Diversity of vascular variants and xylem structure across the Convolvulaceae and its systematic implications

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Abstract: Convolvulaceae is the family of flowering plants that is popularly known as the morning glory family due to its beautiful flowers that open in the morning. The family is dominated by non-self-supporting, herbaceous vines, climbers, or woody lianas while erect growth forms are rare. Its members are widely distributed worldwide (both in tropics and temperate climates) and more often cultivated for various purposes such as the aesthetic importance of flowers, as a food crop and for folk/traditional medicines. The present study characterizes several such anatomical traits that are ontogenetically unique or exclusively reported in Convolvulaceae, and if reported in other eudicots then the feature is rare. Along with common anatomical traits, the present study compiles several features that are extremely rare or hitherto unreported for any other eudicots. Some of them are unifacial and bifacial natures of the intraxylary cambium, formation of interxylary cambium, neo-formed (external) vascular cylinders, isolated xylem strands, perforated rays, radial sieve elements and formation of phloem wedges. Detailed ontogeny of vascular variants, stem anatomy, structure and composition of vascular elements is correlated with eco-physiology. These alterations are attributed to histological alterations towards the climbing habit to cope with narrow stems in cross sectional view, for fulfilling the demand *vs.* supply of water, minerals and photosynthate supply from source to sink. In conclusion, members of the family are well adapted to the climbing habit and various ecological conditions starting from aquatic habitat to pure desert conditions. Different members show all possible combinations of multiple vascular variants for the survival under various growth forms, biogeographic regions or extreme habitats like aquatic, water logging, rocky substratum, and xerophytic conditions. All these features may also have taxonomic implications, but not much anatomical data is available on the family because much of the diversity is still hidden in plants distributed in the tropics. As per recent trends, merging or separating the taxa exclusively based on molecular tool is a fashion that neglects the basic principles of plant biosystematics.

Introduction

Stem or wood anatomy is one of the important disciplines that provide additional diagnostic characters for the delimitation of taxa and helps in the identification of specimens in the absence of the reproductive structure. Mathieu (1853) used wood anatomy to describe the forest trees in his inspiring book entitled *Florae Foresteire* (Maiti *et al.,* 2012). Subsequently, series of books (Bureau, 1864; De Bary, 1884; Schenck, 1893; Solereder, 1908; Pfeiffer, 1926; Metcalfe & Chalk, 1950, 1983; Carlquist, 1988, 2001) and research articles (Carlquist & Hanson, 1991; Dobbins, 2005; Pace *et al.,* 2009; Pace & Angyalossy, 2013; Bastos *et al.,* 2016; Cunha Neto *et al.,* 2020; Quintanar-Castillo & Pace, 2022; Yang *et al.,* 2023) appeared on the anatomy in relation to systematics. Perusal of above cited literature indicates that plant anatomy has pivotal role in plant systematics but

anatomical data is not available on all the growth forms. Particularly, stem and wood anatomy of climbing species has always been neglected and have received relatively less attention as compared to trees (Letcher & Chazdon, 2009; Isnard & Silk, 2009) may be due to lack of commercial value of their wood. Exclusive books on "*Biology of Vines*" (Putz & Mooney, 1991) and "*Ecology of Lianas*" (Schnitzer *et al.*, 2015), presented chapters specifically devoted to anatomy of liana/climbing plant. Despite anatomy being one of the oldest disciplines, a perusal of the literature indicates that the anatomy of climbing species has always been neglected by earlier researches (Rajput *et al.*, 2017) due to their relatively less economic significance as compared to timber trees (Carlquist, 2009). Therefore, the present study deals with stem anatomy of selected species of Convolvulaceae that is dominated by the climbing members.

Recently, the family Convolvulaceae has attracted the interest of various researchers worldwide, and the use of molecular tools has contributed significantly to understand the family's phylogeny. According to Stefanovic *et al.* (2003), the family is monophyletic and it is a sister family to the Solanaceae (Wood *et al.*, 2022). Application of molecular tools, although played a pivotal role in understanding the phylogeny of Convolvulaceae, sometimes it contradicts the aims and objectives of systematics. One of the main objectives of systematics is to facilitate the identification of organisms. For example, from anatomical and habit points of view *Argyreia* and *Ipomoea* stand distinct from each other but *Argyreia* Lour. is merged with *Ipomoea* L. (Muñoz-Rodriguez *et al.*, 2019), based only on molecular data without considering other morphological, anatomical or other characters. Similarly, the genus *Merremia* is separated into *Camonea* Raf., *Decalobanthus* Ooststr., *Distimake* Raf., and *Merremia* Dennst. ex Endl. (Simões & Staples, 2017) by leaving some of the species as unresolved (Wood *et al.*, 2022). Subsequently, Pisuttimarn *et al.* (2023), shifted *Camonea vitifolia* (Burm.f.) A.R.Simões & Staples as *Distimake*

vitifolius (Burm.f.) Pisuttimarn & Petrongari. Therefore, there is a need to include data from various fields like anatomy, cytology, embryology, phytochemistry etc., instead of single character. Unfortunately, comparable information on stem histology is either scanty or unavailable while some species are overlooked by the earlier researchers (Ramoliya *et al.*, 2022; Kapadane *et al.*, 2023).

The family Convolvulaceae consists of 59 genera and nearly 1900 species that are found in the tropics and warm temperate regions (Staples & Traiperm, 2017). It is dominated by climbing members including some herbs and shrubs (Simões & Staples, 2017) with few trees or small trees (Wood *et al.*, 2022). From them, nearly 160–170 species are distributed in the Indian subcontinent, which includes 90 species of *Ipomoea* (including seven cultivated species of *Ipomoea* and genus *Argyreia*) as the major genera, followed by 12 species of *Cuscuta* L., eight species of *Merremia* Dennst. ex Endl. and *Erycibe* Roxb., in each, while other taxa are represented by less than eight species (Wood *et al.*, 2022). Recently, Cunha Neto (2023), classified vascular variants into: i) procambial, ii) cambial variant and iii) ectopic cambia. Based on ontogeny, a similar classification and much simplified classification *i.e.*, primary (promeristem/procambium) and secondary (originating from the secondary tissue) variant was proposed by Patil *et al.* (2011). Histologically, all the representatives are unique and most of them are characterised by the presence of vascular variants like successive cambia, inter- and intraxylary phloem, inter-and intraxylary cambia, functionally inverse cambia, isolated strands of xylem in conjunctive tissues, non-lignified parenchyma wedges or furrowed xylem (Metcalfe & Chalk, 1950; Obaton, 1960; Fukuda, 1967; Lowell & Lucansky, 1986; Carlquist & Hanson, 1991; Rajput *et al.*, 2008, 2014 a-c 2021, 2024; Terrazas *et al.*, 2011; Lekhak *et al.*, 2018; Rajput *et al.*, 2021; Ramoliya *et al.* 2022, Kapadane *et al.*, 2023; Lawand *et al.*, 2023). However, some of them like *Bonamia, Breweria,*

Convolvulus, *Ipomoea fistulosa*, *Mina* and *Wilsonia* (Carlquist & Hanson, 1991), *Neuropeltis* and *Prevostea* (Obaton, 1960) and *Cressa* (Rajput *et al.*, 2014c), *Argyreia capitiformis* (Poir.) Ooststr., *A. coonoorensis* W.W.Sm. & Ramaswami, *A. hirsuta* Wight & Arn., *A. involucrata* C.B.Clarke and *A. pilosa* Wight & Arn. (Kapadane, 2022) lack successive cambia while the presence of intraxylary phloem is universal in all the members investigated so far. In addition to above mentioned characters, *Argyreia nervosa* (Burm.f.) Bojer differs from other species and is characterised by the presence of medullary bundles. Therefore, the main aim of the present investigation is to make a comprehensive report on the occurrence of vascular variants and structure of the secondary xylem documented so far from the family Convolvulaceae.

Terminology used in the present work

Wood and bark description follow the terminology of the International Association of Wood Anatomists (IAWA Committee, 1989) and IAWA Bark features (Angyalossy *et al.*, 2016). Some of the specialized terms are mentioned as follows:

External vascular cylinders or neo-formed vascular cylinders: Term neo-formation is adopted from Acevedo-Rodríguez (2015), and Angyalossy *et al.* (2012, 2015), to refer to the multiple cambia developed around the original vascular cylinder, in cortex or phloem of stems and roots.

Fibriform vessels: The term fibriform vessels is adopted from Carlquist (1984), to refer to the vessel elements that are narrow in diameter like tracheid but possess small perforation plates at terminal, sub-terminal ends or on the lateral walls.

Intraxylary phloem (also referred to as *internal or perimedullary phloem*): Phloem developed on the inner margin of the protoxylem; in other words, phloem that develops on the periphery of the pith.

Intraxylary cambium: Segment of cambium that initiates on the inner margin of the protoxylem and intraxylary proto-phloem on the periphery of the pith (Carlquist, 1988, Carlquist & Hanson, 1991). Functionally, this cambium may be unidirectional (producing only phloem centripetally or both xylem and phloem in the same direction *i.e.*, centripetally) or may be bidirectional that produce secondary xylem externally and phloem in centripetal in direction.

Interxylary phloem (also referred to as *included phloem*): The term interxylary phloem is adopted from Carlquist (2013) to refer to the strands of phloem that are enclosed within the secondary xylem of stem and phloem that is formed from the single vascular cambium.

Interxylary cambium: A small segment of cambium that initiate by dedifferentiation of non-lignified parenchyma embedded within the secondary xylem (Patil *et al.*, 2011; Pace *et al.*, 2018; Rajput *et al.*, 2014a, 2023).

Inverse cambium: The term inverse cambium is used to describe the segments of cambium that produce secondary xylem (centrifugally) and phloem (centripetally) in inverse polarity as compared to regular vascular cambium (Rajput *et al.*, 2008). Inverse cambium initiates from the conjunctive tissues located on the inner margin of the secondary xylem formed by successive cambia

Isolated xylem strands: Isolated xylem strands are referred to the lignified xylem elements (fibres and vessels) deposited by dedifferentiation of conjunctive tissues located between the adjacent successive cambia. These strands lack phloem and are exclusively composed of secondary xylem elements (Kapadane *et al.*, 2023).

Non-lignified parenchyma wedges: Non-lignified axial (and radial) parenchyma wedges are the discreet variant portions of the secondary xylem that derive from the small segments of the

vascular cambium due to the shift of the regular cambial activity that deposits non-lignified parenchyma (both axial and radial) towards the xylem side after the formation of few lignified elements of the secondary xylem (Thacker *et al.,* 2024).

Vascular variants: The term vascular variant is adopted from Cunha Neto (2023). Vascular variants are defined by their developmental origin (instead of anatomical patterns), allowing the classification of alternative vascular ontogenies into three categories: (i) *procambial variants*, (ii) *cambial variants* and (iii) *ectopic cambia*. Each category includes several anatomical patterns.

Wedge and inter-wedge cambium: Both terms *i.e.,* wedge and inter-wedge cambium are adopted from Quintanar-Castillo & Pace (2022). Wedge cambium is the small segment of the vascular cambium that produce parenchyma wedges (*i.e.,* non-lignified parenchyma externally and internally) while inter-wedge cambium is the portion of the cambium located between adjacent wedges. These cambial portions that deposits regular secondary xylem and phloem are referred to as inter-wedge segments of the cambium.

Materials and methods

Samples from different representatives of the family Convolvulaceae were collected from different biogeographic zones of India (Table 1). The voucher specimens of these samples is deposited in herbarium of Shivaji University Kolhapur (SUK) and BARO herbarium of the M.S. University of Baroda, Vadodara. To obtain various developmental stages, samples were collected at various heights from five individuals of each species. Collected stem pieces were trimmed into suitable size and fixed immediately in FAA (Berlyn & Miksche, 1976). After 24-36 hours of fixation in in FAA, they were transferred to 70% alcohol for further processing and storage. Depending on stem diameter and sample hardness, they were sectioned either

with the help of sliding (Leica SM2010R) and rotary (Leica RM 2035) microtome. Details of the histological preparations, sectioning, staining, dehydration and mounting of the permanent slides is described in detail in our earlier studies (Ramoliya et al., 2022; Kapadane et al., 2023; Rajput et al., 2024).

Results and Discussion

The family Convolvulaceae is dominated by the climbing members while herbs and shrubs (Simões & Staples, 2017) and trees or small trees are rare (Wood *et al.*, 2022). An increase in stem diameter in some species of the Convolvulaceae is achieved by regular secondary growth while several taxa show the presence of various kinds of vascular variants such as successive cambia, inter- and intraxylary phloem, inter- and intraxylary cambia, functionally inverse cambia, neo-formed vascular cylinders, isolated strands of xylem in conjunctive tissues, non-lignified parenchyma wedges and furrowed xylem (Schenck, 1893; Metcalfe & Chalk, 1950; Carlquist & Hanson, 1991; Rajput *et al.*, 2008; Terrazas *et al.*, 2011; Rajput *et al.*, 2021, 2024; Kapadane *et al.*, 2023; Ramoliya *et al.*, 2022). Recently, Cunha Neto (2023) proposed the term vascular variants to growth forms (*i.e.*, procambial variants, cambial variants and ectopic cambia) that deviates from the typical growth trajectories of the putative ancestors of seed plants. An attempt was made to fit the vascular variants of the Convolvulaceae in the classification proposed by Cunha Neto (2023). As per the concept of vascular variants, procambial variants include primary intraxylary phloem (observed in all the members investigated) and medullary bundles (in *Argyreia nervosa*), cambial variants include formation of successive cambia, inter- and intraxylary cambium, functionally inverse cambia while formation of vascular (neo-formed) cylinders external to pericycle in *Camonea kingii* (Prain) A.R.Simões & Staples, *C. umbellata* (L.) A.R.Simões & Staples, *Distimake*

Table 1. List of species, place of collection, type of vascular variants reported and references for detailed information: Intraxylary phloem (IP), Intraxylary cambium (IC), Interxylary phloem (IxP), Interxylary cambium (IxC), Inverse cambium (InC), Isolated Xylem Strands (IXS), Medullary bundles (MB); Parenchyma wedges (PW); Neo-formed vascular cylinders (NVC), Regular secondary growth (RSG), Successive cambia (SC).

SL. No.	Name of the Taxon	Place of collection	Type of vascular variants	References for detailed information
1	*Argyreia boseana* Santapau & Patel	Amba forest, Tillari Nagar, Radhanagari	SC, IP, IC, InC, IxP, IXS, PW	Kapadane, 2022
2	*A. capitiformis* (Poir) Ooststr.	Damparengpui (Mizoram)	RSG, IP, PW	Kapadane, 2022; Lawand *et al.*, 2023
3	*A. coonoorensis* W.W.Sm. & Ramaswami	Coonoor, Ooty	RSG, IP, PW	Kapadane, 2022; Lawand *et al.*, 2023
4	*A. elliptica* (Roth) Choisy	Panhala (Maharashtra)	SC, IP, IC, InC, IxP, IXS, PW	Kapadane *et al.*, 2023; Lawand *et al.*, 2023
5	*A. hirsuta* Wight & Arn.	Choral Ghat (Karnataka)	RSG, IP, PW	Kapadane, 2022; Lawand *et al.*, 2023
6	*A. involucrata* C. B. Clarke	Patgaon, Maharashtra	RSG, IP, PW	Kapadane, 2022
7	*A. lawii* C. B. Clarke	Bababudhan (Karnataka)	SC, IP, IC, InC, IxP, PW	Kapadane, 2022; Lawand *et al.*, 2023
8	*A. nervosa* (Burm. f.) Bojer	Junagadh (Gujarat)	SC, MB, IP, IC, IxP, PW	Kapadane *et al.*, 2019
9	*A. pilosa* Wight & Arn.	Tirthahalli (Karnataka)	RSG, IP, PW	Kapadane, 2022; Lawand *et al.*, 2023
10	*Camonea kingii* (Prain) A.R.Simões & Staples	Radhanagari (Maharashtra)	RSG, IP, IC, PW,	Ramoliya *et al.*, 2022; Rajput *et al.*, 2024
11	*C. umbellata* (L.) A.R.Simões & Staples	Ruyad Village, Bhandara, Maharashtra	RSG, IP, IC, IxC, PW,	Ramoliya *et al.*, 2022; Rajput *et al.*, 2024
12	*Convolvulus prostratus* Forssk. (Synonym: *Convolvulus deserti* Hochst. & Steud. Ex Baker & Randle)	MSU Campus, Vadodara (Gujarat)	RSG, IP, PW	Unpublished
13	*Cressa cretica* L.	Bhavnagar (Gujarat)	RSG, IP, IC	Rajput *et al.*, 2014c
14	*Distimake aegyptius* (L.) A.R.Simões & Staples	Dinodhar, Kachchh (Gujarat)	SC, IP, IC, PW	Ramoliya *et al.*, 2022; Rajput *et al.*, 2024
15	*D. dissectus* (Jacq.) A.R.Simões & Staples	Bhorkheda, Shivaji Univ. Kolhapur (Maharashtra)	SC, IP, IC, IxC, PW	Ramoliya *et al.*, 2022; Rajput *et al.*, 2024
16	*D. quinquefolius* (L.) A.R.Simões & Staples	Botanical Garden, MSU campus, Vadodara (Gujarat)	SC, IP, IC, InC, PW	Ramoliya *et al.*, 2022; Rajput *et al.*, 2024
17	*D. vitifolius* (Burm.f.) Pisuttimarn & Petrongari	Dharampur, Dang Forest (Gujarat)	RSG, IP, IC, IxC, NVC, PW	Ramoliya *et al.*, 2022; Rajput *et al.*, 2024
18	*Evolvulus alsinoides* (L.) L.	MSU Campus (Gujarat)	RSG, IP	Unpublished
19	*Hewittia malabarica* (L.) Suresh	MSU Campus (Gujarat)	SC, IP, IC, IxP, NVC, PW	Rajput *et al.*, 2021

SL. No.	Name of the Taxon	Place of collection	Type of vascular variants	References for detailed information
20	*Ipomoea aquatica* Forssk.	Dabhoi, on the way to Poicha, Banni (Gujarat)	RSG, IP	Thacker, 2024
21	*I. carnea* Jacq. (*I. fistulosa*)	Dabhoi, Vadodara (Gujarat)	RSG, IP	Unpublished
22	*I. corymbosa* (L.) Roth (*Turbina corymbosa* (L.) Raf.)	Lead Bot. Garden, Shivaji University, Kolhapur	SC, IP, InC, IC, PW	Lekhak *et al.*, 2018
23	*I. eriocarpa* R.Br.	Banni (Kachchh, Gujarat)	SC, IP, IC, IxP, PW	Thacker *et al.*, 2024
24	*I. hederifolia* L.	Dediapada, Netrang (Gujarat)	SC, IP, IC, IxP, PW	Rajput *et al.*, 2008, 2013
25	*I. obscura* (L.) Ker Gawl	Bhorkheda (Maharashtra)	SC, IP, PW	Rajput *et al.*, 2014a
26	*I. pes-caprae* (L.) R.Br.	Veraval, Somnath (Gujarat)	SC, IP, PW	Rajput et *al.* 2014b
27	*I. muricata* (L.) Jacq. (*I. turbinata*)	Bhorkheda (Maharashtra)	SC, IP, InC, PW	Rajput & Gondaliya, 2017
28	*Jacquemontia pentanthos* (Jacq.) G.Don	MSU Campus, Vadodara (Gujarat)	RSG, IP, PW, IxP,	Unpublished
29	*Merremia emarginata* (Burm.f.) Hallier.f.	Bhorkheda (Maharashtra); Lafani (Gujarat)	RSG, IP, PW	Ramoliya *et al.*, 2022; Rajput *et al.*, 2024
30	*M. hederacea* (Burm.f.) Hallier.f.	Shahada (Maharashtra), Vadodara (Gujarat)	SC, IP, IC, InC, PW	Ramoliya *et al.*, 2022; Rajput *et al.*, 2024
31	*Operculina turpethum* (L.) Silva Manso	Patanvav, Vadodara (Gujarat)	SC, IP, IC, PW	Ramoliya, 2024
32	*O. ventricosa* (Bertero) Peter (*O. tansaensis* Santapau & V.Patel)	Tansanea Lake (Maharashtra)	RSG, IP, PW	Ramoliya, 2024
33	*Rivea hypocrateriformis* (Desr.) Choisy	Bhorkheda (Maharashtra)	SC, IP, PW	Rajput, 2016
34	*Xenostegia tridentata* (L.) D.F. Austin & Staples	TBGRI, Thiruvanantpuram (Kerala)	RSG, IP, PW	Ramoliya, 2024
35	*Seddera latifolia* Hochst. & Steud.	Dinodhar, Kachchh (Gujarat)	RSG, IP	Unpublished

quinquefolius (L.) A.R.Simões & Staples, *D. vitifolius* (Burm.f.) Pisuttimarn & Petrongari, *Hewittia malabarica* (L.) Suresh, and formation of isolated strands of xylem strands exclusively composed of lignified xylem elements in *Argyreia elliptica* (Roth) Choisy may be treated as ectopic cambia. Initiation of multiple cambial variants or differential activity of the vascular cambium resulted in the formation of various shapes and outline of the stems in cross-sectional view. Therefore, stems may be oval circular, variously lobbed or flat like ribbon. However, these shapes may be a conserved character, as in the case of *Argyreia boseana* Santapau & V.Patel while in taxon like *Ipomoea corymbosa* (L.) Roth, same stem sample may share various conformation in transverse view (Fig. 1a–f; 2a–f). The occurrence of circular stem conformations is a common

feature in most of the vascular plants but other forms of the stem outlines may be associated with the climbing habit and that may be helpful for twisting/entwining the stem around the supporting host/object (Rajput *et al.*, 2012).

In all the species enlisted in Table 1, initiation of procambium from the apical tissue led to the development of several vascular bundles. The number of vascular bundles vary from species to species; all these vascular bundles were interconnected by forming an interfascicular cambium and established a complete ring of the vascular cambium. (Fig. 3a). Like most of the eudicots, this cambium was functionally regular and deposited the secondary xylem internally and the secondary phloem externally (Fig. 3a). As mentioned in Table 1, considerable number of species of Convolvulaceae increases their stem diameter by regular (single vascular cambium remains function throughout the life) secondary growth while others show various kinds of vascular variants. In herbaceous members like *Argyreia capitiformis* (Poir.) Ooststr., *A. coonoorensis* W.W.Sm. & Ramaswami, *A. hirsuta* Wight & Arn., *A. involucrata* C.B.Clarke, *A. pilosa* Wight & Arn. (Lawand *et al.*, 2023), *Convolvulus deserti* Hochst. & Steud. ex Baker & Rendle, *Cressa cretica* L. (Rajput *et al.*, 2014c), *Evolvulus alsinoides* (L.) L., *Ipomoea aquatica* Forssk. (unpublished data), *I. kotschyana* Hochst. ex Choisy and *Merremia emarginata* Hallier f. stems were thin with narrow diameter and showed regular secondary growth. Majority of them are either prostrate or spreading on ground and most often die during drier part of the year. In contrast, species like *Camonea kingii* (Prain) A.R.Simões & Staples, *Distimake dissectus* (Jacq.) A.R.Simões & Staples, *D. vitifolius* (Burm.f.) Pisuttimarn & Petrongari, *Jacquemontia pentanthos* (Jacq.) G.Don, *Ipomoea carnea* Jacq., *Operculina ventricosa* (Bertero) Peter and *Seddera latifolia* Hochst. & Steud. are perennial though possessed relatively thick (*i.e.*, 10–15 mm) stems, they were characterised

by the presence of regular secondary growth. They showed regular secondary growth except for the presence of the intraxylary phloem. Based on the present study and a perusal of the literature, it is observed that all members of the family are characterised by the presence of intraxylary phloem as a consistent feature while other types of variants may be species specific and may be present or absent depending on their growth forms. Besides formation of the intraxylary phloem from the procambial derivatives, additional intraxylary sieve elements differentiate from the mature pith cells (Tamaio *et al.*, 2021; Ramoliya *et al.*, 2022). These later formed sieve elements are secondary in origin; therefore, they neither fit into the procambial variant or other types of vascular variants as described by Cunha Neto (2023). Rest of the taxa mentioned in the Table 1 increase their stem diameter either by forming successive cambia (e.g., *A. cuneata* Ker Gawl., *A. lawii* C.B.Clarke (Fig. 1a), *A. nervosa* (Burm.f.) Bojer (Fig. 1b), *Distimake aegyptius* (L.) A.R.Simões & Staples, *Hewittia malabarica* (L.) Suresh (Fig. 1e), *Ipomoea corymbosa* (L.) Roth (Fig. 1d), *I. eriocarpa* R.Br., *I. hederifolia* L. (Fig. 1f), *I. pes-tigridis* L., *Merremia hederacea* (Burm.f.) Hallier f. (Fig. 2e), *Operculina turpethum* (L.) Silva Manso, *Rivea hypocrateriformis* Choisy (Fig. 2d) and or by forming external vascular cylinders.

Ontogeny of vascular variants: As mentioned earlier, Convolvulaceae is characterized by the presence of regular secondary growth in several species while many of them also showed combination of multiple variants within the same or different species. Their ontogeny and diversity is elaborated as follows:

Successive cambia: Formation of successive cambia has been documented in different species of the Convolvulaceae by various researchers (Schenck, 1893; Pfeiffer, 1926; Metcalfe & Chalk, 1950; Pant & Bhatnagar, 1975; Lowell & Lucansky, 1986, 1990; Carlquist
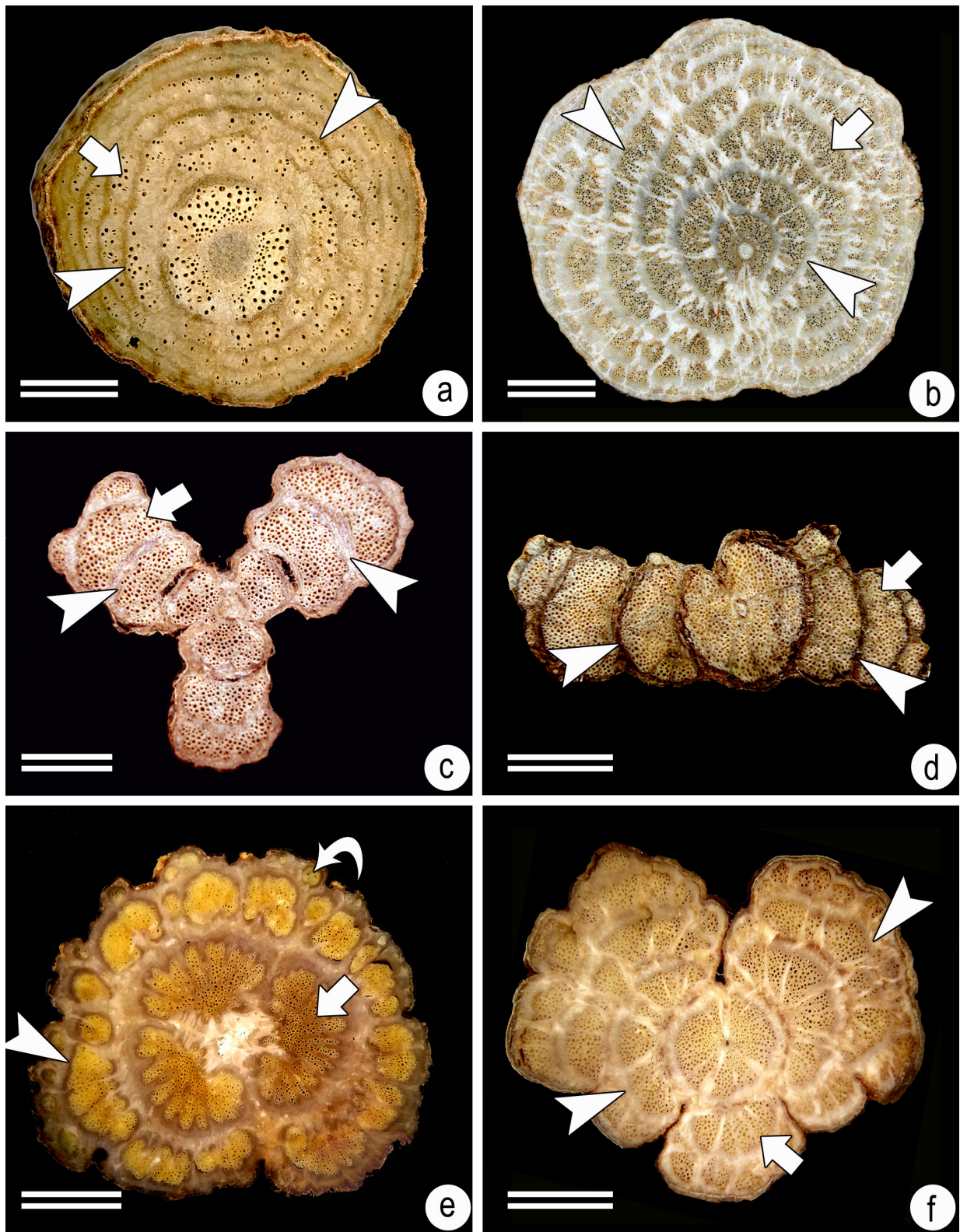
Fig. 1. Stem conformation in cross view of different species of Convolvulaceae. Arrows indicate a secondary xylem formed by the successive cambia while arrowheads indicate successive cambia. **a**.*Argyreia lawii* C.B.Clarke; **b**.*A. nervosa* (Burm.f.) Bojer; **c**.A. *boseana* Santapau & V.Patel; **d**. *Ipomoea corymbosa* (L.) Roth; **e**.*Hewittia malabarica* (L.) Suresh (curved arrow indicates a neo-formed vascular cylinder); **f**. *Merremia hederacea* (Burm.f.) Hallier f.(Scale a, d, f = 5 mm; b = 3 mm; c = 7 mm; e = 10 mm).
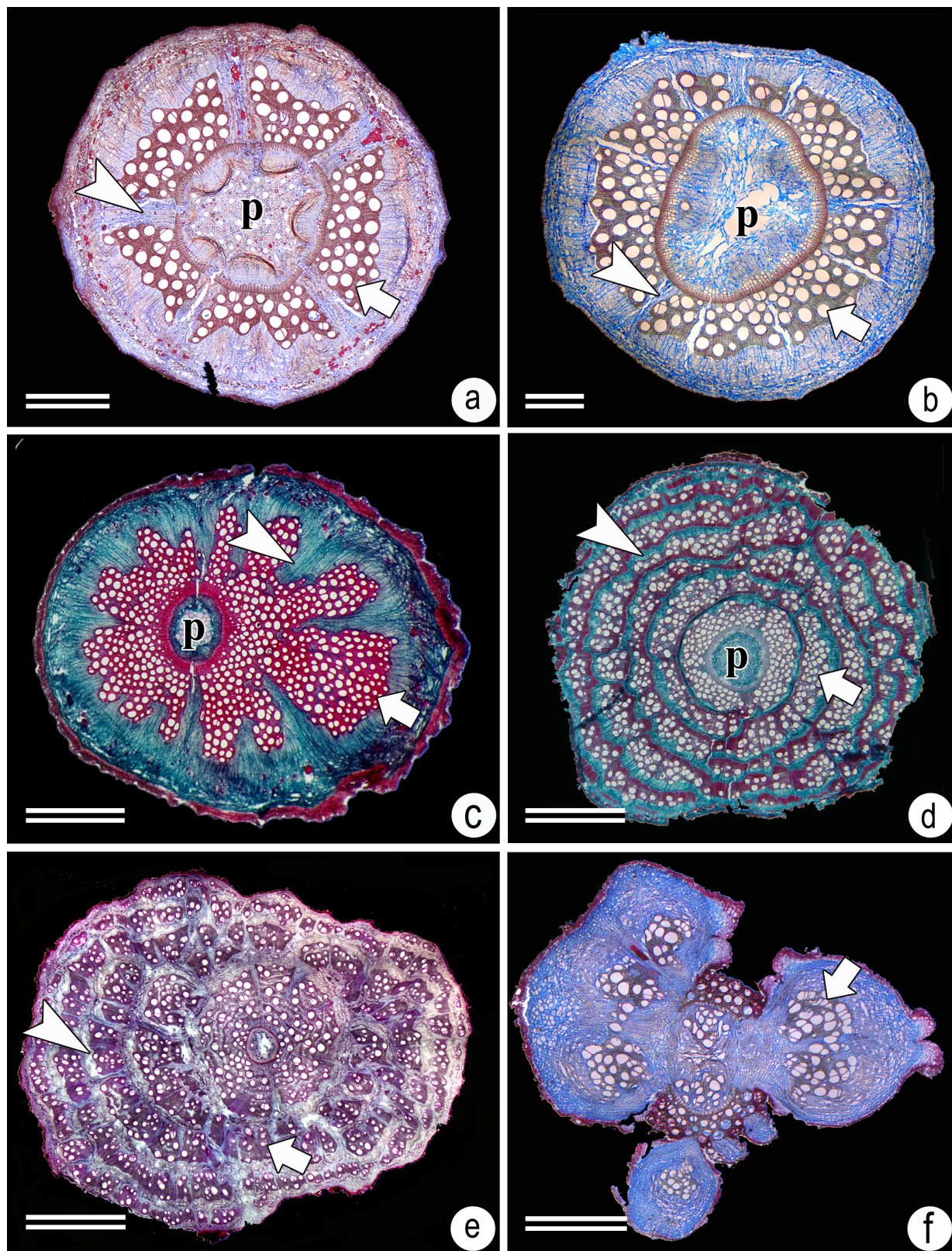
Fig. 2. Microscopic view of stem conformation in different taxa of the Convolvulaceae in transverse view. **a**. *Camonea kingii* (Prain) A.R.Simões & Staples,; **b**. *Operculina tansaensis* Santapau & V.Patel; **c**. *Jacquemontia pentanthos* (Jacq.) G.Don [Note the parenchyma wedge (arrowhead/s) and secondary xylem formed by the interwedge cambium (arrow/s)]; **d**. *Rivea hypocrateriformis* Choisy; **e**. *Ipomoea hederifolia* L. [Note the successive cambia (arrowhead/s) and xylem formed by them (arrow/s)]; **f**. *Xenostegia tridentata* (L.) D.F.Austin & Staples showing irregular development of successive cambial segments (arrow) (Scale: a, b = 1 mm; c, f = 2 mm; d = 4mm; e = 3 mm).

& Hanson, 1991; Kapadane *et al.*, 2023; Lawand *et al.*, 2023; Thacker *et al.*, 2024). The formation of successive cambia is a common feature in various species of *Argyreia, Camonea, Distimake, Hewittia malabarica* and *Ipomoea* (Rajput *et al.*, 2008, 2021, 2024; Lekhak *et al.*, 2018; Kapadane *et al.*, 2023; Thacker *et al.*, 2024). In all these species, as the secondary growth progress further, pericycle parenchyma (*i.e.,* non-lignified thin-walled cells located on the inner margin of pericyclic fibres) dedifferentiates and acquires meristematic activity (Fig. 3b, c). This newly initiated cambium either formed a complete ring or may initiated as small segments that later get interconnected and formed successive rings or anastomosing network of successive cambia (Figs. 1a, b; 2d, e). Subsequent successive cambia initiate from the parenchyma cells positioned external to the phloem formed by the previous cambium (Lowell & Lucansky, 1986; Terrazas *et al.*, 2011; Rajput *et al.*, 2008, 2014a–c, 2021, 2024; Lekhak *et al.*, 2018; Kapadane *et al.*, 2023; Thacker *et al.*, 2024). In species like *Ipomoea corymbosa* (L.) Roth, *I. eriocarpa* R.Br., *I. hederifolia* L. and *I. obscura* (L.) Ker Gawl., the differential activity of small segments of the vascular cambium deposited relatively less or no secondary xylem and phloem in some portions, which consequently resulted in the lobed outline of the stem. Though not a conserved character, in some of the members like *Argyreia boseana*, three segments of successive cambia initiated equidistantly which led to the formation of trilobed stems in transverse view (Fig. 1c). Such trilobed nature of the stems was observed in all samples collected from different locations from different forest regions (Rajput *et al.*, 2017). In contrast, stem samples of the same individual of *Ipomoea corymbosa* (L.) Roth showed various shapes at various height. More frequently, in *I. corymbosa* small segments of the successive cambia initiated only on two opposite lateral sides of the stem (Fig. 1d) that led to the development of flattened stems

(Lekhak *et al.*, 2018). In both cases (*i.e.,* in *A. boseana* and *I. corymbosa*) subsequent successive cambia initiated external to the earlier formed cambial segments so the stems became distinctly trilobed or flat respectively (Rajput *et al.*, 2017; Lekhak *et al.*, 2018). Results of the present study are in agreement with the initiation from the pericyclic parenchyma (Lowell & Lucansky, 1986; Terrazas *et al.*, 2011; Rajput *et al.*, 2008, 2014a-c; Lekhak *et al.*, 2018; Kapadane *et al.*, 2023; Lawand *et al.*, 2023; Thacker *et al.*, 2024).

Neo-formed vascular cylinders: All the members of the Convolvulaceae do not form complete ring of the successive cambia, while in some species like *Camonea kingii, C. umbellata* (Fig. 3e, f), *Distimake vitifolius, D. quinquefolius* and *Hewittia malabarica* (Fig. 1e), neo-formed vascular cylinders initiated external to the regular phloem or outside the pericyclic fibres. Formation of such neo-formed vascular cylinders or neoformations has also been reported in different species other than Convolvulaceae by earlier researchers (Acevedo-Rodríguez, 2015; Angyalossy *et al.*, 2012, 2015; Leal *et al.*, 2020; Rajput *et al.*, 2023). These vascular cylinders were oval to circular or elliptic in outline and fail to interconnect to form a complete ring of the vascular cambium (Rajput *et al.*, 2021, 2024). These vascular cylinders were enclosed within the non-lignified parenchymatous conjunctive tissues. Ontogenetically, these cylinders initiated from the parenchyma cells located external to the phloem formed by the regular vascular cambium (Rajput *et al.*, 2021) or dilating phloem rays. Most often, it is difficult to determine the exact nature of these non-lignified parenchyma (whether phloem parenchyma/ pericyclic parenchyma/cortical parenchyma) that differentiate into neo-formed vascular cylinders. The reason is increasing pressure on earlier formed phloem tissue, parenchyma and cortical cells due to the development of the secondary xylem and phloem and increasing circumference crushed these non-lignified
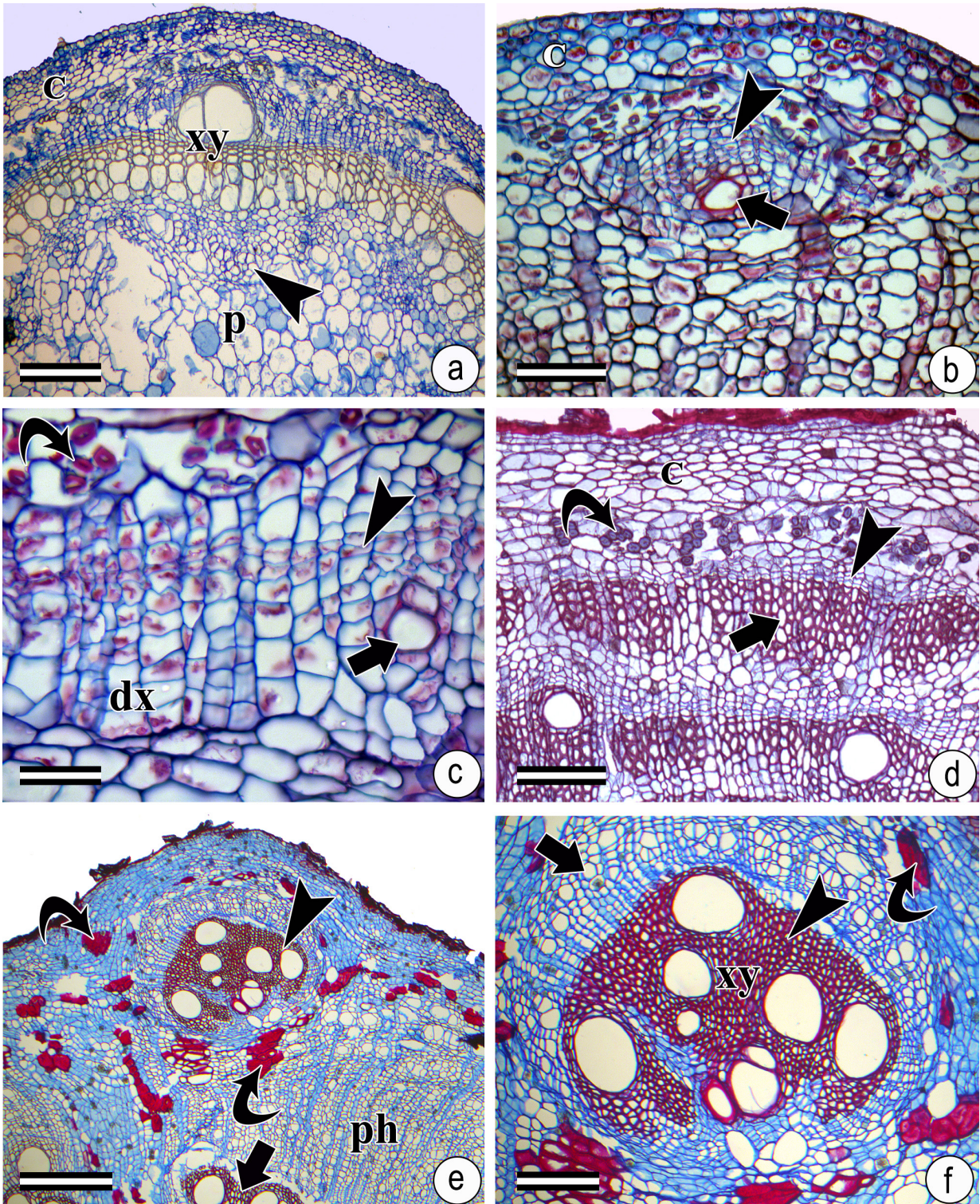
Fig. 3. Transverse view of stems of different species of Convolvulaceae. **a**. Young stem of *Argyreia capitiformis* (Poir.) Ooststr. showing regular secondary growth after the establishment of a complete ring of vascular cambium. The arrowhead indicates intraxylary phloem in the pith region; **b**.Initiation of first successive cambium (arrowhead) in *Merremia hederacea* (Burm.f.) Hallier f.showing recently formed vessel (arrow); **c**. Enlarged view of the initiation of the first successive cambium (arrowhead) and differentiating xylem (dx) and vessel (arrow) in *M. hederacea*. The curved arrow indicates pericyclic fibres; **d**. Bidirectional activity of newly initiated successive cambium (arrowhead) in *Argyreia elliptica* (Roth) Choisy. The arrow indicates the xylem formed from it while the curved arrow indicates pericyclic fibres; **e**.Mature stem of *Camonea umbellata* (L.) A.R.Simões & Staples showing neo-formed vascular cylinder (arrowhead). Note the secondary xylem (arrow) formed by regular vascular cambium. Curved arrows indicate sclerenchyma cells; **f**. Enlarged view of Figure 3E showing the structure and composition of the neo-formed vascular cylinder (arrowhead). The arrow indicates the phloem formed by the cambium of it while the curved arrow shows sclerenchyma cells. (c = cortex, p = pith, xy = xylem, dx= differentiating xylem; ph = phloem; Scale a, d, f = 200 µm, b = 100 µm; c = 50 µm; e = 500 µm).

parenchyma cells. In *Distimake quinquefolius*, *D. vitifolius* and *H. malabarica* it originated from the dilatation of phloem ray parenchyma cells (Rajput *et al.*, 2021, 2024). In thick stems, ray parenchyma cells of the earlier formed phloem underwent dedifferentiation and formed small segments of the cambium (Fig. 4a) that differentiate the secondary xylem and phloem on the either side of it (Fig. 4b). Thus, resulting vascular cylinders secondary xylem and phloem arranged tangentially (Fig. 4c). Besides Convolvulaceae, formation of the neo-formed vascular cylinders is reported in other families like Combretaceae, Euphorbiaceae, Fabaceae, Piperaceae, Rubiaceae, Sapindaceae (Angyalossy *et al.*, 2012, 2015; Leal *et al.*, 2020; Rajput *et al.*, 2022, 2023) in which they originate external to the regular vascular cylinders from the cortical parenchyma cells (Acevedo-Rodríguez, 2015; Angyalossy *et al.*, 2012, 2015; Leal *et al.*, 2020).

Functionally inverse cambia: The occurrence of the secondary xylem and phloem with inverse polarity is observed frequently in the members of the Convolvulaceae (Rajput et al. 2008; Carlquist & Hanson, 1991; Terrazas *et al.*, 2011; Tamaio *et al.*, 2021; Lekhak *et al.*, 2018; Kapadane *et al.*, 2023). In mature stems of species like *Argyreia boseana*, *A. elliptica*, *Distimake vitifolius*, *Ipomoea corymbosa*, *I. hederifolia*, *I. muricata*, *Merremia hederacea* the parenchymatous/ conjunctive tissues located on the inner margin of the secondary xylem formed by the successive cambia dedifferentiated and deposit secondary xylem and phloem in inverse polarity (Fig. 4d). Repeated divisions in these cells resulted in the formation of radial files of cells like vascular cambium (Fig. 5a, b). Functionally this newly initiated cambium was bidirectional and deposited secondary xylem externally and phloem internally *i.e.* showing inverse polarity (Fig. 5b). Besides these Indian species (Rajput *et al.*, 2008, 2021; Lekhak *et al.*, 2018; Kapadane *et al.*, 2023) of the Convolvulaceae, the occurrence of functionally inverse cambia is also reported in other members of the family Convolvulaceae (Carlquist & Hanson, 1991; Terrazas *et al.*, 2011; Tamaio *et al.*, 2021). The reason for the inverse orientation of the secondary xylem and phloem remains unknown. However, molecular studies on *Ipomoea batatas* revealed that expression of *IbMADS3* and *IbMADS4* (*i.e.*, MADS-box genes) in the roots intensify the cambial activity and shows initiation of variant secondary and tertiary meristems (Kim *et al.*, 2002). Similarly, Spicer & Groover (2010) also concluded that miRNA-resistant *Populus*REV transgene expression modifies the vascular tissues differentiation and leads to the initiation of the ectopic cambia in the cortex region which functionally inverse and forms xylem centrifugally and phloem centripetally. Further, Spicer and Groover (2010) suggested that class III HD ZIPs are not only regulating the polarity of the vascular tissue but also initiating cambium from the parenchyma cells. Further, studies are needed on this line to understand the reason for inverse polarity of vascular tissue differentiation.

Formation of non-lignified parenchyma wedges: Parenchyma wedges are radial strips of the secondary xylem composed of non-lignified, thin-walled parenchyma and are formed due to differential activity of the vascular cambium (Rajput *et al.*, 2024a); therefore, considered here as a type of cambial variant. By appearance, these wedges look like phloem wedges as reported in different families such as Bignoniaceae (De Bary, 1884; Schenck, 1893; Dobbins, 1971; Fisher & Ewers, 1989; 1992; Pace *et al.*, 2009, 2011, 2015), Sapindaceae (Rajput *et al.*, 2021), Malpighiaceae (Quintanar-Castillo & Pace, 2022), and Apocynaceae (Rajput *et al.*, 2024b). In all the investigated species, except *Cressa cretica*, *Evolvulus alsinoides*, *Ipomoea acquatica* and *I. carnea*, non-lignified parenchyma wedges were found absent. As listed in Table 1, rest of the species showed the formation of parenchyma wedges, in which 2–5 or more (Fig. 5c) small segments of the vascular cambium (wedge
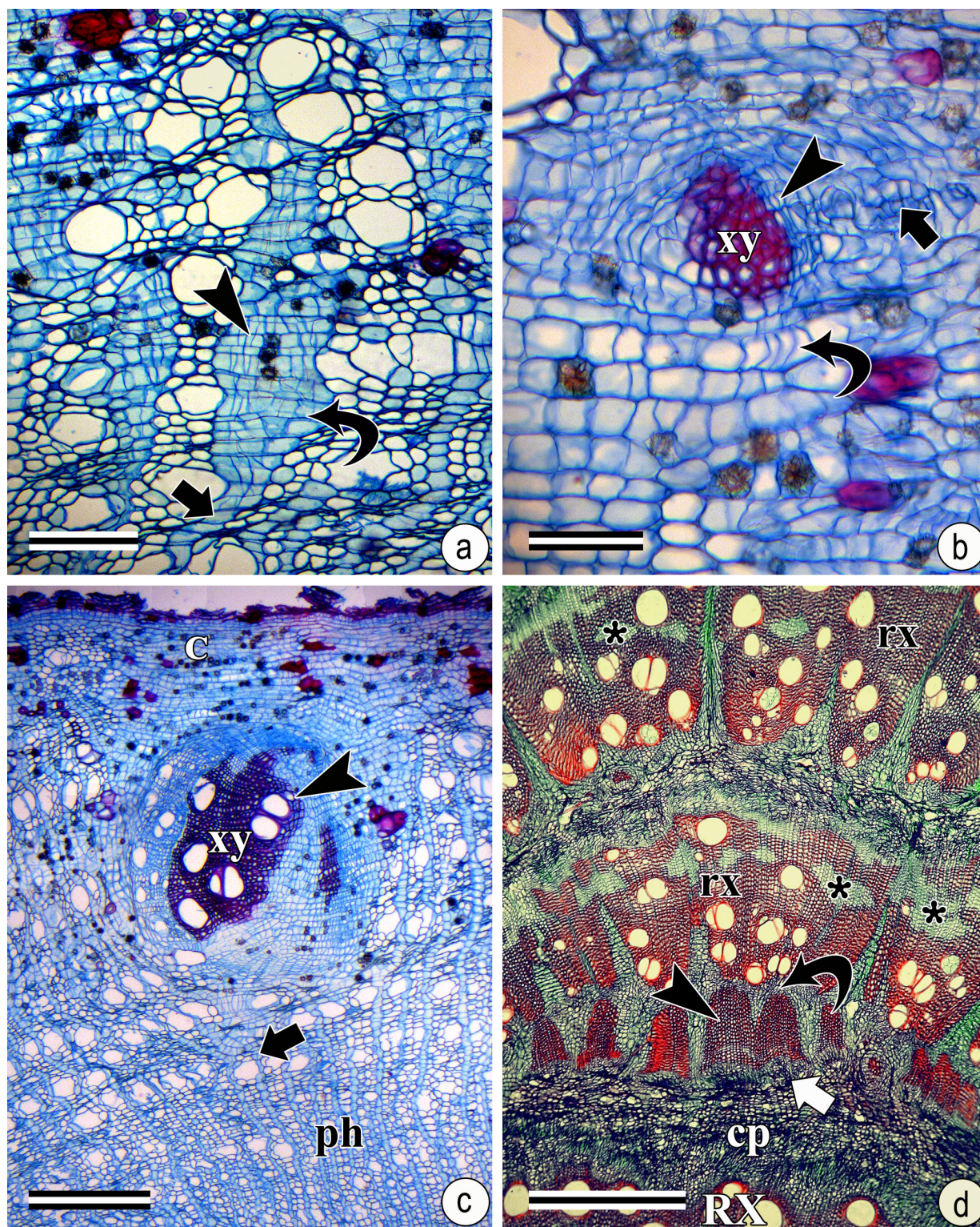
Fig. 4. Transverse view of the mature stems of *Distimake vitifolius* (Burm.f.) Pisuttimarn & Petrongari.(a–c) and *Ipomoea hederifolia* L. (d): **a**. Repeated divisions (arrowhead) in the non-conducting phloem ray cells [Note the width of the phloem ray (arrow) and a portion of it showing repeated divisions (curved arrow)]; **b**. Advanced stage of cell divisions, formation of cambium (arrowhead) and differentiation of the secondary xylem and phloem (arrow) during the formation of neo-formed vascular cylinder. The curved arrow shows divisions in one of the ray cells; **c**.Well-developed neo-formed vascular cylinders (arrowhead). The arrow indicates proliferating phloem ray from which the vascular cylinder initiates; **d**.A mature stem of *I. hederifolia* showing the secondary xylem (arrowhead) and phloem (arrow) formed by the functionally inverse cambium. The curved arrow shows the junction of the regular and inverse xylem (c = cortex, ph = phloem formed by regular vascular cambium,cp = crushed phloem, xy = xylem; rx= xylem formed by functionally regular cambium; Scale: a = 200 μm; b= 100 μm; c, d = 500 μm).

cambium) deposit non-lignified, thin-walled parenchyma on either side (*i.e.*, externally and internally) of the vascular cambium while remaining adjacent segments of the cambium (*i.e.*, inter-wedge cambium) deposit secondary xylem and phloem in regular way (Fig. 5d, e). With the passage of time, the non-lignified cells of the parenchyma wedge underwent dedifferentiation and produced isolated or group of sieve elements (Fig. 5f). In species like *Hewittia malabarica*, *Distimake quinquefolius* and *D. vitifolius*, the parenchyma cells bordering at the lignified xylem elements and parenchyma wedges underwent repeated divisions to form interxylary cambium that produced additional sieve elements (Fig. 6a, b).

Though parenchyma wedges appear like phloem wedges of Bignoniaceae; they differ in their formation (Rajput *et al.*, 2024a). In Bignoniaceae, wedge portion of the cambium either cease to produce secondary xylem internally or functionally became slow while elevates the phloem production externally (Dobbins & Fisher, 1986; Fisher & Ewers, 1989; Pace *et al.*, 2015). In the present study, wedge cambium exclusively produced non-lignified parenchyma on either side of it. With the passage of time, wedge parenchyma underwent dedifferentiation and deposit sieve tube elements. We presume that these wedges may perform similar function as ascribed for the phloem wedges of Bignoniaceae *i.e.,* to increase the stem flexibility and protection of conducting tissues from stem twisting when the plants twine around the supporting host (Dobbins & Fisher, 1986; Fisher & Ewers, 1989; Rowe & Speck, 2005; Quintanar-Castillo & Pace, 2022; Rajput *et al.*, 2024b). Number of parenchyma wedges differ from species to species while in non-scandent species like *Cressa cretica*, *Ipomoea carnea* and *Seddera latifolia* they were found absent. According to Pace *et al.*, (2015), the development of phloem wedges is correlated with the leaf position that regulate the supply

of the growth hormones particularly auxin that may contribute to the development of variant portions of the cambium (Angyalossy *et al.*, 2015; Aloni, 2021; Quintanar-Castillo & Pace, 2022).

Interxylary phloem and interxylary cambium: Interxylary phloem are strands of sieve elements, companion cells and associated axial parenchyma embedded within the secondary xylem. According to Carlquist (2013), interxylary phloem is the product of a single vascular cambium while phloem formed by the successive cambia is embedded within the conjunctive tissue. The present study documents delayed differentiation of the phloem from the axial parenchyma islands that are the product of regular vascular cambium. The secondary xylem of the family is characterized by the presence of the scanty paratracheal parenchyma, which are mostly thick-walled and lignified while thin-walled non-lignified parenchyma cells formed small pockets/patches within it (Fig. 4d). As the stem grew in thickness, non-lignified cells from these parenchyma pockets dedifferentiated into isolated or groups of sieve elements and formed small islands of interxylary phloem (Fig. 5f). Most often these interxylary phloem strands were interconnected by forming radial sieve elements (discussed later). Formation of interxylary phloem is a common feature in several members of the Convolvulaceae and is reported by earlier researchers (Schenck, 1893; Artschwager, 1924; Lowell & Lucansky, 1986, 1990; Carlquist & Hanson, 1991; Rajput *et al.*, 2008; Terrazas *et al.*, 2011; Rajput *et al.*, 2021, 2024; Ramoliya *et al.*, 2022; Kapadane *et al.*, 2023; Lawand *et al.*, 2023).

An advanced stage of interxylary phloem development was observed in species like *Ipomoea pes-caprae*, *Distimake dissectus* and *D. vitifolius.* In the aforesaid species, associated axial parenchyma within the phloem islands and parenchyma of the wedges underwent
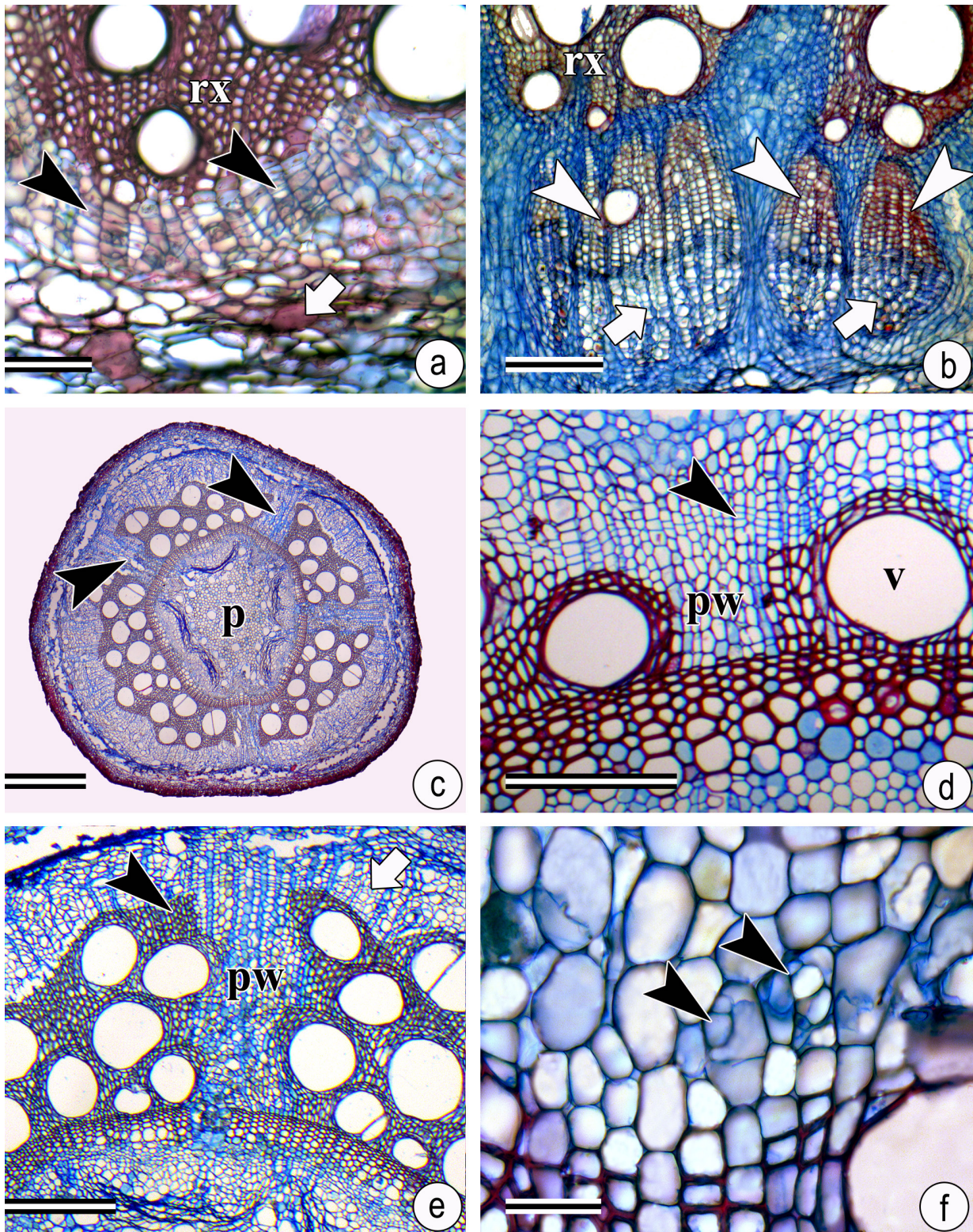
Fig. 5. Transverse view mature stems of *Merremia* Dennst. ex Endl. (a & b), *Camonea* Raf. (c-e) and *Argyreia* Lour.: **a**.Initiation of functionally inverse cambium (arrowheads) in *M. hederacea* (Burm.f.) Hallier f. Arrow indicates crushed phloem; **b**. Thick stem of *M. hederacea* showing secondary xylem (arrowheads) and phloem (arrows) formed by the functionally inverse cambium; **c**. Young stem of *Camonea pilosa* Wight & Arn.showing parenchyma wedges (arrowheads); **d**. Enlarged view of the Figure 5C showing parenchyma wedge. The arrowhead shows a cambium of wedge portion; **e**. Well-developed parenchyma wedge in the relatively thick stem of *C. pilosa*. Note the secondary xylem (arrowhead) and phloem (arrow) formed by the inter-wedge cambium; **f**. *Interxylary phloem* (arrowheads) formed by dedifferentiation of parenchyma cells in *Argyreia nervosa* (rx= xylem formed by the regular cambium,v = Vessel, pw = parenchyma wedges, p = pith; Scale: a = 100 μm; b, d = 200 μm; c = 1 mm; e = 500 μm; f = 50 μm).

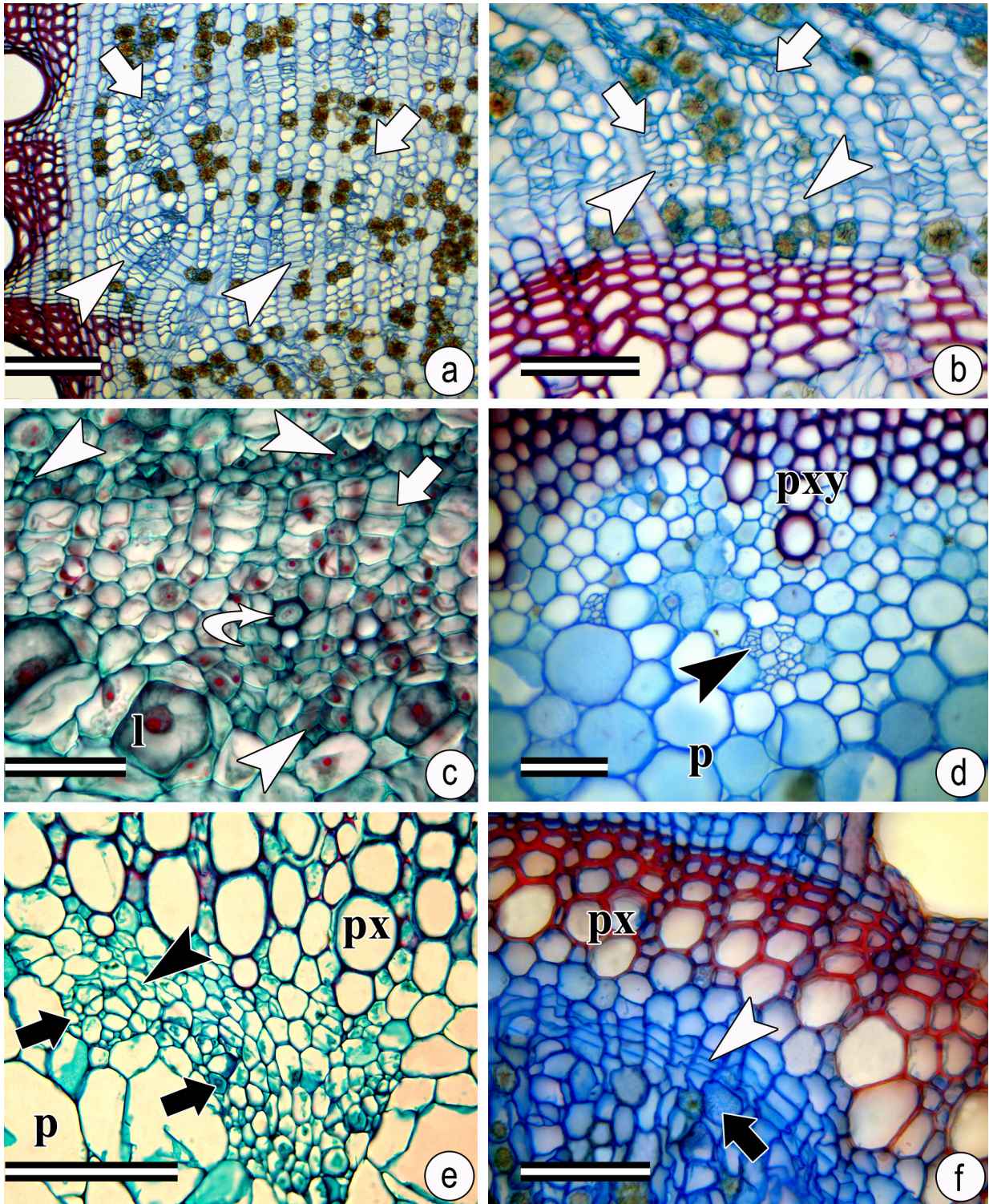**Fig. 6**. Transverse view of thick stems of different species of Convolvulaceae: **a**. Initiation of interxylary cambium (arrowheads) from nonlignified, thin-walled phloem wedge parenchyma in thick stems of *D. vitifolius* (Burm.f.) Pisuttimarn & Petrongari. Arrows indicate sieve elements; **b**. Enlarged view of the secondary xylem of *D. vitifolius* showing initiation of interxylary cambium (arrowheads). Arrows indicate sieve tube elements differentiated from it; **c**. Simultaneous differentiation of regular (upper arrowheads) and intraxylary phloem (lower arrowheads) and initiation of procambium (arrow) in *Ipomoea hederifolia* [The curved arrow shows differentiating protoxylem elements]; **d**. Intraxylary phloem (arrowhead) in *Argyreia nervosa* differentiated from the pith cells. Note that it is located away from the primary xylem; **e**. Initiation of intraxylary cambium (arrowhead) between primary xylem and intraxylary phloem in *Ipomoea hederifolia*. Arrows indicates intraxylary phloem; **f**. Well-developed intraxylary cambium (arrowhead) in *Ipomoea obscura*. Note the intraxylary phloem (arrow). (l = laticifer; p = pith, px = primary xylem; pxy = primary xylem; Scale: a, e = 200 µm; b, d = 100 µm; c, f = 50 µm).

repeated divisions, and formed radial files of meristematic cells referred to as interxylary cambium (Fig. 6a, b). These interxylary cambia were mostly unifacial and exclusively produced interxylary phloem. However, occasionally they became bidirectional and showed deposition of xylem opposite to phloem. These cambia formed as a small segment with no specific orientation and showed tangential, diagonal, or radial orientation. The occurrence of interxylary cambium is a rare feature and is reported in members of families like Fabaceae, Convolvulaceae, Cucurbitaceae and Malpighiaceae (Patil *et al.*, 2011; Pace *et al.*, 2018; Rajput *et al.*, 2014a, 2023). Formation of interxylary phloem (direct by dedifferentiation or from the interxylary cambium) may act as an additional path for the rapid conduction of photosynthate from source to sink (Carlquist & Hanson, 1991; Rajput, 2003; Rajput *et al.*, 2021; Ramoliya *et al.*, 2022; Lawand *et al.*, 2023). Delayed development of interxylary phloem and interxylary cambium may be correlated with the phenological events as their time of initiation is coincides with the flowering and fruit setting (Carlquist & Hanson, 1991; Rajput, 2003; Rajput *et al.*, 2021, 2024a; Ramoliya *et al.*, 2022; Kapadane *et al.*, 2023; Lawand *et al.*, 2023).

Intraxylary phloem: Intraxylary phloem also referred to as internal or medullary phloem (Scott & Brebner, 1891; Hallier, 1893; Metcalfe & Chalk, 1950; Fukuda, 1967; Rajput *et al.*, 2008) are strands of sieve elements, companion cells and associated parenchyma cells located on the inner margin of protoxylem *i.e.,* on the periphery of the pith. The occurrence of intraxylary phloem has been documented in 19 families of eudicots (Carlquist, 1988). Ontogenetically, it may be primary or secondary (Scott & Brebner, 1891; Fukuda, 1967; Lowell & Lucansky, 1986, 1990; Patil *et al.*, 2009; Rajput & Gondaliya, 2017; Lekhak *et al.*, 2018; Kapadane *et al.*, 2019, 2023). Depending on the species, development of intraxylary protophloem may occur before,

simultaneously, or after the formation of regular protophloem (Rajput & Gondaliya, 2017). In the present study, most of the species developed primary intraxylary phloem simultaneous with the regular protoxylem and protophloem (Fig. 6c). However, in *Argyreia nervosa* primary intraxylary phloem was found absent (see Kapadane *et al.*, 2019), while its development was observed only after the initiation of secondary growth (Fig. 6d). In all the species (except *A. nervosa*) primary intraxylary phloem development was observed from the procambial derivatives and subsequently additional intraxylary phloem develop from the marginal pith cells (Fig. 6e). In *A. nervosa* the formation of intraxylary phloem was observed from the mature pith cells only after the initiation of the secondary growth (Fig. 6d). Additional intraxylary phloem deposition took place by the activity of the intraxylary cambium (discussed later). Available literature indicates that ontogenetically intraxylary protophloem may develop from the ground meristem (Esau 1938, pp 362), pericycle or procambial derivatives (Mikesell & Schroeder, 1984; Carlquist, 2001; Patil *et al.*, 2009; Rajput & Gondaliya, 2017; Rajput *et al.*, 2022) or from the marginal pith cells (Carlquist & Hanson, 1991; Carlquist, 2013; Terrazas *et al.*, 2011; Rajput & Gondaliya, 2017; Rajput *et al.*, 2022). In the present study, development of intraxylary phloem was observed from the procambial derivatives, mature pith cells and activity of the intraxylary cambium. The absence of intraxylary protophloem in *A. nervosa* may be associated with the availability of an alternate mechanism *i.e.*, the presence of medullary bundles (discussed later).

Intraxylary cambium: In most of the members (except *Evolvulus alsinoides*, *Merremia emarginata*), thin-walled cells between the protoxylem elements and intraxylary protophloem underwent repeated divisions (Fig. 6e) and formed radial files of meristematic cells like cambial segments (Fig. 6f). Hereafter,

referred to as intraxylary cambium. Its occurrence is frequently observed in families like Convolvulaceae, Apocynaceae and Solanaceae (Fukuda, 1967; Rajput *et al.*, 2013, 2021, 2024a, b; Lekhak *et al.*, 2018; Kapadane *et al.*, 2023). In members of the Convolvulaceae, intraxylary cambium initiates when the individuals attain the flowering stage and fruit setting is in progresses. In the majority of taxa, these cambia were initially unifacial *i.e.*, deposit only phloem internally (Figs. 6f, 7a) but with the passage of time, in species like *Argyreia elliptica, A. boseana, Hewittia malabarica, Ipomoea hederifolia, Ipomoea corymbosa,* the intraxylary cambium became bifacial and deposits xylem externally and phloem internally (Fig. 7b). In thick stems of these species, considerable amount of the secondary xylem and phloem is deposited by these cambial segments (Fig. 7c, d). Such unifacial and bifacial behaviour of the intraxylary cambium in members of the Convolvulaceae has also been reported by earlier researchers (Scott & Brebner, 1891; Fukuda, 1967; Lowell & Lucansky, 1986; Carlquist & Hanson, 1991). Case of *Argyreia nervosa* differs from other members studied so far. In this case secondary intraxylary phloem develops from the mature pith cells that are located away from the pith margin (Fig. 6d). A weak intraxylary cambium initiated from the marginal pith cells located at the periphery of the pith (Fig. 7e, F) that deposit significant amount of intraxylary phloem. The earlier formed intraxylary phloem gets crushed while the functional phloem was present adjacent to the cambium (Fig. 7e, f). A unique behaviour of the intraxylary cambium was observed in *Ipomoea turbinata* (Rajput & Gondaliya, 2017). In this case the intraxylary cambium deposited xylem and phloem in the same direction instead of producing on the either side of the intraxylary cambium (Rajput & Gondaliya, 2017). We believe that the formation of inter- and intraxylary phloem may facilitate rapid conduction of photosynthate

for a higher demand versus supply during the reproductive phase of the lifecycle. We presume that both types of phloem are embedded within the secondary xylem (*i.e.*, interxylary phloem) and on the pith margin (*i.e.* intraxylary phloem); therefore, may act as the safest way for the conduction of photosynthate to safeguard it from sap-feeding and bark damaging insects, animals or from desiccation during the drier part of the year.

Medullary bundles: Medullary bundles are the vascular bundles/cylinders located in the pith region or in the central ground tissue of the plants and are composed of xylem and phloem separated by vascular cambium (Cutler *et al.*, 2007; Beck, 2010). As mentioned above, *Argyreia nervosa* differs from the rest of the members of the family Convolvulaceae by the presence of medullary bundles and absence of the intraxylary protophloem and showed delayed development secondary intraxylary phloem and intraxylary cambium takes only after the initiation of the secondary growth. Like primary intraxylary phloem, in *A. nervosa* development of medullary bundles initiates concomitant to the regular protoxylem and protophloem (Fig. 8a, b). These medullary bundles initiated from a group of meristematic cells located within the ground tissue, which showed initiation of phloem differentiation first or may simultaneously produce protoxylem and protophloem (Fig, 8a). Several such medullary bundles initiated throughout the pith region and showed secondary growth by depositing the secondary xylem and phloem (Fig. 8b, c). Distribution of medullary bundles in dicots is a rare feature and its occurrence is recorded in 49 families (Metcalfe & Chalk, 1983) while *A. nervosa* is the only taxon from the Convolvulaceae (Pant & Bhatnagar, 1975; Kapadane *et al.*, 2019) that showed the presence of medullary bundles. However, there is a possibility of the presence of medullary bundles in a greater number of species but unfortunately
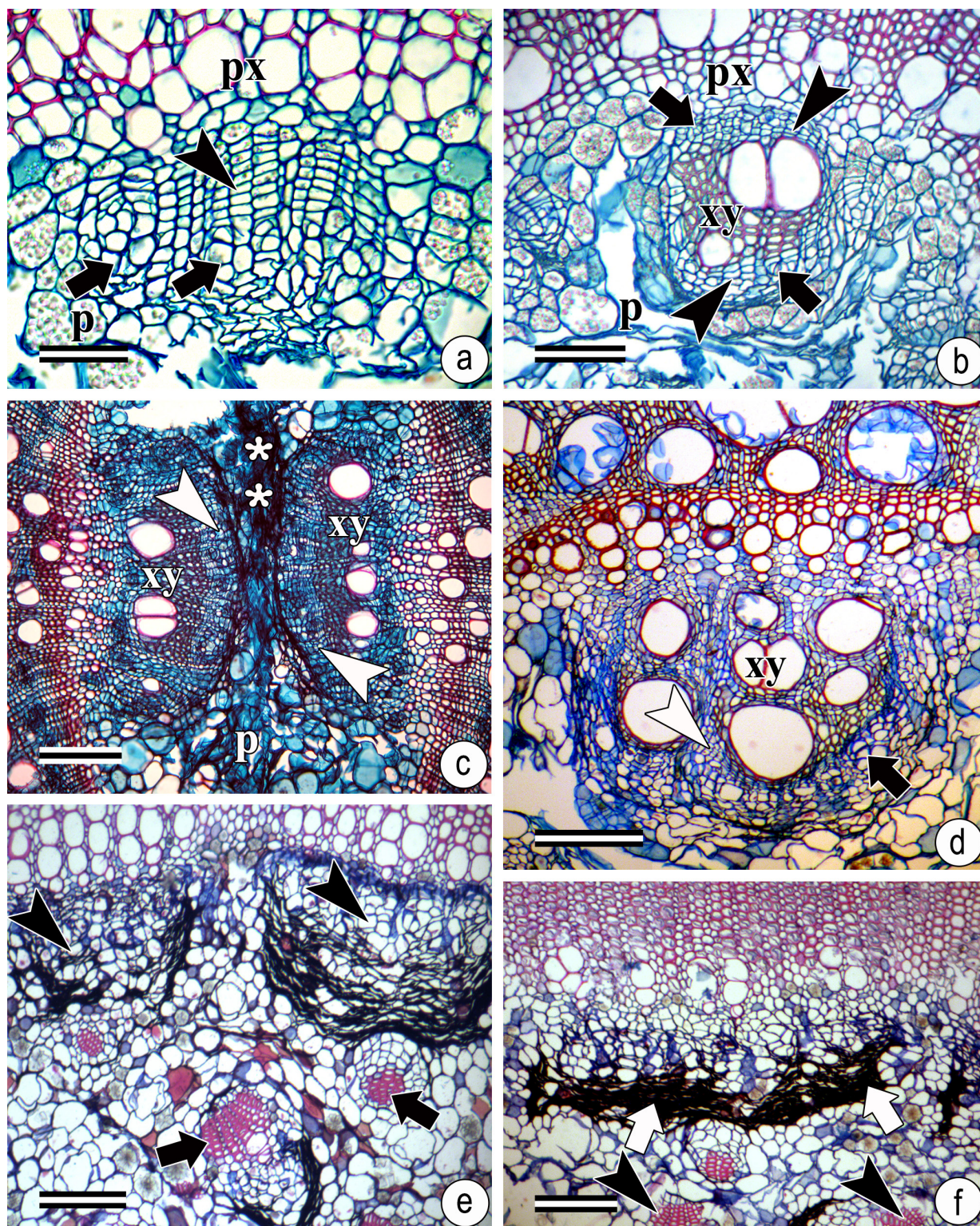
Fig. 7. Transverse view of the mature stem of various species of Convolvulaceae showing activity of intraxylary cambium: **a**. Thick stem of *Cressa cretica* showing unifacial intraxylary cambium (arrowhead). Arrows indicate phloem formed from it; **b**: Bifacial activity of intraxylary cambium (arrowheads) showing recently formed xylem and phloem (arrows) in *I. eriocarpa*; **c**. The pith portion of the thick stem of *Ipomoea hederifolia* is completely occupied by the intraxylary phloem (arrowheads) and xylem (xy) produced by the bifacial intraxylary cambium. Note the crushed portion of the pith (asterisks); **d**. The pith portion of the pith showing the secondary xylem (xy) and phloem (arrows) produced by the intraxylary cambium (arrowhead) in *Ipomoea* sp.; **e**. Secondary intraxylary phloem (arrowheads) formed by the intraxylary cambium in *Argyreia nervosa*. Arrows indicates medullary bundles; f. Relatively thick stem of *Argyreia nervosa* (Burm.f.) Bojer showing the crushed intraxylary phloem. Note the quantity of crushed phloem (arrows) and medullary bundles (arrowheads) (p = pith, px = protoxylem, p = pith, xy = xylem; px = protoxylem; Scale a = 50 μm; b = 100 μm; c–f = 200 μm).

no information is available on the majority of the species of *Argyreia*. Though, it is one of the largest genera of the family comprising about 135 species (Staples & Traiperm, 2017), histological information is available only on few species (Kapadane, 2022; Lawand *et al.*, 2023).

Isolated strands of xylem: One of the interesting and unique features is the formation of isolated strands of the lignified elements in the fully grown thick stems (composed of 4–6 successive rings of cambium) of *A. elliptica* (Fig. 9a–d). These strands were exclusively composed of fibres and narrow vessels while phloem formation was found absent. The parenchyma cells (conjunctive tissue) between the secondary xylem formed by the successive cambia underwent swelling prior to cells division (Fig. 9a, b). Subsequently these non-lignified cells underwent dedifferentiation and divided repeatedly in various angles (Fig. 9a, b) and differentiated directly into xylem elements like fibres and fibriform vessels while phloem cells were found absent (Fig. 9c, d). These strands were oval to circular or elliptic, irregular in size and orientation (Fig. 9b, c). As the growth progressed further, most often they were completely or partially encircled by radial files of meristematic cells like cambium (Fig. 9d). The formation of such isolated strands was reported for the first time in *Argyreia elliptica* (Kapadane *et al.*, 2023). A similar behaviour of the conjunctive tissue located between the successive rings of cambia is also reported in *Ipomoea corymbosa* by Lekhak *et al.* (2018). However, it differs from the *A. elliptica* by the production of both xylem and phloem that are separated by small segments of the vascular cambium (Lekhak *et al.*, 2018). The reason for its occurrence remains unknown, but it may be associated with mechanical strength and the provision of stem flexibility like an electric cable.

Radial sieve elements and perforated ray cells: The occurrence of perforated ray cells and sieve elements in the rays of the secondary xylem was reported often in the Convolvulaceae (Fig. 9e, F). Perorated ray cells were observed in all the investigated species while radial sieve elements were observed only in species showing the presence of successive cambia. Perforated ray cells were isodiametric cells or similar in dimension to the xylem ray cells except for the presence of a simple perforation and bordered pits on the radial walls like vessels (Rajput *et al.*, 2013; Lekhak *et al.*, 2018). Its occurrence was reported for the first time by Chalk & Chattaway (1933) in several taxa belonging to various families. Subsequently, they were reported by several researchers (McLean & Richardson, 1973; Nazma & Vijendra Rao, 1981; Ceccantini & Angyalossy-Alfonso, 2000; Serdar *et al.*, 2004; Merev *et al.*, 2005; Sonsin *et al.*, 2008; Rajput *et al.*, 2013, 2021, 2024a; Lekhak *et al.*, 2018; Kapadane *et al.*, 2023) in the members of the phylogenetically unrelated families. The exact role of these perforated ray cells remains unknown but it is believed that they may be related with short distance, rapid translocation of water and minerals (Sonsin *et al.*, 2008; Lekhak *et al.*, 2018).

Differentiation of the radial sieve elements (Fig. 9f) in the xylem rays was often noticed in the species that formed interxylary phloem while found absent in *Seddera latifolia*, *Evolvulus alsinoides* and *Convolvulus prostratus*. They were similar to- or relatively narrower in diameter and slightly longer than the ray cells except for the presence of simple sieve plate and a companion cell at the corner. These sieve elements were connected end to end to form radial columns to connect the adjacent interxylary phloem islands or they joined with the axial sieve elements to form an anastomosing network. Formation of such radial sieve elements has been documented in members of the other families (Rajput & Rao, 1997; Rajput, 2004a, b; Gondaliya & Rajput, 2017; Rajput *et al.*, 2022; Patil *et al.*, 2024). However, their occurrence is a rare feature and is reported in herbs, shrubs, trees,

and climbers that belong to phylogenetically unrelated families (Rajput *et al.*, 2022; Patil *et al.*, 2024). As reported by Aloni (2021) hormones play a crucial role in the regulation of the xylem and phloem differentiation. In longitudinal view, their development is recorded in uni- to multiseriate rays and may be positioned at the ray margin or in the centre of rays. Available literature indicates that in species like *Adansonia digitata* (Rajput, 2004a, b; Rajput *et al.*, 2022), *Suaeda monoica* (Lev Yadun & Aloni, 1991), *Merremia hederacea* (Rajput *et al.*, 2024a) some of the ray cells also show meristematic activity and initially differentiate only sieve elements followed by the secondary xylem elements, which results into the formation of radial vascular
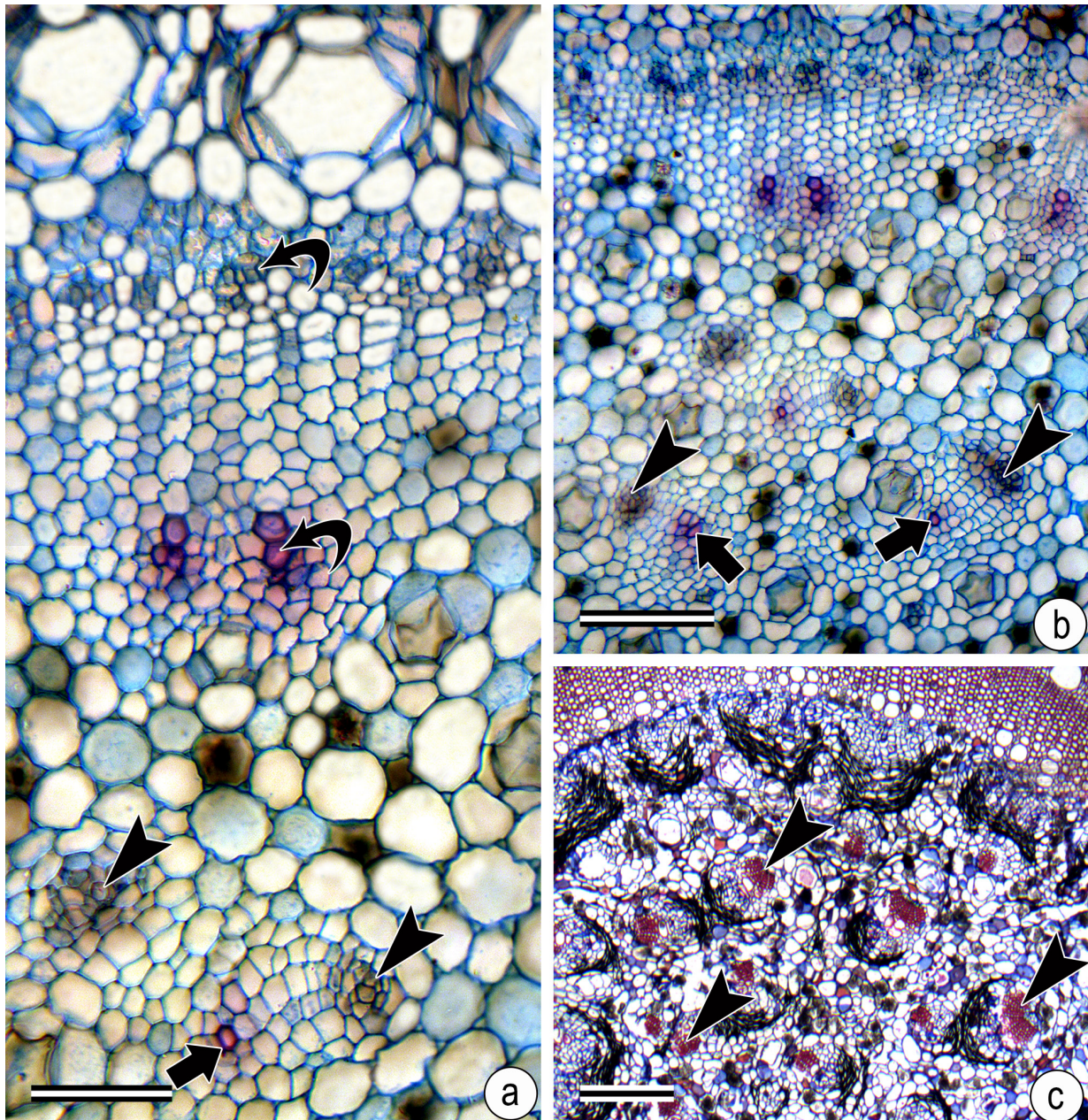

Fig. 8. Transverse view of young and mature stems of *Argyreia nervosa* (Burm.f.) Bojer showing various stages of medullary bundles development: **a**. Young stem initiation of medullary bundle development. Note the differentiating phloem (arrowheads) and the xylem (arrow) indicate the differentiating xylem of the medullary bundle. The curved arrow (upper) showing protoxylem and the lower curved arrow show differentiating protoxylem; **b**. Low magnification view of the young stem showing several initiating medullary bundles. Note the phloem (arrowheads) and xylem (arrows) of differentiating medullary bundles; **c**. Thick stem showing several medullary bundles (arrowheads) (Scale a = 50 μm; b = 200 μm; c = 500 μm).
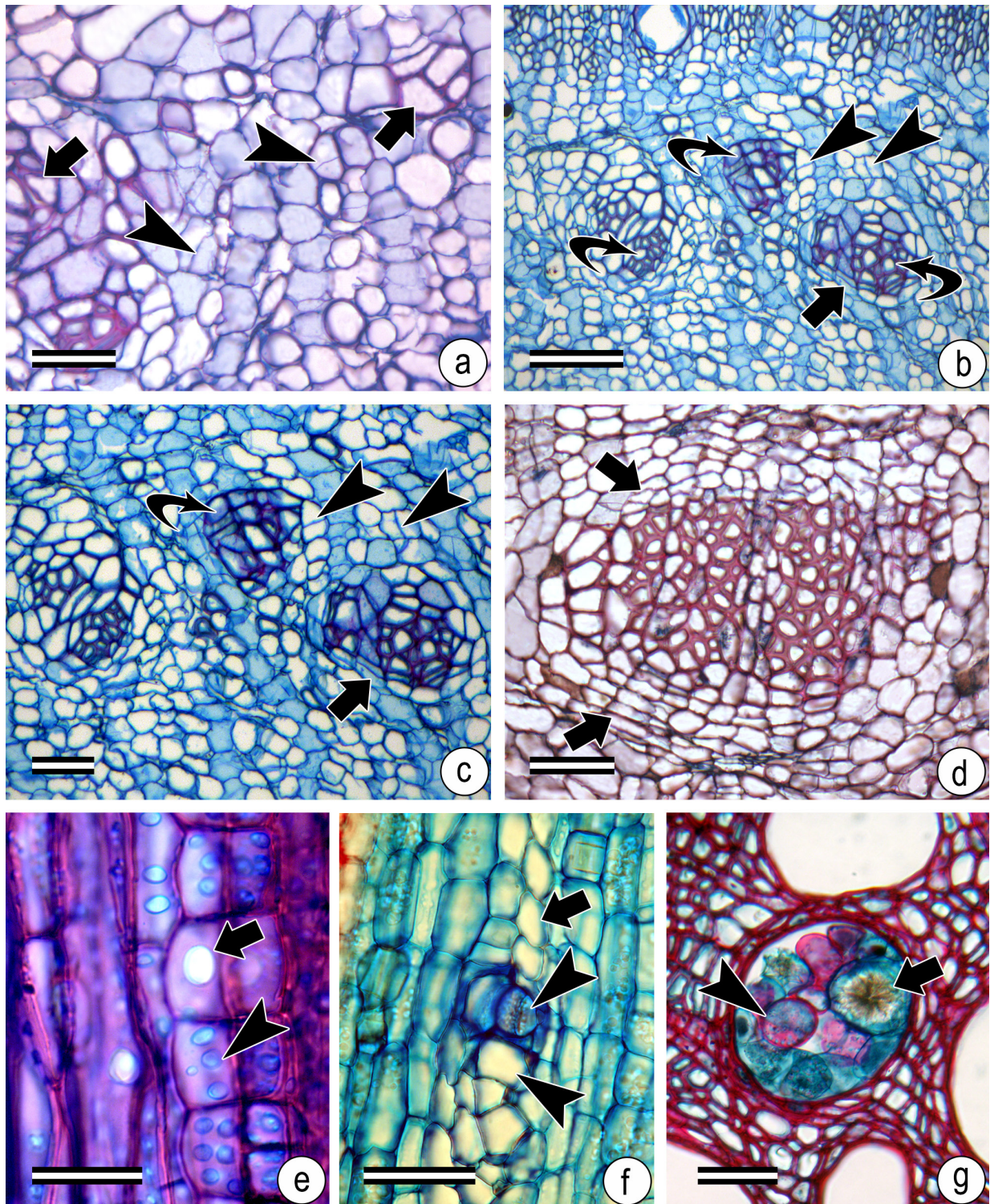
Fig. 9. Transverse view of thick stems of *Argyreia elliptica* (Roth) Choisy (a–d), *A. boseana* Santapau & V.Patel (e), *Ipomoea hederifolia* (f) and *Rivea hypocrateriformis* Choisy (g): **a**. Portion of the conjunctive tissue showing divisions (arrowheads) and differentiation of the xylem cells (arrows). Note the swelling of cells followed by division (arrowheads); **b**. Differentiating isolated strands (curved arrows). Note the swelling of cells before cell division (arrowheads) while the arrow indicates radially arranged meristematic cells surrounding the lignified cells; **c**. Enlarged view of Figure 9b showing structure and composition of isolated strands (curved arrow). Note the swelling of cells before cell division (arrowheads) while the arrow indicates radially arranged meristematic cells surrounding the lignified cells; **d**. One of the isolated strands shows only the xylem. Note the meristematic cells (arrows) and absence of phloem external to the xylem; **e**. Perforated ray cells (arrow) in the secondary xylem. Note the simple pits on the lateral walls adjacent to ray cells (arrowhead); **f**. Radial sieve elements (arrowheads) in the xylem rays. The arrow indicates ray cells; **g**. One of the vessels in the secondary xylem showing deposition of druses (arrow) and thin-walled as well as lignified tyloses (arrowhead) (Scale bar: a, c, d, g = 100 µm; b = 200 µm; e, f = 50 µm).

cylinders in the rays (Rajput, 2004a, b; Rajput *et al.*, 2022). Looking to their radial orientation and formation of an anastomosing network with the adjacent interxylary phloem islands, it indicates they are crucial in interconnecting the phloem islands for the rapid translocation of photosynthate from source to sink. A similar conclusion has also been ascribed to the presence of the radial sieve elements in our earlier studies (Rajput, 2004a, b; Rajput *et al.*, 2022).

Sclerified Tyloses: The formation of tyloses is a common feature in most of the climbing species. They are the occlusions in vessel lumens which are formed in response to protrusion of the protoplast of adjacent parenchyma (may be axial or ray) cells associated with the vessels (De Micco *et al.*, 2016). All the investigated climbing species of the family showed formation of tyloses that look like balloons. The tyloses observed in the species of *Camonea*, *Distimake*, *Ipomoea* and *Merremia* were thin-walled, nonlignified and showed accumulation of starch and prismatic crystals or druses. In contrast, *Rivea hypocrateriformis* showed the formation sclereids and lignified tyloses (Fig. 9g). However, available literature (De Micco *et al.*, 2016) indicates that species like *Dicranostylis ampla* and *Maripa nicaraguensis* of the Convolvulaceae also possess thick-walled lignified tyloses or sclereids (Rajput, 2016; Moya *et al.*, 2019). It is considered that tyloses are commonly formed in response to ageing of the plants during the heartwood formation (De Micco *et al.*, 2016) or as a defense mechanism in response to fungal infection and stem injury (VanderMolen e*t al.*, 1987, Sun *et al.*, 2008, De Micco *et al.*, 2016; Moya *et al.*, 2019). As the family is dominated by climbing members, in which the formation of heartwood has not been reported so far. Therefore, we believe the development of tyloses in the members of the Convolvulaceae may be associated with the defense mechanism and storage of ergastic substances like starch and crystals.

Conclusion: Members of the family Convolvulaceae showed both regular secondary growth and the formation of multiple vascular variants. The presence of combination of multiple cambial variants within the same species is an indication that the members are well adapted to the climbing habit. Moreover, the stem anatomy of different members belonging to different tribes of the family has unique stem anatomy, but unfortunately, it is neglected and not much histological information is available on them. Therefore, delimiting the taxa or their merging based on a single character like molecular sequencing is an indication of imposing artificial classification by neglecting the basic aims of the plant systematics that tries to generate phylogenetic relationships within the taxa. However, the limitations of the present study are lack of plant material from different tribes or lack of data that hinders the full proof conclusion. We believe that each genus (*i.e., Ipomoea* and *Argyreia*) is distinct while there are species that share transitional characters. Further studies are needed on a greater number of species that are yet to be investigated from a histological point of view.

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