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COMPARATIVE ANATOMICAL STUDY ON LATICIFERS IN SIX APOCYNACEAE SPECIES

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Abstract

This study examined six species from three subfamilies of Apocynaceae – *Cynanchum thesiodes*, *Periploca sepium*, *Cynanchum chinense*, *Asclepias curassavica*, *Metaplexis japonica*, and *Catharanthus roseus* to characterize the structure and distribution of laticifers, providing an anatomical basis for further research on laticifer biology and Apocynaceae taxonomy. Using comparative anatomical methods, we investigated the type, size, and distribution of laticifers in stems and leaves. In stems, all species possessed non-articulated, unbranched laticifers located in both the cortex and pith. In leaves, *C. thesiodes*, *P. sepium*, and *C. chinense* contained articulated, branched laticifers; *A. curassavica* and *M. japonica* had non-articulated, branched laticifers with Y-shaped branches; and *C. roseus* exhibited articulated, unbranched laticifers. Across all species, leaf laticifers were mainly distributed within the spongy mesophyll and outside the palisade cells, with occasional occurrence external to the phloem.

Key words: Apocynaceae; Laticifer; Microstructure

Introduction

The family Apocynaceae, belonging to the order *Gentianales* within the eudicots, comprises approximately 415 genera and over 4,500 species according to the latest classification (Endress *et al*., 2014). Members of Apocynaceae are widely distributed and exhibit diverse growth forms, including trees, shrubs, herbs, and lianas (Ollerton *et al*., 2019). Laticifers are a common anatomical feature in this family and have long been regarded as important morphological traits in phylogenetic studies (Vega, 2002).

Laticifers are tubular structures specialized for latex secretion and are generally classified into two structural types: articulated and non-articulated (Chaffey, 2007; Fahn, 1988). Non-articulated laticifers consist of a single elongated cell that develops concurrently with organ growth, lacking transverse walls. During development, the nucleus may fragment or the cytoplasm may form multinucleated cells without wall partitioning, often producing branched systems extending throughout the plant (Fahn, 1988). In contrast, articulated laticifers comprise a series of laticiferous cells in which the intervening cell walls dissolve, forming a continuous network that allows latex to move freely between cells (Fahn, 1988; Metcalfe, 1967). Both types can occur in anastomosed or non-anastomosed forms (Farrell, 1991).

Historically, laticifers in Apocynaceae were considered predominantly non-articulated (Fahn, 1988; Metcalfe, 1967). However, more recent studies have documented the presence of articulated laticifers in certain taxa (Demarco *et al*., 2006; Lopes *et al*., 2009), leading to ongoing debate regarding their differentiation and classification. Whether laticifer characteristics can serve as reliable taxonomic markers remains unresolved. Notably, the APG IV classification incorporates all former Asclepiadaceae species into Apocynaceae (Bremer *et al*., 2016), further underscoring the need for detailed anatomical investigations.

In this study, we examined the secretory structures in the stems and leaves of six Apocynaceae species representing three subfamilies – *Cynanchum thesiodes*, *Asclepias curassavica*, *Cynanchum chinense*, *Metaplexis japonica*, *Periploca sepium*, and *Catharanthus roseus*. Comparative anatomical analyses were conducted to determine the type, structure, and distribution of laticifers. Based on observed similarities and differences, we propose an anatomical framework for distinguishing laticifer types and discuss their potential implications for the taxonomy of Apocynaceae.

Material and Methods

Plant material: Specimens of *Metaplexis japonica* (Thunb.) Makino, *Cynanchum thesiodes* (Freyn) K. Schum., *Cynanchum chinense* R. Br., and *Periploca sepium* Bunge were collected from Yunqiu Mountain, Shanxi Province, China (E111°01′, N35°44′; altitude 650–1580 m) between July and September 2018. *Asclepias curassavica* L. and *Catharanthus roseus* (L.) G. Don were cultivated in the experimental garden of Shanxi Normal University from July to October 2018.

Methods: Fresh samples were fixed in 2.5% glutaraldehyde prepared in 0.1 M phosphate buffer (pH 7.2) for 6 h at 4°C. The samples were then placed under vacuum to remove trapped air, rinsed three times with phosphate buffer, and post-fixed in 1.0% osmium tetroxide for 6–12 h at 4°C. Following fixation, specimens were dehydrated through a graded acetone series and embedded in SPI-812 resin at 60°C for three days. Semi-thin sections (1–2 μm) were prepared using a Leica RM2265 rotary microtome and stained with 0.05% toluidine blue O in citrate buffer.

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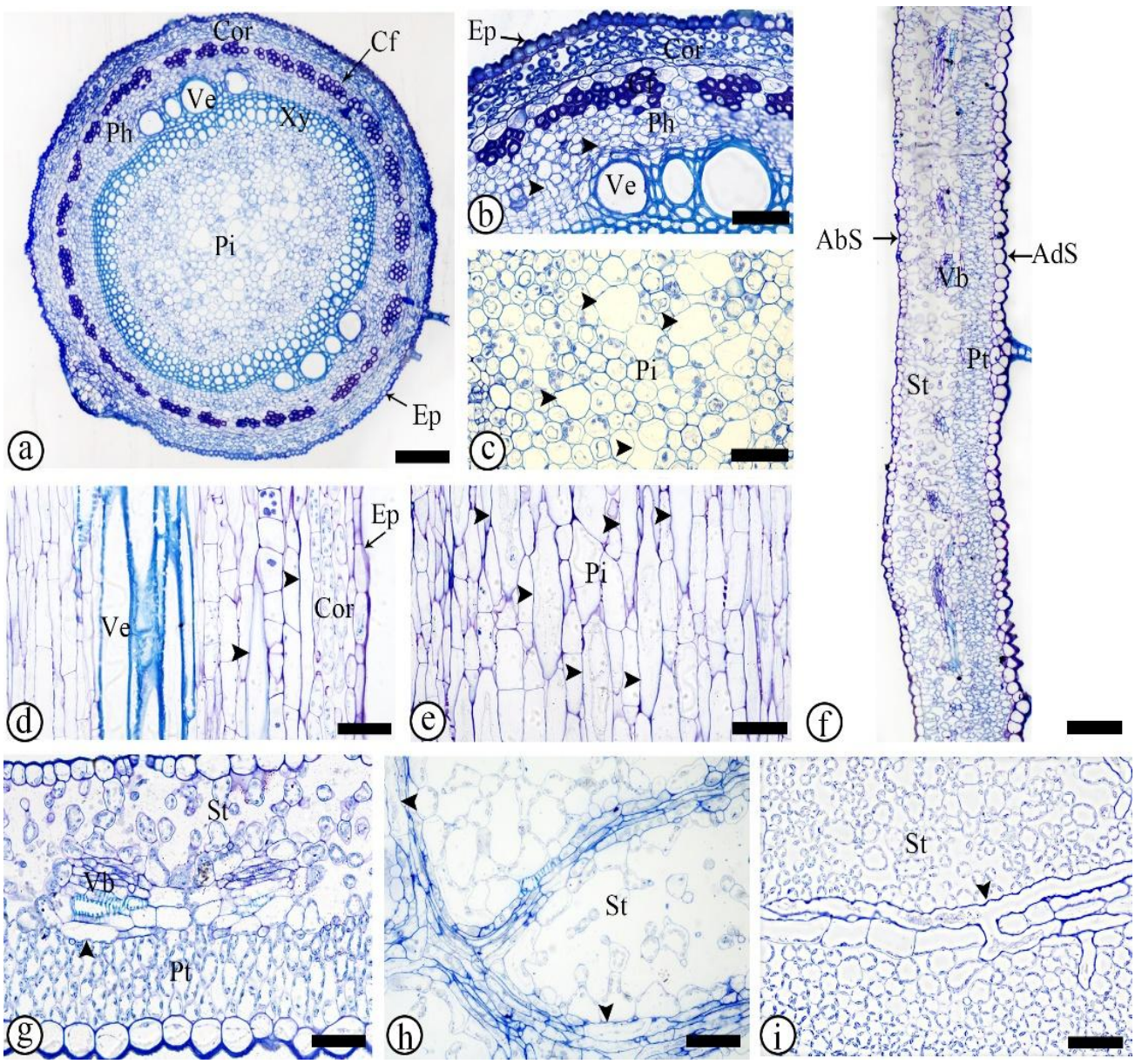

Fig. 1. Microstructure of *C. thesiodes*.
a: Cross section of the stem, bar=386 μm; b: Microstructure of epidermis, cortex, and vascular bundle in stem, bar=180 μm; c: Microstructure of pith in stem, bar=177 μm; d: Longitudinal section of the stem showing non-articulated laticifers in cortex, bar =148 μm; e: Longitudinal section of pith showing non-articulated laticifers, bar=153 μm; f: Cross section of the leaf, bar=362 μm; g: Microstructure of spongy mesophyll, palisade cells, and vascular bundle, bar =141 μm; h: Parallel section of leaf, bar=139μm; i: Branched laticifers in leaf, bar=147 μm. Abbreviations: AbS: Abaxial Side; AdS: Adaxial Side; Cf: Cortical fiber; Cor: Cortex; Ep: Epidermis; Ph: Phloem; Pi: Pith; Pt: Palisade cell; St: Spongy mesophyll; Vb: Vascular bundle; Ve: Vessel; Xy: Xylem. Arrows indicate laticifers.

Sections were examined and photographed using an Olympus BX41 microscope equipped with a digital imaging system. Laticifer dimensions in the pith and cortical regions were measured using Image-Pro Plus 6.0 software. For each species, 20 cross-sectional samples were analyzed to determine laticifer area and diameter. Data were statistically processed using OriginPro 8.0.

Results

Microstructure of *C. thesiodes*: The laticifers in the stem of *C. thesiodes* were identified as non-articulated, unbranched types, distributed throughout both the cortex and pith. On cross sections, the lumen shape was irregular (Fig. 1b), with a volume larger than that of parenchyma cells containing starch granules (Fig. 1c). In longitudinal sections of the stem, cortical laticifers appeared as long tubular structures measuring (432.1±6.5) μm in length, exhibiting invasive growth at the tips (Fig. 1d). The laticifers in the pith were comparatively larger, with an average diameter of (60.1±5.5) μm (Fig. 1e). In the leaves, the laticifers were articulated and branched, predominantly located in the external phloem of the veins, accompanying vascular bundles (Fig. 1g). They were mainly distributed within the spongy mesophyll (Fig. 1h), with the branches typically exhibiting a Y-shaped pattern (Fig. 1i).

Microstructure of *A. curassavica*: Laticifers in the stem of *A. curassavica* were non-articulated and unbranched,

primarily distributed in the cortex with occasional presence in the pith. The laticifer lumen was smaller than that of parenchyma cells containing starch granules (Fig. 2b) and exhibited an irregular shape (Fig. 2c). In longitudinal sections, laticifers in the pith appeared as long tubular structures with invasive growth at the tips and contained abundant crystalline particles (Fig. 2d). Compared to those in the pith, cortical laticifers were smaller in diameter, averaging 9.1 μm (Fig. 2e). In the leaves, laticifers were non-articulated but branched, sparsely distributed in the external phloem and palisade cells (Fig. 2f), with most scattered throughout the spongy mesophyll interior. The laticifers ran parallel to the veins without meta-isomerism, and their branches displayed a Y-shaped morphology (Fig. 2h).

Microstructure of *C. chinense*: Laticifers in the stem of *C. chinense* were non-articulated and unbranched, distributed throughout both the cortex and pith. The laticifer lumen appeared hexagonal in cross section and was surrounded by large parenchyma cells (Fig. 3b). The pith laticifers contained a greater amount of cellular contents (Fig. 3c). In longitudinal section, the pith laticifers were long tubular structures measuring approximately 968.3 μm (Fig. 3d). The diameter of cortical laticifers (40.0 μm) did not significantly differ from those in the pith (Fig. 3e). Leaf laticifers were articulated and branched, with a few located in the external phloem (Fig. 3f) and most distributed within the spongy mesophyll and near the exterior of palisade cells. The articulated laticifer branches exhibited a characteristic Y-shaped structure (Figs. 3g–h).

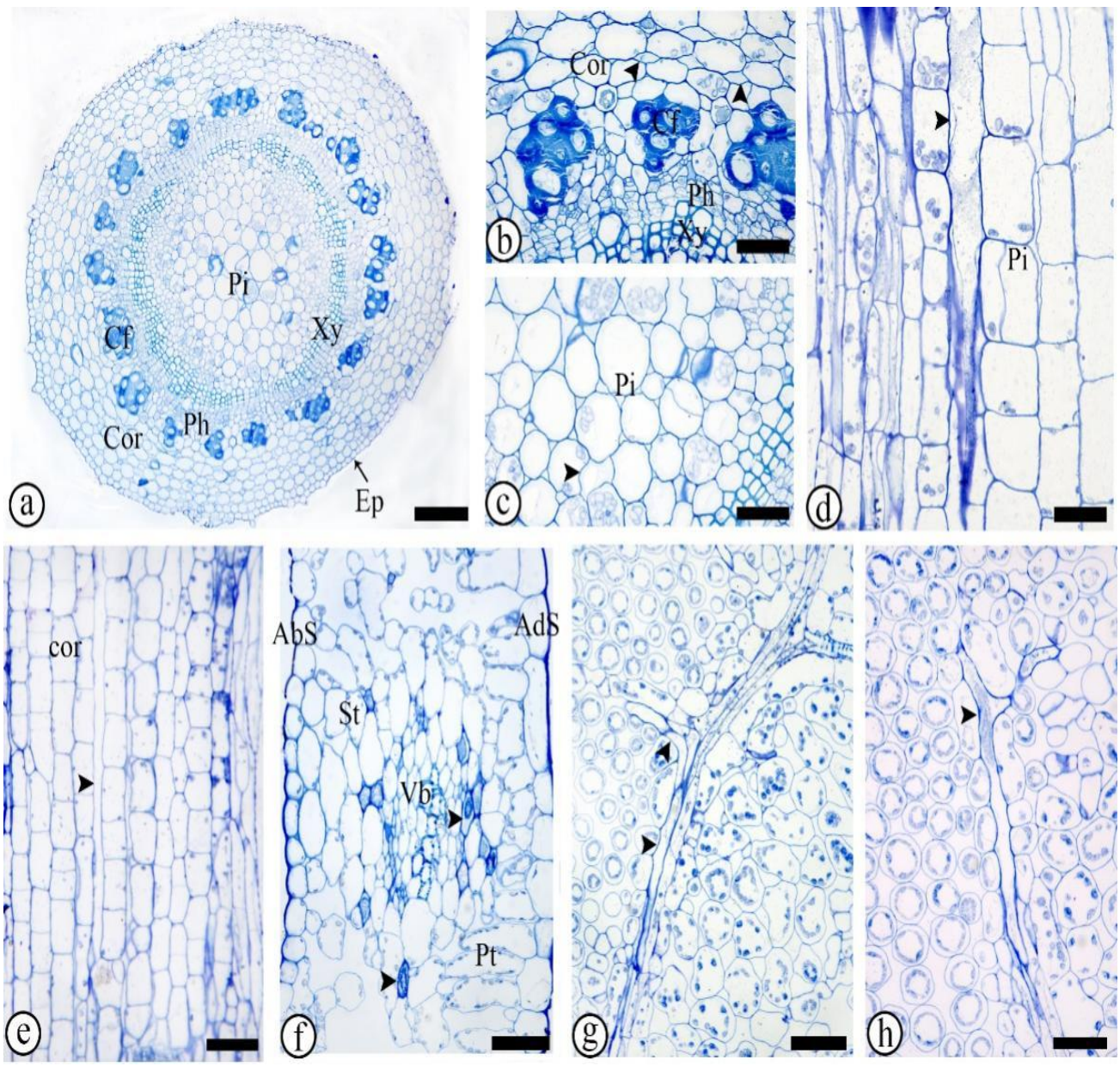

Fig. 2. Microstructure of *A. curassavica.*
a: Cross section of *A. curassavica*, bar=509μm; b: Microstructure of epidermis, cortex and vascular bundle in stem, bar=187μm; c: Microstructure of pith in stem, bar=184μm; d: show the invasive growth of laticifer, bar=172μm; e: Longitudinal section of cortex, show the nonarticulated laticifer, bar=232μm; f: Cross section of leaf, bar=143μm; g: Parallel section of leaf, bar=125μm; h:Branched laticifer in leaf, bar=99μm. Abbreviations: AbS: Abaxial Side; AdS: Adaxial Side; Cf: Cortical fiber; Cor: Cortex; Ep: Epidermis; Ph: Phloem; Pi: Pith; Pt: Palisade cell; St: Spongy mesophyll; Vb: Vascular bundle; Xy: Xylem. Arrow show the laticifer.

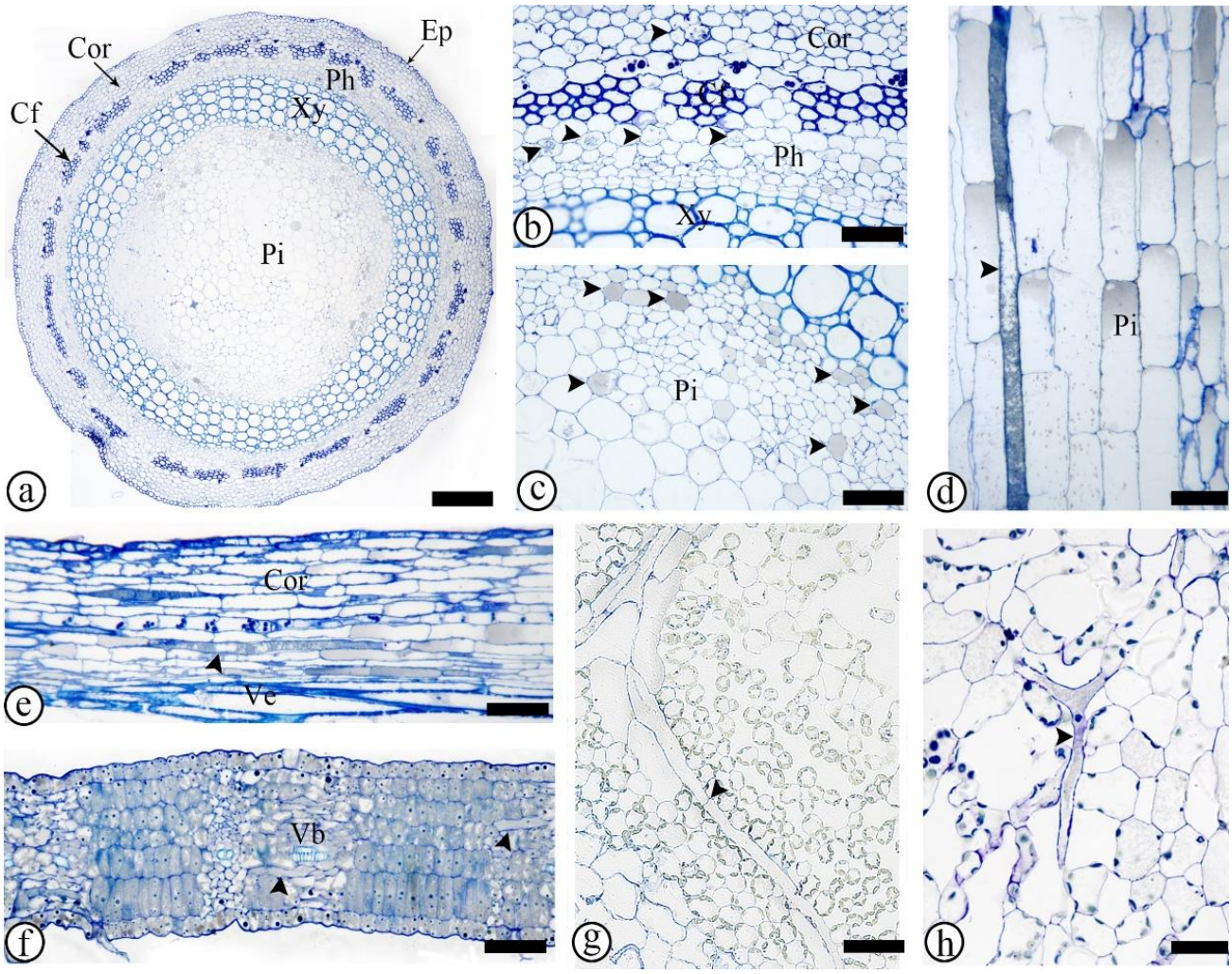

Fig. 3. Microstructure of *C. chinense.*
a: Cross section of stem, bar=599μm; b: Microstructure of epidermis, cortex and vascular bundle in stem, bar=164μm; c: Microstructure of pith in stem, bar=163μm; d: Longitudinal section of pith, show the nonarticulated laticifer, bar=224μm; e: Longitudinal section of cortex, show the nonarticulated laticifer, bar=242μm; f: Cross section of leaf, bar=117μm; g: Parallel section of leaf, show the articulated laticifer, bar=127μm; h: Show the branched laticifer in leaf, bar=105μm. Abbreviations: Cf: Cortical fiber; Cor: Cortex; Ep: Epidermis; Ph: Phloem; Pi: pith; Vb: Vascular bundle; Ve: Vessel; Xy: Xylem. Arrow show the laticifer.

Microstructure of *M. japonica*: Laticifers in the stem of *M. japonica* were non-articulated and unbranched, predominantly found in the cortex with some occurrence in the pith. The lumen exhibited a hexagonal shape and was surrounded by large parenchyma cells containing starch granules (Fig. 4b). Compared to cortical laticifers, those in the pith were larger in size (Fig. 4c). Longitudinal sections revealed that pith laticifers were relatively short tubular structures (~14 μm) with invasive tip growth and contained abundant crystalline particulate matter (Fig. 4d). Cortical laticifers had a larger cross-sectional area averaging 18 μm² (Fig. 4e). In the leaves, laticifers were non-articulated and branched, sparsely located external to the phloem alongside vascular bundles (Fig. 4f), with most distributed around the exterior of palisade cells and within the spongy mesophyll interior. The branches were slender, Y-shaped, and non-articulated (Figs. 4g–h).

Microstructure of *P. sepium*: In *P. sepium*, stem laticifers were non-articulated, unbranched, and scattered throughout the cortex and pith. The cortex laticifer lumens were irregular in shape (Fig. 5b), whereas pith laticifers exhibited a hexagonal cross section (Fig. 5c). Longitudinal sections showed shorter laticifers in the cortex with invasive growth at the tips (Fig. 5d), and longer tubular laticifers in the pith measuring approximately 94.7 μm (Fig. 5e). Leaf laticifers were articulated and branched, primarily located within the spongy mesophyll interior, with branches exhibiting a Y-shaped pattern (Fig. 5g). A minority of laticifers were found external to the phloem and palisade cells (Fig. 5h).

Microstructure of *C. roseus*: Laticifers in the stem of *C. roseus* were non-articulated and unbranched, distributed in both cortex and pith. Cross-sectional views showed irregularly shaped laticifer lumens containing distinct inclusions (Figs. 6b–c). Longitudinal sections revealed long tubular laticifers in the pith with invasive tip growth (Fig. 6d). Cortical laticifers had a larger cross-sectional area (~29 μm²) compared to those in the pith (Fig. 6e). Leaf laticifers were mostly non-branched and articulated, with a few located outside the phloem and palisade cells (Fig. 6f). The majority were distributed within the spongy mesophyll interior (Figs. 6g–h).

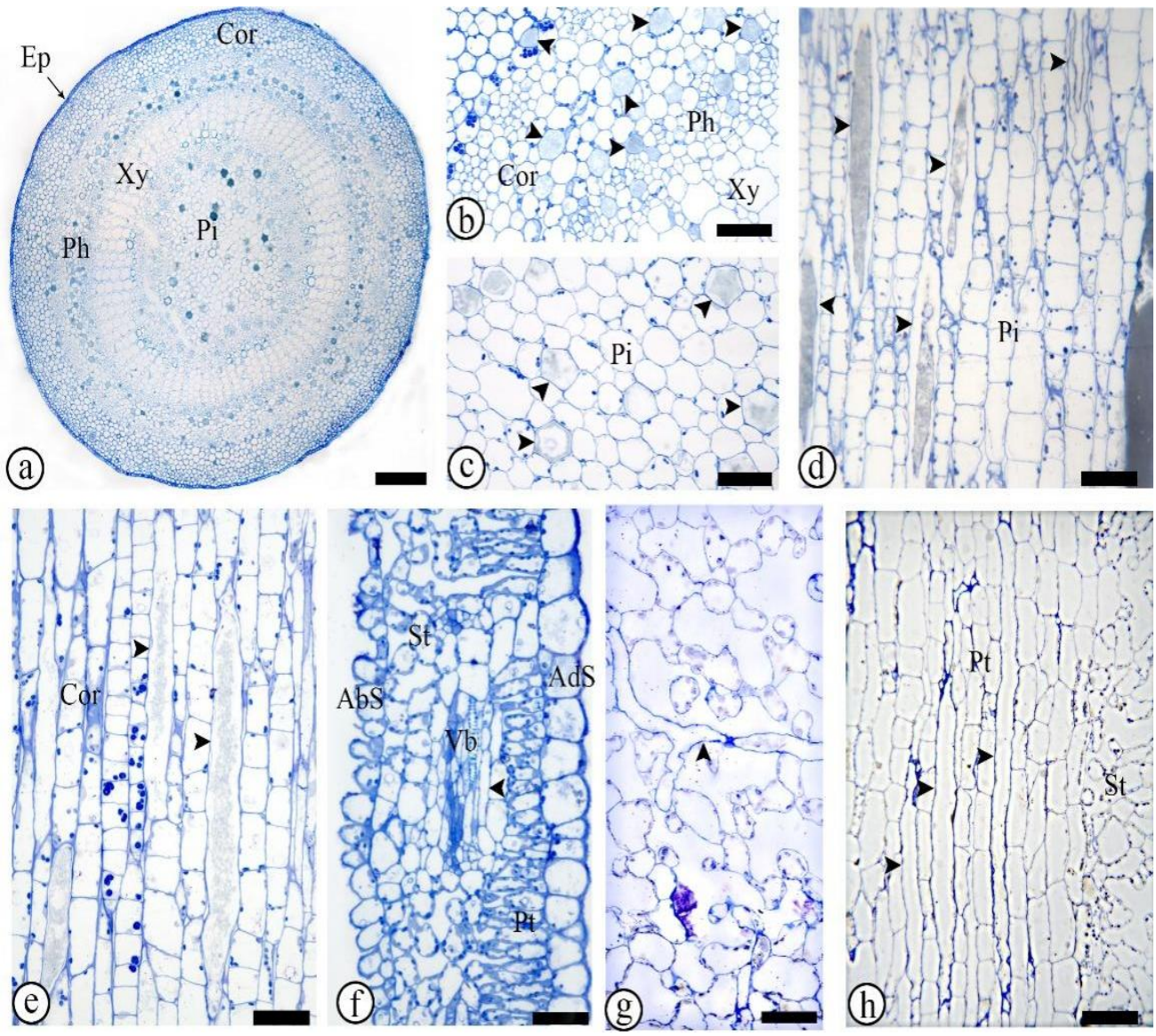

Fig. 4. Microstructure of *M. japonica*
a: Cross section of stem, bar=769μm; b: Microstructure of cortex and vascular bundle in stem, bar=182μm; c: Microstructure of pith in stem, bar=178m; d: Longitudinal section of pith, show the nonarticulated laticifer in pith, bar=227μm; e: Longitudinal section of cortex, show the nonarticulated laticifer in cortex, bar=133μm; f: Cross section of leaf, bar=133μm; g: Parallel section of leaf, show the branched laticifer, bar=133μm; h: Show the nonarticulated laticifer in leaf, bar=259μm. Abbreviations: AbS: Abaxial Side; AdS: Adaxial Side; Cor: Cortex; Ep: Epidermis; Ph: Phloem; Pi: Pith; Pt: Palisade cell; St: Spongy mesophyll; Vb: Vascular bundle; Xy: Xylem. Arrow show the laticifer.

Discussion

Secretory tissues are widely distributed in vascular plants and represent one of the five major tissue systems. These tissues synthesize, store, or release specialized organic and inorganic secondary metabolites, which can be retained within the plant body, exuded to intercellular spaces, or secreted externally (Fahn, 1988). Based on this functional criterion, secretory structures are classified as external or internal, with laticifers representing a prominent type of internal secretory structure. Laticifers occur in more than 12,500 plant species across 22 families (Chaffey, 2007) and exhibit diverse developmental origins, resulting in notable structural variation (Farrell & Mitter, 1991). This diversity has been recognized as an important morphological indicator for phylogenetic analyses (Hagel *et al*., 2008; González. 2022).

The family Apocynaceae, belonging to *Gentianales* within the asterid clade, comprises approximately 415 genera and over 4,500 species (Endress *et al*., 2014; Bremer *et al*., 2016). In this family, laticifers are a defining feature and have been reported in both woody and herbaceous taxa (Chaffey, 2007; Naidoo, 2020). For decades, Apocynaceae laticifers were considered exclusively non-articulated (H, 1908), a view supported by anatomical studies in multiple genera (Mahlberg, 1961; Murugan, 1987; Inamdar *et al*., 1988; Roy & De, 1992; Appezzato-da-Glória & Estelita, 2014; Sacchetti, 1999; Serpe *et al*., 2001; Souza, 2021). However, more recent research has challenged this paradigm. Articulated laticifers have been identified in *Forsteronia australis* and *F. bicuspidata* (DeMarco *et al*., 2006), and in Mandevilla (Lopes *et al*., 2009), providing the first confirmed evidence of this type within the family.

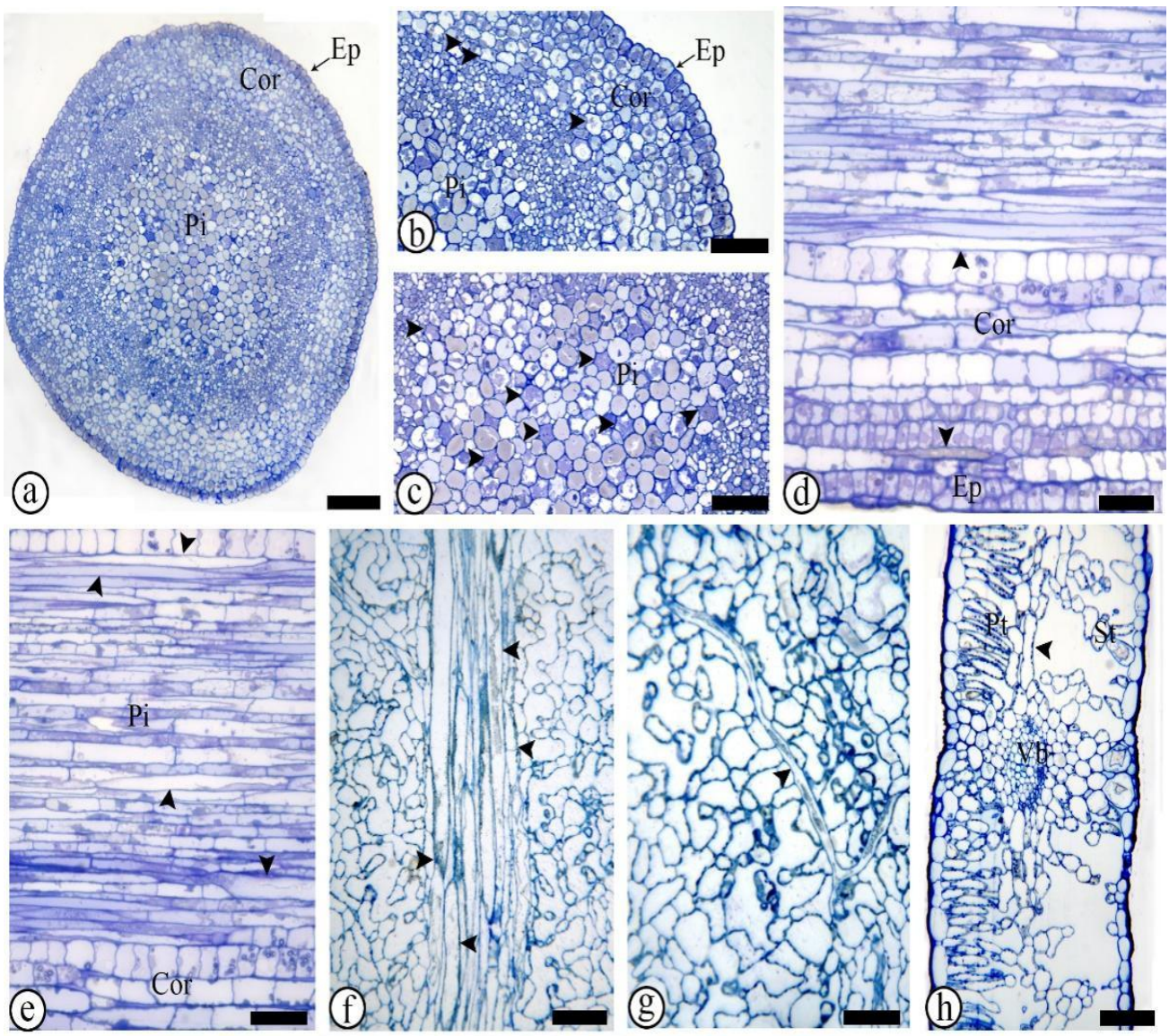

Fig. 5. Microstructure of *P. sepium.*

a: Cross section of stem, bar=298μm; b: Microstructure of epidermis and cortex in stem, bar=160μm; c: Microstructure of pith in stem, bar=159m; d: Longitudinal section of cortex, show the nonarticulated laticifer, bar=107μm; e: Longitudinal section of pith, show the nonarticulated laticifer, bar=127μm; f: Parallel section of leaf, show the articulated laticifer, bar=286μm; g: Show the branched laticifer in leaf, bar=189μm; h: Cross section of of leaf, show the laticifer outside of plisade cell, bar=260μm. Abbreviations: Ep: Cor: Cortex; Epidermis; Pi: Pith; Pt: Palisade cell; Vb: St: Spongy mesophyll; Vascular bundle. Arrow show the laticifer.

Our results further expand this understanding. Among the six species examined, we found both articulated and non-articulated laticifers, with variation in branching patterns (branched vs. unbranched) and organ-specific distribution. For example, leaves of *Cynanchum thesiodes*, *Periploca sepium*, and *C. chinense* possessed articulated branched laticifers, whereas leaves of *Asclepias curassavica* and *Metaplexis japonica* exhibited non-articulated branched laticifers with Y-shaped branching. Such structural variability aligns with the diversity reported in Apocynaceae, which ranges from simple non-articulated forms to complex articulated networks (Arruda *et al*., 2019), likely reflecting functional adaptation to ecological pressures such as herbivory (Konno & Agrawal, 2021).

The taxonomic implications of these findings are significant. Historically, laticifer type was not considered a reliable diagnostic character within Apocynaceae due to perceived uniformity (non-articulated type). However, our comparative data indicate that laticifer architecture may correlate with phylogenetic subdivisions, particularly at the subfamily or tribal level. This is consistent with the suggestion that secretory structure traits, when combined with molecular phylogenies, can provide robust synapomorphies for clade delimitation (Rando & Pirani, 2021). Moreover, the occurrence of articulated laticifers in some taxa may represent either an independent evolutionary acquisition or a retained ancestral condition within Gentianales (González. 2022).

Functionally, the branching patterns and distribution of laticifers may also have adaptive significance. Y-shaped branching in non-articulated laticifers, as seen in *A. curassavica* and *M. japonica*, may facilitate rapid latex flow to wounded sites, enhancing defense against herbivores and pathogens (Agrawal & Konno, 2020). In contrast, articulated laticifers in leaves could provide more

extensive defensive coverage across the mesophyll tissue, potentially deterring both chewing and piercing–sucking insects (Diego, 2014).

In summary, our findings confirm that laticifer diversity in Apocynaceae is greater than previously recognized, encompassing multiple structural types and branching forms within a single family. These anatomical traits, in conjunction with molecular evidence, hold promise for refining the taxonomy of Apocynaceae and for elucidating evolutionary patterns in latex-producing plants. Future research integrating developmental genetics, chemical profiling of latex, and expanded taxon sampling will be essential to fully resolve the phylogenetic and ecological significance of laticifer diversity in this family.

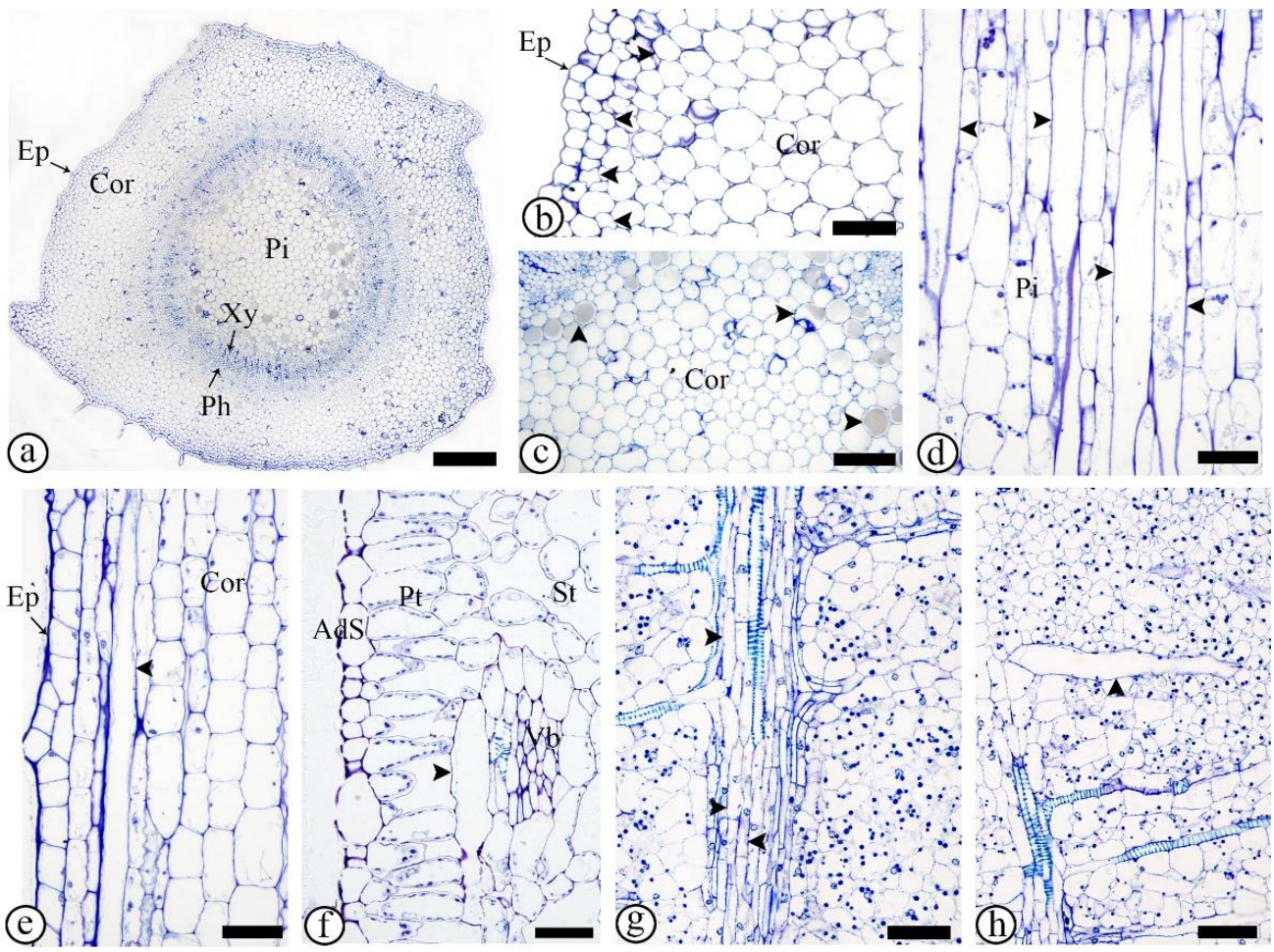

Fig. 6. Microstructure of *C. roseus.*
a: Cross section of stem, bar=889μm; b: Microstructure of epidermis and cortex in stem, bar=173μm; c: Microstructure of pith in stem, bar=340μm; d: Longitudinal section of pith, show the nonarticulated laticifer, bar=128μm; e: Longitudinal section of cortex ,show the nonarticulated laticifer, bar=136μm; f: Cross section of leaf, bar=140μm; g: Parallel section of leaf, show the articulated laticifer, bar=130μm; h: Show the nonbranched laticifer in leaf, bar=143μm. Abbreviations: AdS: Adaxial Side; Cor: Cortex; Ep: pidermis; Ph: Phloem; Pi: pith; Pt: Palisade cell; St: Spongy mesophyll; Vb: Vascular bundle; Xy: Xylem. Arrow show the laticifer.

Acknowledgements

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