caryophyllales) em Aguascalientes, México. Act. Bot. Mex. 127:e1673.

Scheck, H. 1893. Beiträge zur Biologie und Anatomie der Lianen im Besonderen der in Brasilien einheimische. Belträge zur Anatomie der Lianen. In: Schimper, AFW; Fischer, G. Botanische Mittheilungen aus der Tropens. Gustav Fischer, Jena, Germany.

Schwallier, R., B. Gravendeel, H. De Boer, S. Nylinder, B.J. Van Heuven, A. Sieder, & F. Lens. 2017. Evolution of wood anatomical characters in *Nepenthes* and close relatives of Caryophyllales. Ann. Bot. 119:1179–1193.

Schweingruber, F.H. 2011. Atlas of stem anatomy in herbs, shrubs and trees. Springer-Verlag, Berlin, Germany.

Spellenberg, R. 2003. Nyctaginaceae. In: Flora of North America Editorial Committee, eds. Flora of North America north of Mexico. Oxford University Press, New York, New York, U.S.A. Pp. 14–74.

Spellenberg, R. 2012. Nyctaginaceae. In: N. Holmgren, P.K. Holmgren, J.L. Reveal, et al., Intermountain Flora Vol. 2, pt. A, New York Botanical Garden, Bronx, New York, U.S.A. Pp. 574–604.

Standley, P.C. 1931. The Nyctaginaceae and Chenopodiaceae of northwestern South America. Field Mus. Nat. Hist., Bot. Ser. 11:171–254.

Stevenson, D.W. & R.A. Popham. 1973. Ontogeny of the primary thickening meristem in seedlings of *Bougainvillea spectabilis*. Amer. J. Bot. 60:1–9.

Struwig, M., S.J. Siebert, & E.S. Klaassen. 2011. Nyctaginaceae. Bothalia 2:289–292.

Sukhorukov, A.P., M.V. Nilova, M.J. Moore, E.F.S. Rossetto, & N.A. Douglas. (In Press). Anatomical Diversity and evolution of the anthocarp in Nyctaginaceae. Bot. J. Linn. Soc.

Syvertsen, J.P., G.L. Nickell, R.W. Spellenberg, & G.L. Cunningham. 1976. Carbon reduction pathways and standing crop in three Chihuahuan Desert plant communities. S. W. Naturalist 21:311–320.

Turner, B.L. 1994. Revisionary study of the genus *Allionia* (Nyctaginaceae). Phytologia 77:45–55.

Voznesenskaya, E.V., N.K. Koteyeva, G.E. Edwards, & G. Ocampo. 2010. Revealing diversity in structural and biochemical forms of C_4 photosynthesis and a C_3–C_4 intermediate in genus *Portulaca* L. (Portulacaceae). J. Exp. Bot. 61:3647–3662.

Waterfall, U.T. 1946. Observations on the desert gypsum flora of southwestern Texas and adjacent New Mexico. Amer. Midl. Naturalist 36:456– 466.

Willson, J. & R. Spellenberg. 1977. Observations on anthocarp anatomy in the subtribe Mirabilinae (Nyctaginaceae). Madroño 24:104–111.

Ziemińska, K., M. Westoby, & I.J. Wright. 2015. Broad anatomical variation within a narrow wood density range—a study of twig wood across 69 Australian angiosperms. PLoS ONE 10:e012489.