RESEARCH ARTICLE

Comparative wood anatomy and origin of woodiness in subfamilies Secamonoideae and Asclepiadoideae (Apocynaceae)

Vicky Beckers,[1,2] Alessandro Rapini,[3] Erik Smets[1,2] & Frederic Lens[1,2]

1 *Naturalis Biodiversity Center, P.O. Box 9514, 2300 RA Leiden, The Netherlands*
2 *Plant Sciences, Institute of Biology Leiden, Leiden University, Sylviusweg 72, 2333BE Leiden, The Netherlands*
3 *Programa de Pós-graduação em Botânica, Departamento de Ciências Biológicas, Universidade Estadual de Feira de Santana, Av. Transnordestina s.n., Novo Horizonte, 44036-900, Feira de Santana, Bahia, Brazil*
Address for correspondence: *Vicky Beckers, vicky.beckers@naturalis.nl*

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Abstract The Apocynaceae subfamilies Secamonoideae and Asclepiadoideae have undergone several transitions during their evolution with regard to growth form and degree of woodiness. In this study, we present a wood anatomical overview of both subfamilies that complements previous work on the remaining Apocynaceae. Detailed microscopic wood descriptions using light and scanning electron microscopy were performed on 60 species that cover most Secamonoideae genera and all major woody Asclepiadoideae lineages. Our observations are in line with subfamilial and (sometimes sub)tribal delimitations. Furthermore, we present for the first time an overview of the estimated number of shifts from herbaceousness to (phylogenetically) derived woodiness in Asclepiadoideae, along with a derived woody species list with distribution and habitat information. In total, at least 168 derived woody species resulting from at least 28 independent woodiness transitions were found, with drought possibly being one of the main drivers of most of these transitions.

Keywords ancestral woodiness; derived woodiness; dogbane family; lianas; microscopic wood anatomy; milkweeds

Supporting Information may be found online in the Supporting Information section at the end of the article.

INTRODUCTION

Whereas Secamonoideae is a relatively small subfamily, with approximately 160 species belonging to eight genera that are native to the Paleotropics and Australia (Endress & al., 2019), Asclepiadoideae are much more species rich. With about 3400 species, 181 genera, 17 subtribes, and 5 tribes (Asclepiadeae, Ceropegieae, Eustegieae, Fockeeae, Marsdenieae), this subfamily comprises 60% of all species in the family Apocynaceae (Fishbein & al., 2018; Endress & al., 2019). In addition to its ancestral African distribution (Bitencourt & al., 2021), four independent radiation events occurred in the Americas (Rapini & al., 2003), of which one – the species-rich MOG-clade comprising subtribes Metastelmatinae, Oxypetalinae and Gonolobinae, along with five smaller subtribes of the tribe Asclepiadeae – includes 75% of all American Asclepiadoideae species (Rapini & al., 2007; Silva & al., 2012; Endress & al., 2019) (Fig. 1). While Secamonoideae are mostly woody with predominantly lianescent or twining growth forms and to a lesser extent also small erect shrubs (Endress & al., 2019), Asclepiadoideae are defined by a wide array of non-woody life forms. Most of them are climbing, with erect species in African and several Neotropical genera (Endress & al., 2019), succulent life forms common in Ceropegieae, and epiphytes restricted to Marsdenieae (see Fig. 1 for an overview of recognised clades within Asclepiadoideae with information on woodiness and growth form). These various woody and non-woody growth forms thrive in habitats ranging from lowland rainforests in Southeast Asia to diverse, often disturbed, forests and shrublands in Africa and the Americas (Endress & al., 2019). Interestingly, Bitencourt & al. (2021) observed a family-wide evolutionary transition in habitat occupation, ranging from stable and dense tropical rainforests typical of the rauvolfioids and some apocynoids, towards highly diverse twining lineages in more open, semi-arid, pioneer vegetation in Asclepiadoideae.

The evolution of growth forms in Apocynaceae is of particular interest. The rauvolfioid grade is almost entirely woody and ranges in size from small to medium-sized understorey trees and shrubs to tall canopy trees (reaching up to 80 m) and lianas (Endress & Bruyns, 2000; Middleton, 2007). Also, the apocynoid grade, and the subfamilies Periplocoideae and Secamonoideae of the APSA clade are predominantly woody, although the plants are on average considerably smaller. This

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means that the ancestral life form of the family is undoubtedly (ancestrally) woody with a reduction of plant height across the phylogenetic backbone of the family (Fishbein & al., 2018; Endress & al., 2019). This decrease in size is further continued in the predominantly non-woody Asclepiadoideae, with some shrubby species in Marsdenieae and Ceropegieae, possibly representing a retention of the ancestral woody condition, while most other woody species in Asclepiadoideae are widely scattered in the remaining lineages. The latter woody species are the result of evolutionary shifts from an herbaceous towards a (phylogenetically) derived woody growth form, which has happened multiple times (Fishbein & al., 2018). Consequently, derived woodiness should be treated as an evolutionary reversion (Porter & Crandall, 2003), a phenomenon that is best known from (sub)tropical oceanic islands (Carlquist, 1974; Lens & al., 2013a; Hooft van Huysduynen & al., 2021; Zizka & al., 2022). An overview of derived woodiness in Asclepiadoideae is, however, not available, making any taxonomic, evolutionary and biogeographic assessments of derived woodiness in the family – i.e., species identity, number of transitions, growth form variation and global distribution – unknown. In addition to the presence of ancestrally as well as derived woody species in Asclepiadoideae, the wood anatomical variation in both Asclepiadoideae and Secamonoideae is poorly known. There are only a few published wood descriptions on the erect growth form of less than 10 African and Asian species (Saint-Laurent, 1932; Messeri, 1938; Vigodsky-De Philippis, 1938; Tabatabai & Soleymani, 1964; Cellai, 1967, 1971; Fahn & al., 1984; Jagiella & Kürschner, 1987; Yaman & Tumen, 2012), which makes a wood anatomical overview of Secamonoideae and Asclepiadoideae valuable.

The presence of derived woodiness and the variation in growth forms makes Apocynaceae particularly interesting for comparative wood anatomical studies. Moreover, the phylogenetic backbone of the family is well-resolved (Endress & Bruyns, 2000; Livshultz & al., 2007; Livshultz, 2010; Endress & al., 2014; Fishbein & al., 2018), which allows searching for wood anatomical synapomorphies at the tribal level, as demonstrated by two review papers on the rauvolfioid and apocynoid grades, and subfamily Periplocoideae (Lens & al., 2008, 2009a). Next, the spectacular variation of (woody) growth forms in Apocynaceae offers the opportunity to assess the role of growth form on wood anatomical traits. For instance, pronounced differences in vessel grouping patterns (mainly solitary vs. densely grouped) and presence/absence of vessel dimorphism (co-occurrence of few wide and many narrow

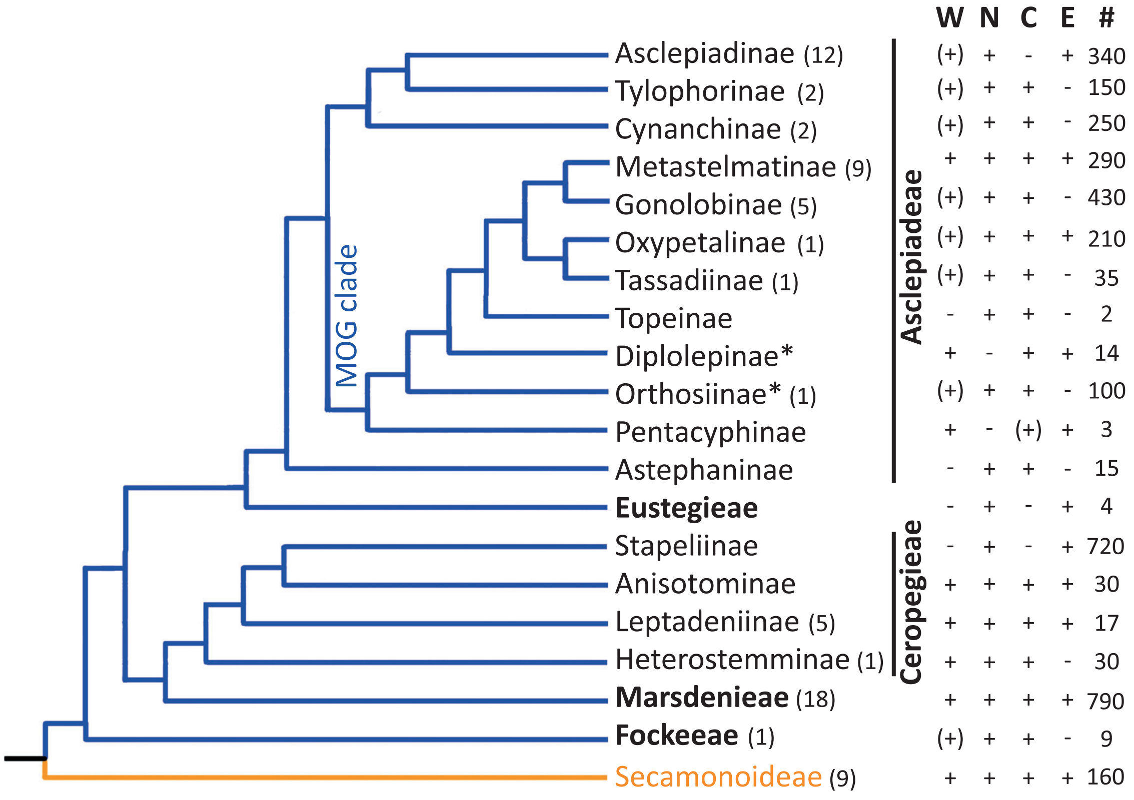

Fig. 1. Cladogram showing phylogenetic relationships of Secamonoideae (orange) and tribes (in bold) and subtribes of Asclepiadoideae (blue), with information on growth form. Relationships after Fishbein & al. (2018) using the plastid phylogeny under constraint of the plastome phylogeny (their Appendix S10), with inclusion of Topeinae based on Keller & Liede-Schumann (2017). W = woody, N = non-woody, C = climbing, E = erect, # = species number per clade, + = present, - = absent, (+) = occasionally present. Numbers within parentheses after clade names are the number of wood samples included in this study. Subtribes not recovered as monophyletic in the plastid phylogeny are indicated with an asterisk (*). Number of species per clade and growth form were extracted from Endress & al. (2019).

vessels) provided wood anatomical support for multiple independent transitions from an erect towards a climbing growth form in the family (Lens & al., 2008, 2009a).

Other relevant transitions represent multiple independent shifts towards derived woodiness (Fishbein & al., 2018). Carlquist (1962) was the first to identify the presence of primary xylem traits that extended in the later-formed secondary xylem of derived woody species. The anatomical traits that he observed, such as absence of rays, abundance of square to upright cells in rays, and/or presence of flat or decreasing length-on-age curves for vessel elements, were originally considered to represent paedomorphosis (Carlquist, 1962; Dulin & Kirchoff, 2010) or protracted juvenilism (Lens & al., 2009b, 2012), and were thought to only occur in the wood of derived woody species. More recently, the term peramorphosis was used to interpret this phenomenon more correctly (Olson, 2020; Onyenedum & Pace, 2021), and has been described in several wood anatomical studies that included derived woody species (Lens & al., 2009b, 2012, 2013b; Kidner & al., 2016; Neupane & al., 2017). However, there is growing evidence that this phenomenon is not restricted to the wood anatomy of derived woody species, but is more related to specific growth forms, such as rosette trees, stem succulents, slender-stemmed shrubs, lianas or species with sympodial growth forms that may be either ancestrally or derived woody (Carlquist, 2009, 2012; Lens & al., 2013b; Olson, 2020; Onyenedum & Pace, 2021).

The main objectives of this paper are to: (1) describe the wood anatomical diversity in all the major ancestrally and derived woody clades in subfamilies Secamonoideae and Asclepiadoideae, (2) search for a set of wood anatomical synapomorphies at the (sub)tribal level within Asclepiadoideae, (3) investigate the impact of the climbing growth form and derived woodiness on wood anatomical traits, and (4) compile a dataset of derived woody species in Asclepiadoideae along with information about growth form, habitat and global distribution, phylogenetic position and estimated number of evolutionary transitions.

■ MATERIALS AND METHODS

The variety in growth form displayed by the Asclepiadoideae made it difficult to assess the degree of woodiness for many species. In this study, the following definition of Kidner & al. (2016) was used: woody species are species that produce a distinct wood cylinder in the stem extending towards the upper parts (i.e., shrubs, trees, lianas), but excluding species with only a woody base (woody herbs or suffrutescent species). In total, 67 mature wood specimens belonging to 60 species and 35 genera, covering 4 out of 8 Secamonoideae genera, 4 out of 5 Asclepiadoideae tribes, 8 out of 11 subtribes of Asclepiadeae, and 2 out of 4 subtribes of Ceropegieae (Fig. 1) as recognised by Fishbein & al. (2018) were sampled from the wood collections of Naturalis (Lw, Uw, WAGw), Tervuren (Tw), Kew (Kw), and Madison (MADw, SJRw), or generously donated by Apocynaceae experts. Appendix 1 includes voucher information of all the samples included in this study.

The wood samples were subsequently sectioned, stained and mounted according to the standard protocol in Lens & al. (2005a), and described following the “IAWA list of microscopic features for hardwood identification” (IAWA Committee, 1989). For the total density of rays, we opted to measure the density of uniseriate and multiseriate rays separately because this division is informative in Apocynaceae (Lens & al., 2008, 2009a). Non-lignified zones in the secondary xylem (which mostly represent non-lignified rays, but also fibres and parenchyma, and may include interxylary phloem as explained below) were categorised according to terminology used in Carlquist (2001) for cambial variants. The terms “lobed” and “furrowed” were adopted to circumscribe the variation in non-lignified tissue across Asclepiadoideae as observed in cross sections, the term “isolated” was applied for the non-lignified areas typical of Leptadeniinae, and “phloem wedges” was adopted from Chery & al. (2020). Interxylary phloem was not easily defined in Asclepiadoideae, because: (1) sieve tubes are scarcely present or not observed in the non-lignified tissue within the wood cylinder, and (2) we observed mostly dried xylarium samples in which the non-lignified tissue was partly damaged due to desiccation. We therefore only mentioned the presence of interxylary phloem when sieve tube cells were observed, and referred to “non-lignified tissue” instead of “non-lignified xylem” or “interxylary phloem” when the distinction is not clear. In some families, like the Malpighiaceae, druses are common in chambered axial parenchyma cells of the secondary phloem (Pace & al., 2018), and the presence of these mineral inclusions in the non-lignified tissue within the secondary xylem cylinder could be used as an additional argument for the presence of interxylary phloem (M.R. Pace, pers. comm.). Although druses are common in the non-lignified tissues of the wood cylinder in Asclepiadoideae, we opted to not use this criterion because of the mixed presence of other crystal types in the same tissue. With regard to (lignified) xylem fibres, two types were recognised in this study: (1) fibres with few simple to minutely bordered pits (2–3 μm in horizontal size) primarily in radial walls (libriform fibres), and (2) fibres with more abundantly and more conspicuously bordered pits (3–6 μm in horizontal size) in radial and tangential walls (fibre-tracheids). In contrast to Lens & al. (2008, 2009a), we did not score tracheid presence due to the limited presence or even absence of this character in Secamonoideae and Asclepiadoideae.

We observed the anatomical slides with a Leica DM2500 light microscope (LM) equipped with a Leica DFC425 C digital camera. Additionally, anatomical slides from five juvenile wood samples were made and used for illustrative purposes (full wood descriptions of these samples are in suppl. Table S1). Scanning electron microscopic (SEM) observations were carried out on nine samples to visualise fine-scale anatomical traits. For these SEM observations, longitudinal and radial surfaces were sectioned and treated with household bleach, subsequently dehydrated in 50%, 70% and 95% ethanol, air-dried (Lens & al., 2005a), coated with platinum-palladium in a Quorum Q150TS sputter coater, and observed with a Jeol JSM-7600F Scanning Electron Microscope at 5 kV. Maceration slides at every 1 mm from pith to cambium were made for four samples according to Franklin (1945) and stained with astra blue (0.5 g astra blue and 2 g tartaric acid in 100 ml water). The length of 10 vessel elements was measured for each maceration slide to reconstruct length-on-age curves of ancestral and derived woody species.

Statistics on quantitative wood characters was done in R v.4.1.1 (R Core Team, 2021) with packages ggplot2 v.3.3.6 (Wickham, 2016), dunn.test v.1.3.5 (Dinno, 2017), and viridis v.0.6.2 (Garnier & al., 2021). Differences between the means of climbing and non-climbing were calculated with a Mann-Whitney test, and differences between the means of the three Asclepiadoideae tribes were calculated with a Kruskal-Wallis test (Bonferroni corrected) because all data was non-normally distributed and of unequal class sizes. Calculations of vessel statistics were done including (very narrow) fibriform vessels, except when mentioned otherwise.

Information about the identity of derived woody species and the estimated number of shifts from herbaceousness towards derived woodiness in Asclepiadoideae was summarised in a genus and species database, based entirely on information from published phylogenies and growth form retrieved from the literature (e.g., Floras, taxonomic revisions). Our derived woodiness database provides additional level of detail with regard to species identity and estimated number of shifts towards derived woodiness at the genus and/or tribal level compared to the ancestral state reconstructions of Fishbein & al. (2018 – appendices S14 and S16: reconstruction of herbaceous, basally woody [which we regarded as not woody enough according to the definition of Kidner & al., 2016, hence herbaceous] and woody states within the Apocynaceae). In order to ensure that only woody species are included according to the definition of Kidner & al. (2016), we deemed species woody enough when they were referred to as “trees”, “shrubs”, “woody lianas”, etc., but excluded species that were described as having an intermediate herbaceous-woody growth form, as exemplified by the terms “basally woody”, “suffrutescent twiner”, “herbaceous shrub” and so forth. To provide an estimate of the number of independent evolutionary transitions towards woodiness, we searched for the phylogenetic position of woody species in Asclepiadoideae in various phylogenies and visually counted the shifts according to the parsimony principle using a conservative approach (suppl. Fig. S1). For instance, when one or several closely related species are nested within an otherwise herbaceous clade or a clade consisting of species having only a woody stem base (cf. Fishbein & al., 2018), we counted one shift.

■ RESULTS

Wood anatomical descriptions of the Secamonoideae and Asclepiadoideae are given below. Numbers without parentheses are ranges of means, while numbers between parentheses represent minimum or maximum values. A summary of the most informative wood anatomical characters per (sub)tribe is given in Table 1, and Figs. 2–5 present a visualisation of the wood anatomical variation observed. The complete wood descriptions are given in suppl. Table S1, as are five descriptions of juvenile wood samples; statistical support for the quantitative wood traits can be found in suppl. Fig. S2. Figure 6 shows a selection of derived and ancestral woody length-on-age curves of vessel elements. Below the wood anatomical descriptions, a summary is given of the estimated number of evolutionary shifts in Asclepiadoideae from an herbaceous to a derived woody growth form (additional information on taxonomic position, growth form, habitat, geographic distribution, and literature on individual species is listed in suppl. Table S2; visualisation of these shifts in published phylogenies can be found in suppl. Fig. S1).

Secamonoideae (Fig. 2; 8 spp.). — Growth ring boundaries distinct (Fig. 2A) to indistinct. Wood diffuse-porous. Vessel density (17–)60–90(–180)/mm^2, with some species showing low vessel density (average of 30/mm^2 – *Genianthus micranthus*, *Secamone afzelii*, *Secamonopsis madagascariensis* and *Secamone racemosa*), and others high vessel density (mean 110/mm^2 – remaining *Secamone* samples); vessels predominantly solitary with occasional tangential multiples of 2–3 and/or clusters of 3–6 (Fig. 2B), occasional clusters up to 10 in *Genianthus micranthus*, *Secamonopsis madagascariensis* and *Secamone racemosa*; vessel perforation plates exclusively simple (Fig. 2E). Intervessel pits alternate, mostly 5–7 μm in horizontal diameter and vestured (Fig. 2F), up to 10 μm in *Secamone stuhlmannii*; vessel-ray pits similar in shape and size to intervessel pits (Fig. 2G). Tangential diameter of vessels (20–)70–120(–180) μm, some species have wider vessels (mean 100 μm – *Genianthus micranthus*, *Secamone afzelii*, *Secamonopsis madagascariensis* and *Secamone racemosa*) and others have more narrow vessels (mean 50 μm – remaining *Secamone* samples, Fig. 2D); vessel elements (150–)220–480(–550) μm long. Fibre-tracheids 500–790 μm in length, up to 1000 μm in *Secamone emetica*, *Secamonopsis madagascariensis* and *Secamone racemosa*; fibre-tracheids often thin- to thick-walled, but thin-walled in *Genianthus micranthus* (Fig. 2B), *Secamone afzelii* and *Secamonopsis madagascariensis*. Axial parenchyma mostly scanty paratracheal (Fig. 2D), but also diffuse apotracheal parenchyma in *Secamone racemosa*, and diffuse-in-aggregates and short apotracheal bands of 2–3 cells wide in *Genianthus micranthus*; 2–6 cells per axial parenchyma strand (Fig. 2D). Rays 1–2–3(–6)-seriate (Fig. 2C,D), uniseriate rays more abundant than multiseriate rays (5–14 vs. 0–6 rays/mm, Fig. 2C); uniseriate ray height 80–500 μm, up to 1500 μm in *Genianthus micranthus* and up to 1000 μm in *Secamone racemosa*; multiseriate ray height 210–630 μm, up to 1500 μm in *Genianthus micranthus* and up to 1000 μm in *Secamonopsis madagascariensis*; procumbent and/or square-upright cells in uniseriate rays, procumbent body ray cells and 1–2(–5) rows of upright-square marginal ray cells in multiseriate rays (Fig. 2G), 1–4(>4) rows of upright marginal ray cells in *Secamone racemosa*; rays partially non-lignified in *Secamone racemosa*, *Secamonopsis* *madagascariensis* and *Secamone elliptica*. Prismatic crystals sporadically observed in chambered ray and axial parenchyma cells of *Genianthus micranthus*, in chambered axial parenchyma of *Secamone filiformis* and in chambered ray cells of *Secamonopsis madagascariensis*. Laticifers observed in rays of *Secamone emetica*, *Secamonopsis madagascariensis* and *Secamone racemosa* (Fig. 2D). No interxylary phloem observed; intraxylary phloem observed in samples where the pith was included.

Asclepiadoideae (Figs. 3–5; 52 spp.). — Growth ring boundaries distinct to indistinct. Wood diffuse-porous, semi-ring-porous in *Calotropis procera*. Vessels predominantly solitary across the subfamily (Fig. 3D,F,G), with additional radial multiples of 2–18(–60) and/or clusters of 3–10(–30) in Marsdenieae (Fig. 3A), with additional tangential multiples of 2–3(6) and/or clusters of 3–7(–12) in Ceropegieae (Fig. 3B) and most Asclepiadeae clades, except for Asclepiadinae, which has a scarcity of solitary vessels and abundance of radial multiples of 2–10(–30) and clusters of 3–15(–30); (tendency to) a dendritic pattern in a few species of Marsdenieae and Asclepiadinae (Fig. 3C); vessel perforation plates exclusively simple. Intervessel pits often 4–8 µm in horizontal diameter (Fig. 4H), additionally up to 10 µm in *Cynanchum viminale*, additionally (pseudo)scalariform up to 15 µm in *Fockea angustifolia* (Fockeeae) and *Matelea pedalis* (Asclepiadeae), and <4 µm in Metastelmatinae (Asclepiadeae); vessel-ray pits alternate, similar in shape and size to intervessel pits (Fig. 5B), except (pseudo)scalariform up to 15 µm in *Fockea angustifolia* (Fockeeae); vessel dimorphism observed in all tribes (Fig. 4E), but most abundant in Marsdenieae; vessel diameter (10–)20–180(–380) µm, significant difference between climbing (mean 105 µm) and non-climbing (mean 55 µm) species; vessel density (1–)30–60(–190) mm^{2}, significantly lower in Ceropegieae (mean 11/mm^{2}) compared to Marsdenieae (mean 55/mm^{2}) and Asclepiadeae (mean 55/mm^{2}), but no significant difference between climbing and non-climbing samples; vessel element length (70–)190–450(–1500) µm, significantly longer in climbing Asclepiadoideae species (mean 330 µm, compared to 265 µm in non-climbing), significantly shorter in Asclepiadeae (mean 270 µm) compared to Marsdenieae (mean 355 µm); a general trend towards a reduced vessel element length is apparent across Asclepiadoideae (suppl. Fig. S2); relatively long vessel elements in *Hoya spartioides* (Marsdenieae – often longer than 600 µm), *Cynanchum viminale* and *Gonolobus parviflorus* (Asclepiadeae – often longer than 550 and 500 µm, respectively); relatively short vessel elements (often 150–370 µm) in Asclepiadinae and Metastelmatinae (Asclepiadeae – Fig. 4D,F). Fibre length (150–)440–780(–1300) µm, significantly shorter fibres in Asclepiadeae (mean 460 µm) compared to Marsdenieae (mean 730 µm), and a general trend towards a reduced fibre length observed in Asclepiadoideae (suppl. Fig. S2); mostly libriform fibres observed with additional fibre-tracheids or occasional species with only fibre-tracheids in Marsdenieae and Asclepiadeae (Fig. 4D), fibre-tracheids dominant in Ceropegieae, intermediate fibre types present in various species across the subfamily additionally to the libriform fibres and fibre tracheids; fibres generally thin- to thick-walled (Fig. 3A), but often

Table 1. Summary of taxonomically informative wood characters in Secamonoideae and studied (sub)tribes of the Asclepiadoideae.

	Secamonoideae (9)	Marsdenieae (18)	Leptadeniinae (5)	Metastelmatinae (9)	Asclepiadinae (12)
Vessel distribution pattern	S	S, C2–40	S	S	RM2–10, C3–20
Vessel element length (µm)	320–380	310–350	270–300	160–250	220–270
Fibre length (µm)	570–760	640–860	600–720	340–410	480–810
Fibre cell wall thickness	T–T	T	T	T–T, VT	T
Axial parenchyma distribution	Sc	Sc, (V)	Sc, B1–10	–	Sc, (V)
Axial parenchyma strand (nr. cells)	2–5	2–5	2–4, 1	2–4	2–4
Uniseriate ray height (µm)	150–290	240–400	170–290	130–170	180–280
Multiseriate ray width (nr. cells)	2–4	2	6–12	–	2–3
Multiseriate ray density (per mm)	2–6	5–15	1–4	–	2–7
Homocellular ray composition	–	Sq/U	–	Sq/U	Sq/U
Heterocellular ray composition	P+1–2U	P+1–2Sq/U	P+1–2Sq/U	–	P/Sq+1–2U
Non-lignified tissue variants	–	(L, W)	I	F	–
Mineral inclusions	(PC)	(PC, D)	PC	PC	–

Vessel distribution pattern (C = clusters, RM = radial multiples, S = mainly solitary), fibre cell wall thickness (T = thin, T–T = thin to thick, VT = very thick), axial parenchyma distribution (B = banded apotracheal, Sc = scanty paratracheal, V = variable between apotracheal diffuse and/or diffuse-in-aggregates, and/or vasicentric paratracheal), ray composition (P = procumbent, Sq = square, U = upright, / = and/or, + = distinguishing body ray cells from marginal ray cells), non-lignified tissue variants (F = furrowed, I = isolated zones, L = lobed, W = phloem wedges), mineral inclusions (D = druses, PC = prismatic crystals). () = occur in a significant proportion of species but not all. The quantitative wood characters are presented as the interquartile range of the mean values per clade.

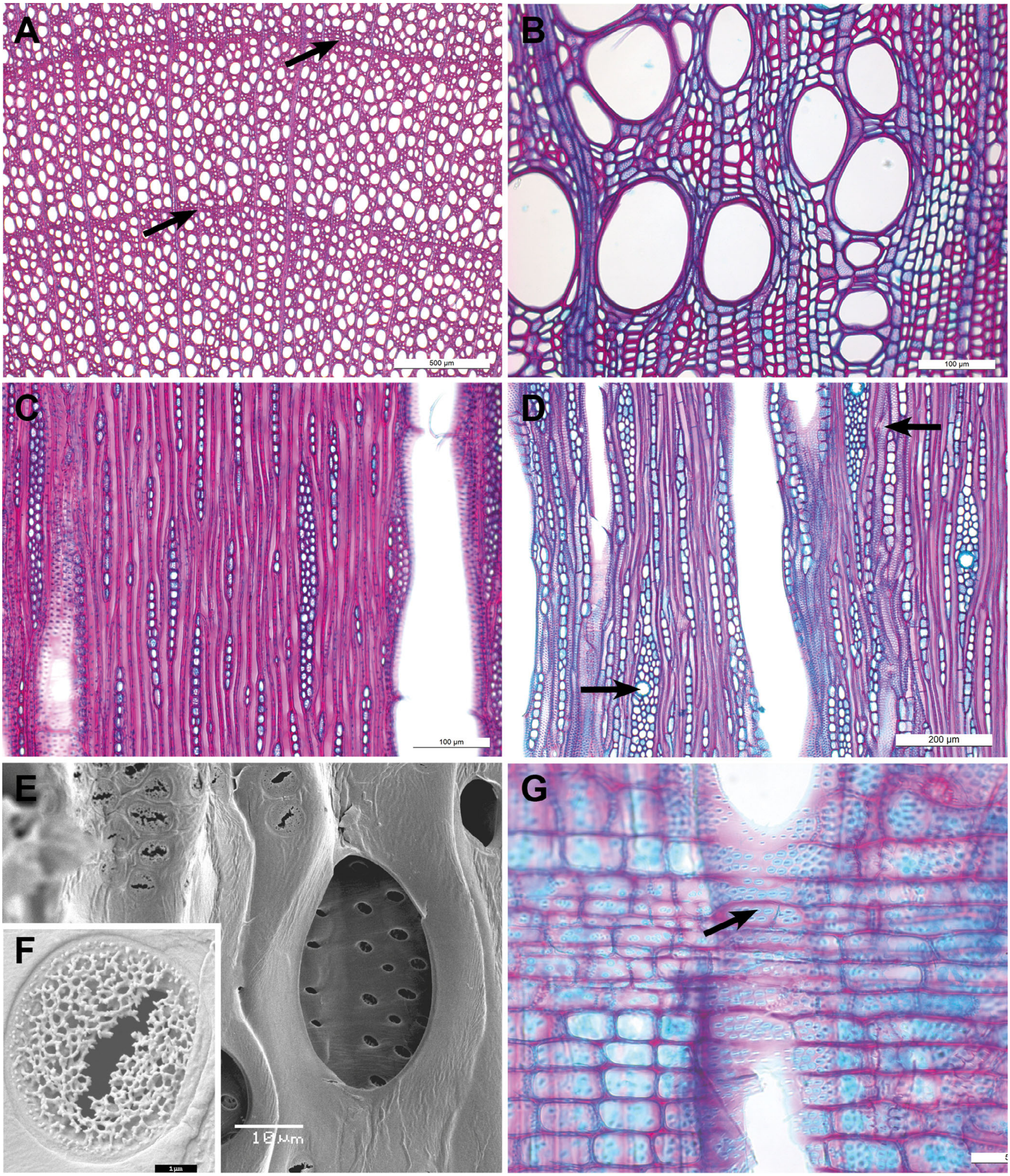

Fig. 2. Wood anatomical variation of Secamonoideae based on LM sections (A–D, G) and SEM surfaces (E, F). **A**, *Secamone stuhlmannii*: transverse section with distinct growth ring boundaries (arrows), delimited by thick-walled narrow fibres; **B**, *Genianthus micranthus*: transverse section with vessel clusters; **C**, *Secamone afzelii*: tangential section with uniseriate ray density exceeding that of multiseriate rays; **D**, *Secamonopsis madagascariensis*: tangential section showing narrow fibriform vessels (arrow pointing to the left), and laticifers in multiseriate rays (arrow pointing to the right); **E** & **F**, *Genianthus micranthus*: radial surface showing simple vessel perforation plates (**E**) and a detail of an intervessel pit with vestures occluding the pit chamber on a tangential surface (**F**); **G**, *Secamonopsis madagascariensis*: radial section showing a multiseriate ray consisting of procumbent body cells and several rows of square-upright marginal cells and bordered vessel-ray pits (arrow).

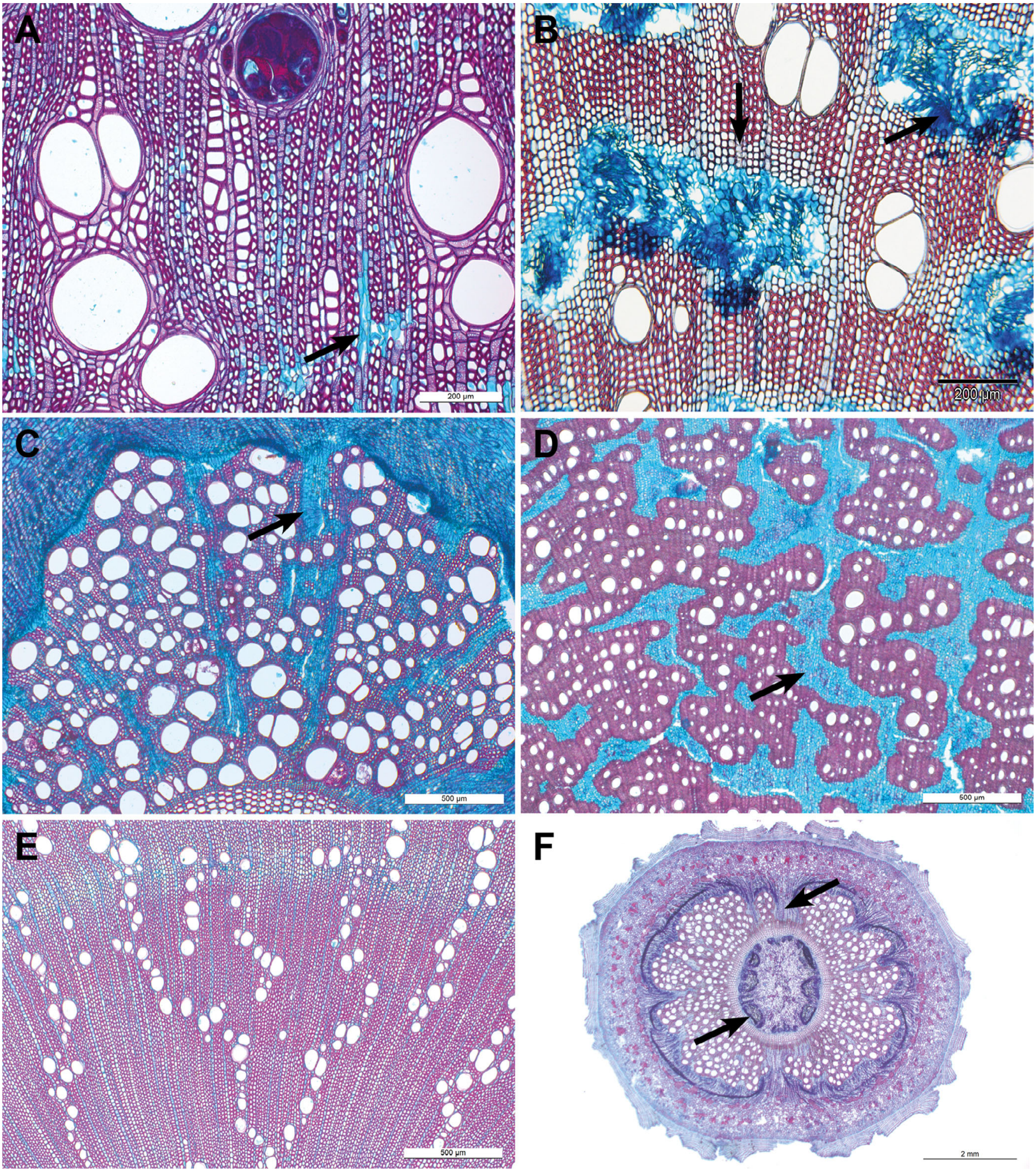

Fig. 3. Asclepiadoideae wood cross sections showing vessel and parenchyma distribution and the presence-absence of non-lignified tissues. **A**, *Stephanotis volubilis* (tribe Marsdenieae): vessel clusters including few wide vessels intermingled with many narrow vessels, and non-lignified ray and axial parenchyma portions (arrow); **B**, *Leptadenia pyrotechnica* (subtribe Leptadeniinae): elongated islands of non-lignified tissue with interxylary phloem (tilted arrow) inside wide apotracheal axial parenchyma bands (arrow pointing down); **C**, *Gonolobus parviflorus* (subtribe Gonolobinae): lobed non-lignified tissue (arrow) that consists mostly of rays, without interxylary phloem; **D**, *Ditassa capillaris* (subtribe Metastelmatinae): vessels mainly solitary, thick-walled fibres and furrowed non-lignified tissue with interxylary phloem (arrow); **E**, *Pergularia tomentosa* (subtribe Asclepiadinae): vessels in a dendritic pattern, no non-lignified zones; **F**, *Vincetoxicum sylvaticum* (subtribe Tylophorinae): section of a juvenile stem including pith and intraxylary phloem (arrow pointing to the right) in the centre and distinctive phloem wedges (arrow pointing to the left).

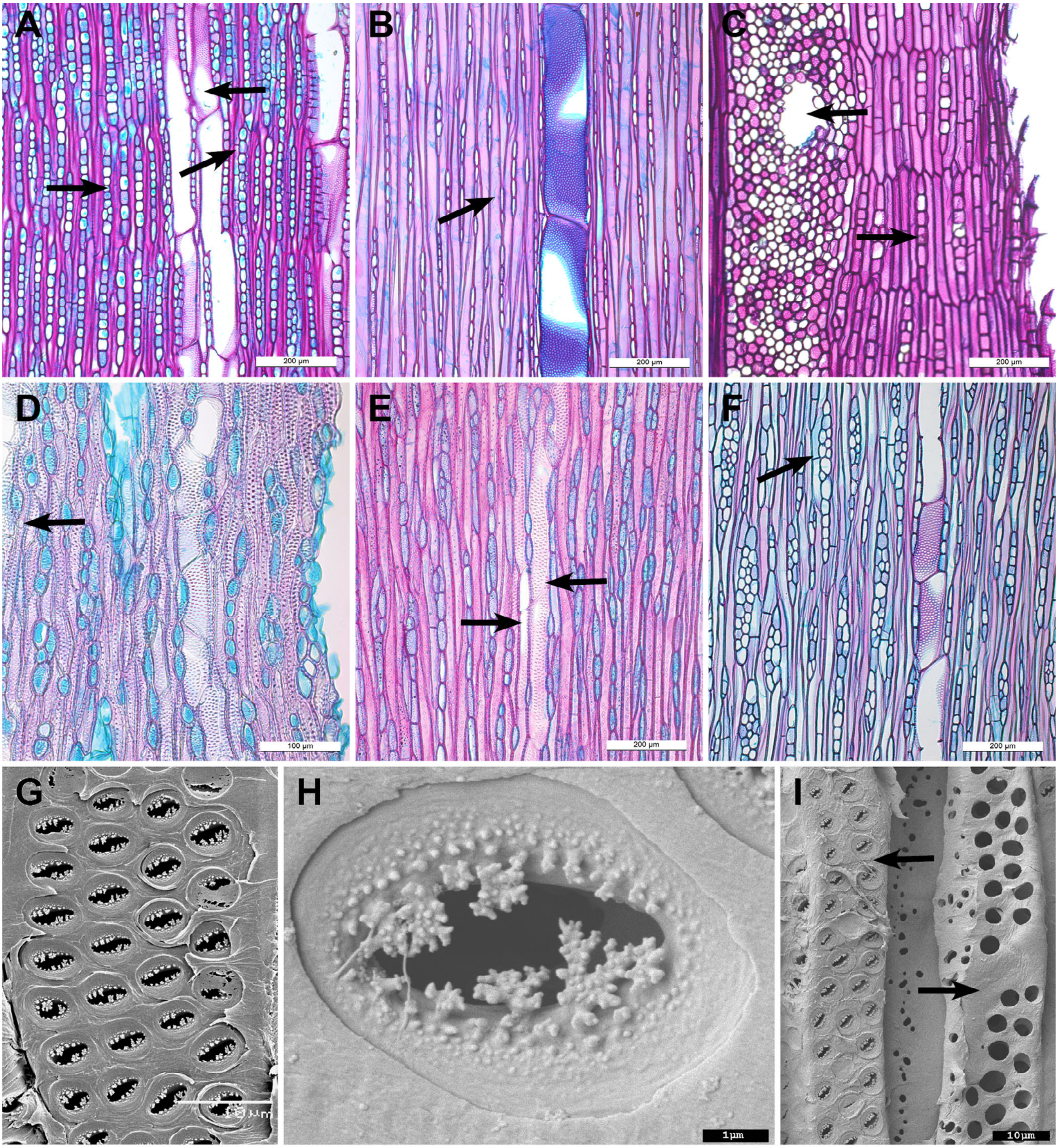

Fig. 4. Asclepiadoideae tangential wood sections showing storied structures, variation in ray parameters and intervessel pitting based on light microscope sections (A–F) and scanning electron microscope surfaces (G–I). **A**, *Stephanotis volubilis* (tribe Marsdenieae): storied rays (arrow pointing to the right), vessel elements (arrow pointing to the left), fibres and axial parenchyma strands (tilted arrow); **B**, *Hoya spartioides* (tribe Marsdenieae): uniseriate rays with thin-walled libriform fibres (arrow); **C**, *Leptadenia pyrotechnica* (subtribe Leptadeniinae): storied fusiform axial parenchyma and narrow uniseriate rays (arrow pointing to the right) near non-lignified tissue (ruptured zone at the right hand side) and laticifer in multiseriate ray (arrow pointing to the left); **D**, *Minaria acerosa* (subtribe Metastelmatinae): short uniseriate rays, and many fibriform vessels (arrow shows simple perforation plate of one such a vessel); **E**, *Kanahia laniflora* (subtribe Asclepiadinae): rays mainly uniseriate, narrow vessels (arrow pointing to the left) and fibriform vessels (arrow pointing to the right); **F**, *Calotropis procera* (subtribe Asclepiadinae): uni- and multiseriate rays and short axial parenchyma strand libriform fibres (arrow); **G**, *Gymnema inodorum* (tribe Marsdenieae): alternate vestured intervessel pits; **H**, *Funastrum clausum* (subtribe Oxypetalinae): detail of vestured intervessel pit; **I**, *Gomphocarpus fruticosus* (subtribe Asclepiadinae): narrow vessel (arrow pointing to the left) with alternate vestured pits and axial parenchyma cell (arrow pointing to the right) with simple non-vestured pits.

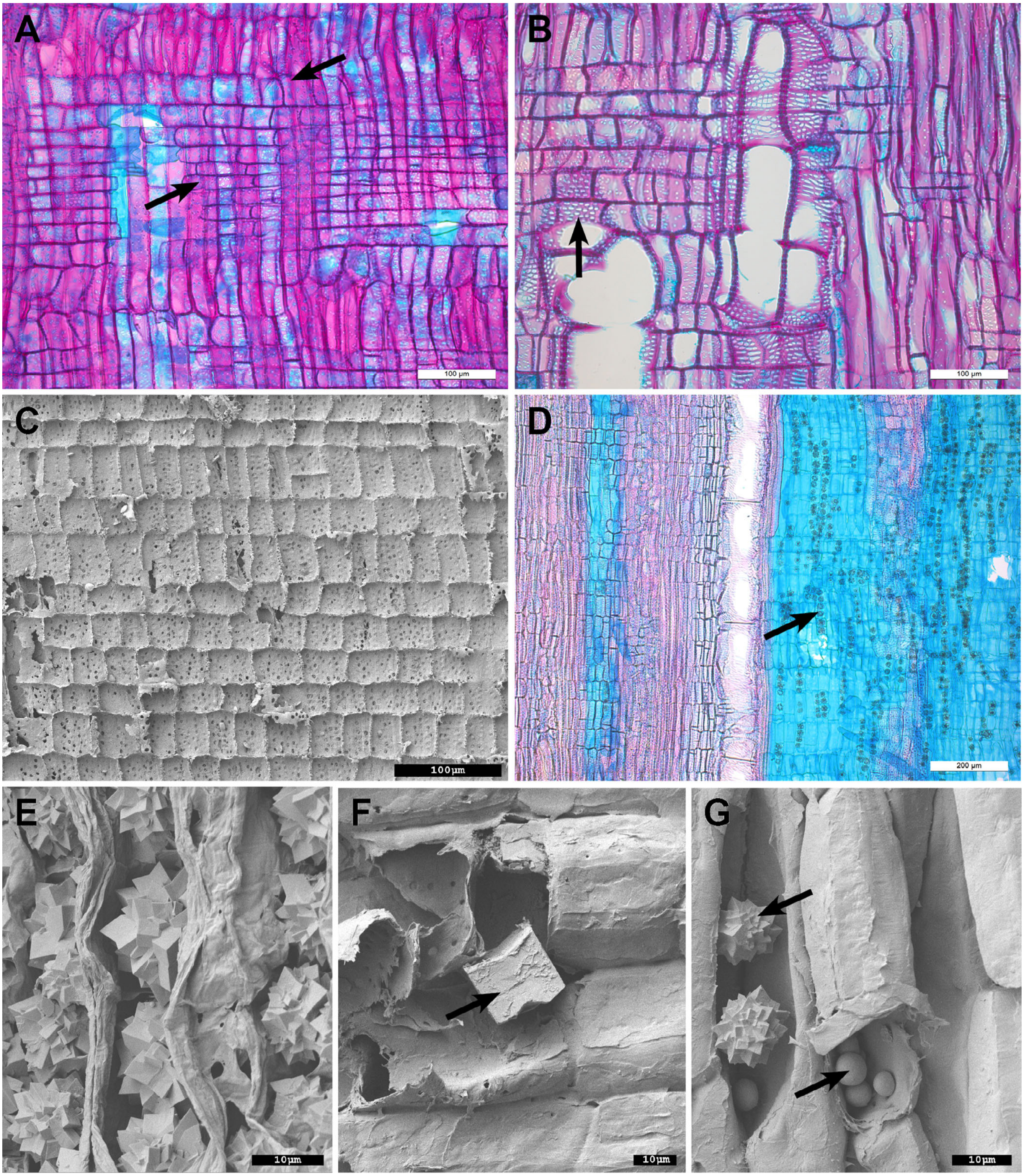

Fig. 5. Asclepiadoideae radial wood sections showing the cellular composition of rays and mineral inclusions, as seen in light microscope sections (A, B, D) and scanning electron microscope surfaces (C, E–G). **A**, *Stephanotis volubilis* (tribe Marsdenieae): procumbent body ray cells (tilted arrow pointing up) with one row of square to procumbent, and upright marginal ray cells (tilted arrow pointing down); **B**, *Calotropis gigantea* (subtribe Asclepiadinae): heterocellular ray consisting of square to upright cells and vessel-ray pits (arrow); **C**, *Gomphocarpus fruticosus* (subtribe Asclepiadinae): heterocellular ray consisting of square, procumbent, and upright cells; **D**, *Heterostemma cuspidatum* (subtribe Heterostemminae): homocellular rays with upright cells containing druses (arrow) in non-lignified part; **E**, *Funastrum clausum* (subtribe Oxypetalinae): detail of druses in non-lignified tissue; **F**, *Leptadenia pyrotechnica* (subtribe Leptadeniinae): prismatic crystal in ray cell (arrow); **G**, *Funastrum clausum* (subtribe Oxypetalinae): ray cells with druses (arrow pointing to the left) and starch granules (arrow pointing to the right).

thin-walled in tribe Marsdenieae and subtribe Leptadeniinae (Ceropegieae), often very thick-walled in subtribe Metastelmatinae (Asclepiadeae – Fig. 3D), and exclusively thin-walled in subtribe Asclepiadinae (Asclepiadeae – Fig. 3E). Axial parenchyma scarce throughout the subfamily, scarce to almost absent in Asclepiadeae; present mostly as scanty paratracheal axial parenchyma, but additional apotracheal bands of 1–10(–20) cells wide in subtribe Leptadeniinae (Ceropegieae – Fig. 3B), scanty and vasicentric paratracheal parenchyma in *Anisopus efulensis*, *Hoya spartioides* and *Ruehssia altissima* (Marsdenieae), and occasionally also additional diffuse and/or diffuse-in-aggregates and/or apotracheal bands of 1–2(–8) cells wide across all clades (Fig. 3A); axial parenchyma strands (1)2–4 cells (Fig. 4A,F), up to 6–8 cells in the epiphytic *Hoya spartioides* (Marsdenieae). Rays uni- and 2-seriate, with no or very few multiseriate rays in Asclepiadeae (Fig. 4D, except subtribe Asclepiadinae – Fig. 4F), and uniseriate rays in combination with exceptionally wide rays of 3–13(–30) cells wide in *Ibatia maritima* (Asclepiadeae), and of 6–12 cells wide in subtribe Leptadeniinae (Ceropegieae – Fig. 4C); more uniseriate rays (7–13(–25)/mm) than multiseriate rays (0–5(–11)/mm); uniseriate rays (10–)120–740(–2600) µm high, exceptionally tall in *Ruehssia rubrofusca* (Marsdenieae – up to 2200 µm) and *Funastrum clausum* (Asclepiadeae – up to 1600 µm), exceptionally short in subtribe Metastelmatinae (Asclepiadeae – up to 450 µm – Fig. 4D); uniseriate ray height significantly lower in tribe Asclepiadeae (mean 235 µm) compared to Marsdenieae (mean 360 µm), a trend towards a reduced uniseriate ray height apparent in Asclepiadoideae (suppl. Fig. S2); multiseriate rays (70–)280–1420(–8500) µm high, exceptionally tall in *Ruehssia* (Marsdenieae – up to 3200 µm), Leptadeniinae (Ceropegieae – up to 4800 µm) and *Vincetoxicum sylvaticum* (Asclepiadeae – up to 8500 µm), and exceptionally short in subtribe Metastelmatinae (Asclepiadeae – up to 550 µm); uniseriate rays in most species of Marsdenieae and Ceropegieae composed of square and/or upright cells (Fig. 5C,D) together with multiseriate rays composed of procumbent and/or square body ray cells with 1–2(–10) rows of square or upright marginal ray cells (Fig. 5A), mostly rays composed of square and/or upright cells in Asclepiadeae, except for procumbent and/or square body ray cells with 1–2(>4) rows of upright marginal ray cells in subtribe Asclepiadinae (Asclepiadeae). Large non-lignified tissue abundantly present in Marsdenieae (phloem wedges – Fig. 3F) and in subtribes Leptadeniinae (Ceropegieae, large isolated zones inside apotracheal parenchyma bands – Fig. 3B) and Metastelmatinae (Asclepiadeae, furrowed – Fig. 3D), and occasionally lobed-shaped (Fig. 3C); interxylary phloem observed in the non-lignified tissue of *Gymnema sylvestre* (Marsdenieae), *Leptadenia arborea*, *L. pyrotechnica* (Ceropegieae – Fig. 3B), and *Blepharodon pictum*, *Ditassa capillaris*, and *Vincetoxicum sylvaticum* (Asclepiadeae). Storied vessel elements, fibres, axial parenchyma

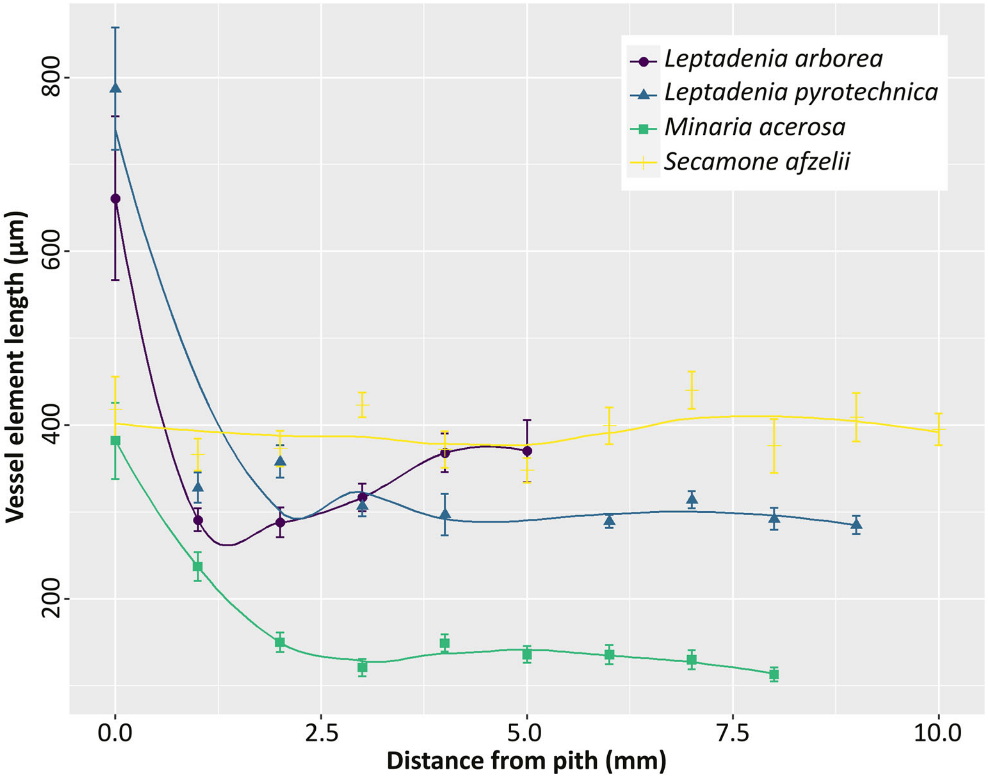

Fig. 6. Length-on-age (LoA) curves of Secamonoideae and Asclepiadoideae vessel elements, showing no clear difference between ancestrally and derived woody species. *Leptadenia arborea* is regarded as a climbing, ancestrally woody species, *Leptadenia pyrotechnica* a non-climbing ancestrally woody species, *Minaria acerosa* a non-climbing derived woody species and *Secamone afzelii* a climbing, ancestrally woody species.

and rays observed in *Gymnema inodorum*, *Gongronemopsis tenacissima* and *Stephanotis volubilis* (Marsdenieae – Fig. 4A), and storied axial parenchyma close to non-lignified tissue in tribes Ceropegieae (Fig. 4C) and Asclepiadeae, except for subtribe Metastelmatinae. Mineral inclusions scarcely observed; prismatic crystals common in lignified and/or non-lignified ray cells of subtribe Leptadeniinae (Ceropegieae – Fig. 5F), common in non-lignified axial parenchyma and ray cells of subtribe Metastelmatinae (Asclepiadeae), and absent in subtribe Asclepiadinae (Asclepiadeae); druses observed in non-lignified tissue of *Anisopus efulensis*, *A. mannii* (Marsdenieae), *Heterostemma cuspidatum* (Ceropegieae), *Funastrum clausum*, *Gonolobus parviflorus* and *Cynanchum ellipticum* (Asclepiadeae – Fig. 5E). Laticifers sporadically present in the subfamily, completely absent in subtribe Metastelmatinae (Asclepiadeae), but common in subtribe Leptadeniinae (Ceropegieae – Fig. 4C).

Estimated number of shifts from a herbaceous towards a derived woody growth form. — We scored 168 species within Asclepiadoideae that are derived woody, representing an estimated number of 28 independent shifts from an herbaceous towards a derived woody growth form in tribes Marsdenieae (6 shifts) and Asclepiadeae (21 shifts) (Appendix 2; suppl. Table S2; suppl. Fig. S1). Derived woody species occur in Asclepiadeae subtribes Gonolobinae (38 species – 2 shifts), Tylophorinae (29 species – 4 shifts), Metastelmatinae (26 species – 2 shifts), Cynanchinae (17 species – 8 shifts), Asclepiadinae (9 species – 2 shifts), Astephaninae (6 species – 0 shifts), Oxypetalinae (5 species – 2 shifts), Diplolepinae (5 species – 0 shifts), Pentacyphinae (1 species – 1 shift), Orthosiinae (1 species – 0 shift), paraphyletic genera-group sister to Asclepiadinae (2 species – 1 shift) and in tribe Marsdenieae (29 *Hoya* species – 6 shifts). Genera that include most species with derived woodiness are *Matelea* (Asclepiadeae subtribe Gonolobinae – 38 species), *Vincetoxicum* (Asclepiadoideae subtribe Tylophorinae – 31 species), *Hoya* (tribe Marsdenieae – 29 species), *Cynanchum* (Asclepiadeae subtribe Cynanchinae – 16 species) and *Minaria* (Asclepiadeae subtribe Metastelmatinae – 15 species). Climbing and non-climbing species are equally represented and species are found in the Americas, Africa, and Asia. Most records of derived woody species are in South American open habitats, such as savannas and campos rupestres highlands, in African dry environments, such as deserts and dry bushlands, on sandy and rocky soils, and in a wide range of Asian habitats, from open and disturbed forest at higher altitudes to moist valleys.

■ DISCUSSION

Our wood anatomical descriptions of Secamonoideae and Asclepiadoideae are in line with previously published wood descriptions (e.g., Fahn & al., 1984; Jagiella & Kürschner, 1987; Yaman & Tumen, 2012). Traits that are uniformly present across ancestrally and derived woody Apocynaceae are vessels with simple perforation plates (Fig. 2E) and alternate vestured intervessel pits (Figs. 2F, 4G–I), vessel-ray pits that are similar in shape and size to the intervessel pits (Fig. 5B), and the presence of intraxylary phloem (Lens & al., 2008, 2009b) (Fig. 3F). In addition, Secamonoideae and Asclepiadoideae woods show diffuse porous wood, solitary vessels in combination with various types of vessel multiples (Fig. 3), non-septate fibres with simple to minutely bordered pits ranging to fibre-tracheids with more distinctly and larger (3–4 μm) bordered pits, axial parenchyma often scanty paratracheal in low quantities and few (2–4) cells per strand (Figs. 3, 4), and uniseriate rays co-occurring with a small number of narrow and short multiseriate rays (Fig. 4). The occurrence of several species showing a large variation of non-lignified areas within the lignified wood cylinder is remarkable and rarely found in other angiosperm families, although not uncommon in lianas (Fig. 3) (Carlquist, 2001; Angyalossy & al., 2015). As far as we know, we describe for the first time the wood anatomy of Neotropical Asclepiadoideae and species of tribe Fockeeae, subtribe Heterostemminae (tribe Ceropegieae) and several subtribes of tribe Asclepiadeae (Cynanchinae, Gonolobinae, Metastelmatinae, Orthosiinae, Tassadiinae, Tylophorinae), making this overview a valuable contribution to further complete our wood anatomical knowledge of the Apocynaceae family.

Wood anatomy reveals taxonomically informative features across subfamilies Secamonoideae and Asclepiadoideae. — The wood anatomy of Secamonoideae (9 descriptions in a fairly woody subfamily of large lianas, small shrubs, and perennial herbs with 160 species – Endress & al., 2019) is distinguishable from that of Asclepiadoideae by a combination of mainly solitary vessels (vs. vessels solitary and/or in vessel multiples), longer vessel elements (mean 340 μm vs. 290 μm), and heterocellular rays with a clear difference between procumbent body ray cells and upright marginal ray cells (vs. more homocellular rays with mostly square to upright cells; Fig. 1; Table 1; suppl. Table S1). Likewise, within the Asclepiadoideae lineages, a set of wood anatomical characters can differentiate between the major clades as defined by molecular data (Fig. 1; Table 1). The woody species studied of tribe Marsdenieae (18 descriptions in an otherwise herbaceous tribe of herbs, vines, lithophytes and epiphytes, with 790 species – Endress & al., 2019), are recognised by a combination of solitary vessels and extensive clusters of 2–40 (–60) vessels (Fig. 3A), thin-walled fibres and the occurrence of phloem wedges produced by a wavy vascular cambium (only for half of the species). Asclepiadeae (33 descriptions in a predominantly herbaceous tribe of 1840 species – Endress & al., 2019 – of which around 140 are woody – Appendix 2; suppl. Table S2) clearly differs from Marsdenieae by the occurrence of shorter uniseriate rays (mean range 170–320 μm vs. 220–410 μm), shorter fibres (400–650 μm vs. 630–850 μm), shorter vessel elements (220–330 μm vs. 320–380 μm) (suppl. Fig. S2), and a much lower abundance of multiseriate rays and axial parenchyma (Fig. 4D–F). In addition to these diagnostic features at the tribal level, the wood anatomy of two Asclepiadeae subtribes studied (Asclepiadinae, Metastelmatinae) can be distinguished from one another as well. Metastelmatinae (9 wood descriptions in a predominantly herbaceous subtribe of suffrutescent twiners, herbs, subshrubs with 290 species – Endress & al., 2019 – of which around 30 are woody – Appendix 2; suppl. Table S2) is one of the best-recognisable clades from a wood anatomical point of view with a unique combination of solitary vessels (Fig. 3D), thicker-walled fibres, virtually no axial parenchyma and no multiseriate rays (Fig. 4D), homocellular rays with square to upright ray cells, and furrowed non-lignified tissue (Fig. 3D). In contrast, species in Asclepiadinae (12 wood descriptions within a subtribe of usually erect herbs of 340 species – Endress & al., 2019 – Appendix 2; suppl. Table S2) typically have extensive grouped vessels in radial vessel multiples of 2–10, often combined with clusters of 3–20 including many narrow fibriform vessels, thin-walled fibres, and no non-lignified tissue (Table 1). In another Asclepiadeae subtribe with woody species (Gonolobinae), our sampling (only 5 wood descriptions in a clade with an estimated 30 woody species – Appendix 2; suppl. Table S2) is not sufficient to make comparative statements with respect to other clades. Also, for tribe Ceropegieae, a sound comparison at the tribal level is not feasible because only two woody genera (*Heterostemma*, *Leptadenia*) from two different subtribes (Heterostemminae, Leptadeniinae) are represented in our study. Interestingly, the three *Leptadenia* species investigated here show a set of wood anatomical traits that is unique for the entire family: banded apotracheal parenchyma of 1–10 cells wide (Fig. 3B), fusiform axial parenchyma (Fig. 4C), wide multiseriate rays of 6–12 cells in width (Fig. 4C), prismatic crystals in chambered ray cells, and isolated zones of non-lignified tissue with interxylary phloem (Gondaliya & Rajput, 2016). Whether or not this set of traits represent a wood anatomical synapomorphy of Leptadeniinae subtribe remains unclear and will become evident when more species are investigated, along with other woody genera (*Neoschumannia*, *Orthanthera*) in tribe Ceropegieae for which currently no wood anatomical descriptions are available. Despite the clear taxonomic signal of wood anatomical traits between Secamonoideae and Asclepiadoideae on the one hand, and across Asclepiadoideae lineages on the other hand, we found no clear evidence to distinguish the Secamonoideae-Asclepiadoideae clade from the rest of the Apocynaceae.

Wood anatomy of climbers vs. non-climbers. — Molecular phylogenies convincingly show that there have been multiple evolutionary transitions from a twining towards an erect growth form in the Apocynaceae (Lahaye & al., 2005; Silva & al., 2012; Fishbein & al., 2018). The opposite transition from an erect towards a climbing growth form has not been found, although climbing lineages were also hypothesised to have evolved multiple times based on the clear difference in vessel grouping patterns between lianescent rauvolfioids (radial multiples), and lianescent apocynoid and Periplocoideae members (large vessel clusters) (Lens & al., 2008, 2009a). Whether or not vessel clusters are typical of all APSA-clade climbers could not be supported with anatomical observations from Secamonoideae and Asclepiadoideae (Figs. 2, 3; suppl. Table S1). The reason for this is that growth form information of Secamonoideae and Asclepiadoideae is often difficult to obtain, not only because information is missing in the literature or on voucher labels, but even more so because the growth form of some species depends on the availability of a nearby host plant, and could, therefore, be either climbing or non-climbing, or be interpreted as an intermediate crawling/scrambling type (e.g., most species in subtribe Metastelmatinae show wood anatomical traits intermediate to a climbing and non-climbing growth form; Fig. 3D).

Although climbers have much narrower stems compared to erect trees, they still support a similar total leaf surface (Castellanos, 1991), which is why most studies find that lianas have wider vessels in order to ensure a more efficient water transport to support transpiration (e.g., Carlquist, 1985; Ewers, 1985; Ewers & al., 1990; Angyalossy & al., 2015). However, Rosell & Olson (2014) found no difference in vessel diameter between climbers and erect species of the same height in their analysis on climbers in 160 angiosperm families, implying that the tip-to-base vessel widening is the same in trees compared to lianas (Olson, 2020). We found that vessels in Asclepiadoideae climbers are significantly wider compared to non-climbers (on average 105 µm vs. 55 µm, respectively – suppl. Fig. S2), even when including for the many narrow fibriform vessels in the climbing species. With respect to vessel density, some studies have found that lianas have more vessels per square millimetre due to vessel dimorphism (Rosell & Olson, 2014; Angyalossy & al., 2015), referring to the co-occurrence of many narrow fibriform vessels (often 10–15 µm wide) that are frequently grouped in close association with wide vessels (up to 700 µm) (Ayensu & Stern, 1958; Angyalossy & al., 2012, 2015; Olson, 2020). Other papers, however, report less vessels per square millimetre (Carlquist, 1975, 1984a,b; Vliet, 1981). This difference may have biological reasons as some lianas only have a very small proportion of narrow vessels (e.g., Marcgraviaceae; Lens & al., 2005b), or may be attributed to misidentification of narrow vessels as fibres or vasicentric tracheids (Carlquist, 1985). Indeed, vessel density patterns in Asclepiadoideae suggest that either including or excluding the narrow fibriform vessels from density measurements can explain this inconsistency in the literature: vessel density is higher in climbers when fibriform vessels are included, but higher in erect species when fibriform vessels are excluded (although these differences are not statistically significant – suppl. Fig. S2). The standardised wood description guideline with its prime objective to identify wood samples (IAWA Committee, 1989) suggests to only measure wide vessels for vessel diameter, but more detailed measurements including the entire vessel diameter range is required when discussing wood anatomical differences between growth forms.

Another major anatomical difference between climbing and non-climbing species is the increased flexibility in liana stems. Whereas trees need to build mechanically strong stems with a huge portion of lignified wood, the liana growth form is facilitated by a higher proportion of parenchyma in the wood that enables a more flexible stem (Carlquist, 1985, 2001; Ewers & al., 1990; Gasson & Dobbins, 1991). Except for the clearly banded apotracheal axial parenchyma pattern in Leptadeniinae, axial parenchyma is scarcely present in Secamonoideae and Asclepiadoideae climbers. Consequently, the increased amount of axial parenchyma in vines as part of a "lianescent vascular syndrome" as proposed by Angyalossy & al. (2015) could not be supported in Asclepiadoideae, as is also the case for other lianescent groups, such as Bignoniaceae (Pace & Angyalossy, 2013). Likewise, we did not observe taller rays, another component of this syndrome. We did, however, observe an increased amount of non-lignified rays or entire non-lignified zones (with or without interxylary phloem) in the wood cylinder of many Asclepiadoideae climbers (Fig. 2A,B,D,F,G), which was also observed by Metcalfe & Chalk (1950), with the exception of non-lignified tissue in erect Metastelmatinae species that often present an intermediate scrambling growth form as mentioned before. We could not confirm the presence of interxylary phloem in most non-lignified tissue of Asclepiadoideae, because the xylarium wood samples studied were completely desiccated, breaking down and tearing the non-lignified tissue. We did observe interxylary phloem in the fresh samples of *Gymnema sylvestre* (Marsdenieae), *Leptadenia lanceolata* (Ceropegieae) and *Vincetoxicum sylvaticum* (Asclepiadeae), and additional species with interxylary phloem were reported by other authors in *L. lanceolata* (Singh, 1943), *L. pyrotechnica* (Gondaliya & Rajput, 2016), and at the base of the stem and/or in the root of *Asclepias obtusifolia*, *A. syriaca* and *Ceropegia* sp. (Solereder, 1908).

The origin of woodiness in Secamonoideae and Asclepiadoideae. — According to the ancestral state reconstructions of Fishbein & al. (2018), it is clear that the common ancestor of Secamonoideae and Asclepiadoideae is woody, and that multiple transitions from the herbaceous towards the woody condition have occurred across Asclepiadoideae. Our study offers new complementary information about the identity, biogeography and number of evolutionary transitions of derived woody species across Asclepiadoideae based on published phylogenetic evidence, taxonomic revisions, and flora treatments. Although the ancestral state of the Asclepiadoideae was calculated to be herbaceous in Fishbein & al. (2018), the presence of woody species in both Marsdenieae and Ceropegieae could be the result of a retention of the ancestrally woody condition, considering that most Marsdenieae and the oldest subtribes of Ceropegieae (Heterostemminae, Leptadeniinae) have at least a woody base as opposed to the few woody species nested within the predominantly herbaceous Asclepiadeae. It is, however, clear that both tribes underwent a general shift from woodiness towards herbaceousness (Meve & al., 2017; Liede-Schumann & al., 2022). Apart from species of Marsdenieae and Ceropegieae that probably retained ancestral woodiness, we estimate the presence of 168 derived woody species in Asclepiadoideae (suppl. Table S2), which are hypothesised to be the result of at least 28 independent shifts from herbaceousness towards derived woodiness (Appendix 2; suppl. Fig. S1). The majority of the derived woody species in this subfamily occupy dry continental habitats, such as the deserts of the Middle East and Namib, the chaparral of southwestern U.S.A. and the savannas in Brazil and eastern Africa (e.g., Rapini & al., 2007; Goyder & al., 2012; Bitencourt & al., 2021 – suppl. Table S2). The abundance of derived woody Asclepiadoideae in (seasonally) dry environments is in line with the drought hypothesis, which states that drought could have been a major evolutionary driver of wood formation (Lens & al., 2013a,b; Zizka & al., 2022). While we cannot be fully confident that drought is the main driver of wood formation in the otherwise predominantly herbaceous Asclepiadoideae lineages, the link between increased woodiness and increased drought tolerance (1) supports earlier studies on the distribution of insular woodiness on the Canary Islands (Lens & al., 2013a; Hooft van Huysduynen & al., 2021), (2) agrees with drought as one of the most important drivers triggering wood formation on islands (Zizka & al., 2022), and (3) is in line with experimental work demonstrating that species with more wood formation or lignification in the stem are better able to prevent the formation and spread of drought-induced gas bubbles inside the water conducting xylem cells (Lens & al., 2013b; Dória & al., 2018, 2019; Thonglim & al., 2020; Frankiewicz & al., 2021). However, we do acknowledge that drought may not be the only potential driver of woodiness and that these drivers may be lineage specific (Carlquist, 1974; Kidner & al., 2016; Neupane & al., 2017; Frankiewicz & al., 2020; Zizka & al., 2022).

From a wood anatomical point of view we cannot make a distinction between ancestrally and derived woody species based on the presence/absence of peramorphic features in the later formed wood. Previous interpretations of this heterochronic xylem syndrome are incorrect and these peramorphic features are more associated with growth form than woody origin (Carlquist, 2009, 2012, 2013; Dulin & Kirchoff, 2010; Lens & al., 2013b; Kidner & al., 2016; Neupane & al., 2017). For instance, most Secamonoideae (ancestrally woody) and many woody Asclepiadoideae (mostly derived woody) show flat or decreasing length-on-age curves (Fig. 6), and Marsdenieae species with ancestral woodiness show rays with predominantly square to upright cells. These traits refer to peramorphosis that was previously erroneously assigned to derived woody species only (Carlquist, 1962).

■ CONCLUSION

This Secamonoideae and Asclepiadoideae overview supports previous statements in the family that wood anatomical traits are taxonomically informative at the subfamily and tribal level (Lens & al., 2008, 2009a). Traits of particular interest are vessel grouping, length of vessel elements and fibres, variation in rays (width, cellular composition), and non-lignified zones in the wood cylinder. From a wood anatomical point of view, there are a number of clades with a unique set of features in the entire family, such as Metastelmatinae (solitary vessels, thick-walled fibres, no multiseriate rays, furrowed non-lignified zones), and *Leptadenia* (wide bands of axial parenchyma including fusiform cells and zones of interxylary phloem, and wide multiseriate rays). Furthermore, the increased flexibility in Asclepiadoideae liana stems does not result from increased axial or ray parenchyma, but from a higher proportion of non-lignified tissue in the wood that may or may not include interxylary phloem. Performing ancestral state reconstructions of phylogenetically informative wood anatomical characters including all larger lineages will be the subject of a follow-up paper where we aim to summarise the phylogenetic trends in the wood anatomy of the entire family. With respect to the origin of woodiness, all the woody Secamonoideae are assumed to be ancestrally woody, which is perhaps also the case for most woody lineages in Marsdenieae and Ceropegieae. The remaining woody Asclepiadoideae species in the tribe Asclepiadeae, as well as the woody *Hoya* species in tribe Marsdenieae are hypothesised to have evolved from herbaceous ancestors. These 168 species with derived woodiness are estimated to be the result of approximately 28 independent shifts from an herbaceous towards a (derived) woody growth form, which may have been triggered by drought.

■ AUTHOR CONTRIBUTIONS

FL, ES and VB designed the research. AR, FL, VB collected the samples. FL and VB conducted the laboratory work and collected the data. VB analysed the data under supervision of AR, ES and FL. All authors interpreted the results. VB wrote the manuscript with participation of AR, ES and FL. — VB, https://orcid.org/0000-0001-7539-3708; AR, https://orcid.org/0000-0002-8758-9326; ES, https://orcid.org/0000-0002-9416-983X; FL, https://orcid.org/0000-0002-5001-0149

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Appendix 1. Voucher information of wood samples used, along with information on the xylarium source and the sample diameter. Values in sample radius (diameter) column are split into the radius of the xylem cylinder and total sample diameter including pith. Species in bold are juvenile samples (descriptions given at end of suppl. Table S1)

Accepted species name	Origin	Voucher	Accession number	Radius (Diameter) (mm)	Source institute
Anisopus efulensis (N.E.Br.) Goyder	D.R. Congo (Yangambi)	*Louis J. 3300*	Tw35026	4 (9)	Africa museum – Tervuren xylarium
Anisopus mannii N.E.Br.	Congo (Kinshasa)	*Louis J. 14203*	WAG0093985	3 (7)	Naturalis Biodiversity Center
Asclepias curassavica L.	Panama (Darien)	*Stern & al. 273*	SJRw54557	3 (8)	U.S. Forest Service Laboratory
Asclepias linaria Cav.	Mexico (Tehuantepec)	*Lamb S.H. s.n.*	Tw32025	Mature	Africa museum – Tervuren xylarium
Asclepias syriaca L.	U.S.A. (Philadelphia)	*Livshultz s.n.*	–	3 (11)	University of Bayreuth
Barjonia cymosa E.Fourn.	Brazil (Mato Grosso)	*Rapini A. 1941*	–	2 (5)	HUEFS – Herbario da Universidade Estadual de Feira de Santana
Blepharodon pictum (Vahl) W.D.Stevens	Brazil (Minas Gerais)	*Rapini A. & Ribeiro P.G. 1932*	–	3 (5)	HUEFS – Herbario da Universidade Estadual de Feira de Santana
Calotropis gigantea (L.) W.T. Aiton	India (Orissa)	Collector unknown	Kw13180	Mature	Royal Botanic Gardens, Kew
Calotropis procera (Aiton) W.T.Aiton 1	Senegal (Casamance)	*De Wolf J. s.n.*	Tw51614	Mature	Africa museum – Tervuren xylarium
Calotropis procera (Aiton) W.T.Aiton 2	Egypt	*Schweinfurth G. 31*	Kw13181	Mature	Royal Botanic Gardens, Kew
Cynanchum ellipticum (Harv.) R.A.Dyer	South Africa (Cape-Grahamstown)	*Liede 2911*	–	5 (8)	University of Bayreuth
Cynanchum viminale (L.) L.	Uganda (Hoimo)	*Dechamps R. & al. 11562*	Tw50942	7 (22)	Africa museum – Tervuren xylarium
Ditassa capillaris E.Fourn.	Brazil (Bahia)	*Rapini A., Ribeiro P.L., Bitencourt C.C.C. 1949*	–	4 (10)	HUEFS – Herbario da Universidade Estadual de Feira de Santana
Ditassa mucronata Mart.	Brazil (Minas Gerais)	*Rapini A., Ribeiro P.L. 1925*	–	2 (5)	HUEFS – Herbario da Universidade Estadual de Feira de Santana
Ditassa retusa Mart.	Brazil (Minas Gerais)	*Rapini A., Ribeiro P.L. 1929*	–	3 (6)	HUEFS – Herbario da Universidade Estadual de Feira de Santana
Fockea angustifolia K.Schum.	South Africa (Cape)	*Frohning U. & H. s.n.*	–	2 (4)	University of Bayreuth
Funastrum clausum (Jacq.) Schltr.	Surinam	*Heyde 564*	U0261429	3 (7)	Naturalis Biodiversity Center
Genianthus micranthus (Roxb.) I.M.Turner	Thailand (Chiang Mai, Doi Sutep-Pui National Park)	*Maxwell J.F. 89-131*	L0085675	5 (11)	Naturalis Biodiversity Center
Gomphocarpus fruticosus (L.) W.T.Aiton	Spain	*Schweingruber F.H. 23*	L0085676	6 (14)	Naturalis Biodiversity Center
Gomphocarpus phillipsiae (N.E.Br.) Goyder	D.R. Congo (Kivu)	*Baudet J.C. 433*	Tw27439	5 (11)	Africa museum – Tervuren xylarium
Gomphocarpus semilunatus A.Rich.	Kenya (Nyanza)	*Plaizier 1345 A.C.*	WAG0370818	6 (12)	Naturalis Biodiversity Center
Gomphocarpus tomentosus subsp. *frederici* (Hiern) Goyder & Nicholas	Angola (Cunene)	*Dechamps & al. 1290*	MADw37409, Tw28363	5 (11)	U.S. Forest Service Laboratory/Africa museum – Tervuren xylarium
Gongronemopsis latifolia (Benth.) S.Reuss, Liede & Meve	Cameroon	*Elad M.E. 466*	WAG.1658496	3 (9)	Naturalis Biodiversity Center
Gongronemopsis tenacissima (Roxb.) S.Reuss, Liede & Meve 1	Thailand (Doi Sutep-Pui National Park)	*Maxwell J.F. 90-567*	L0085681	3 (7)	Naturalis Biodiversity Center

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Appendix 1. Continued.

Accepted species name	Origin	Voucher	Accession number	Radius (Diameter) (mm)	Source institute
Gongronemopsis tenacissima (Roxb.) S.Reuss, Liede & Meve 2	India (Dehra Deen)	Collector unknown	Kw13197	52	Royal Botanic Gardens, Kew
Gonolobus parviflorus Decne.	Brazil (Minas Gerais)	*Rapini A., Souza-Silva R.F. 1293*	–	2 (5)	HUEFS – Herbario da Universidade Estadual de Feira de Santana
Gymnema inodorum (Lour.) Decne.	Philippines	*Ridsdale C.E. 1911*	L0085678	49	Naturalis Biodiversity Center
Gymnema pachyglossum Schltr.	Philippines	*Ridsdale C.E. 1880*	L0085677	5 (8)	Naturalis Biodiversity Center
Gymnema rotundatum Thwaites	Sri Lanka (Weerasooriya, Jayasekera)	*Ridsdale C.E. 1948*	L0625144	6 (12)	Naturalis Biodiversity Center
Gymnema sylvestre (Retz.) R.Br. ex Sm.	D.R. Congo (Katanga)	*Malaisse F. 11168*	Tw39944	10 (25)	Africa museum – Tervuren xylarium
Heterostemma cuspidatum Decne.	Philippines	*Ridsdale C.E. 1876A*	L0085679	3 (8)	Naturalis Biodiversity Center
Hoya lauterbachii K.Schum.	Papua New Guinea	*Jacobs M. 9484*	L0802418, L0693201	4 (10)	Naturalis Biodiversity Center
Hoya spartioides (Benth.) Kloppenb.	Borneo	*Fuchs 21248*	L0085670	87	Naturalis Biodiversity Center
Ibatia maritima (Jacq.) Decne.	Colombia (Atlantico)	*Dugand A. 522-b*	SJRw33805x	5 (11)	U.S. Forest Service Laboratory
Kanahia laniflora (Forssk.) R.Br.	Kenya (Sala Gate, Tsavo East)	*Liede & Newton 3211*	–	3 (7)	University of Bayreuth
Leptadenia arborea (Forssk.) Schweinf.	Cameroon	*Wit P. & Geerling C. 3289*	WAG0111826	7 (15)	Naturalis Biodiversity Center
Leptadenia lanceolata (Poir.) Goyder	Mali (Gao)	*Geerling & Bokdam 2669*	WAG.1658027	7 (15)	Naturalis Biodiversity Center
Leptadenia pyrotechnica (Forssk.) Decne. 1	Egypt	*Cappers R. & Vermeeren C. s.n.*	L0079631	11 (20)	Naturalis Biodiversity Center
Leptadenia pyrotechnica (Forssk.) Decne. 2	Egypt	*Schweinfurth G. 39*	Kw13194	Mature	Royal Botanic Gardens, Kew
Leptadenia pyrotechnica (Forssk.) Decne. 3	Mali (Plateau Bandiagara)	*Doutrelepont H. s.n.*	Tw55461	43	Africa museum – Tervuren xylarium
Macroscepis diademata (Ker Gawl.) W.D.Stevens 1	Mexico	*King R.M. & Soderstrom 5222*	SJRw55304	2 (5)	U.S. Forest Service Laboratory
***Macroscepis diademata* (Ker Gawl.) W.D.Stevens 2**	**Guatemala (Guatemala City)**	***Heyne s.n.***	**–**	**1 (4)**	**University of Bayreuth**
Marsdenia sp.	Mozambique (Tete, Cabora Bassa (futura albufeira) andados)	*Correia M.F., Marques A., Monteiro P. 3984*	WAG.1658949	3 (9)	Naturalis Biodiversity Center
Matelea pedalis (E.Fourn.) Fontella & E.A.Schwarz 1	Brazil (Minas Gerais)	*Rapini, Mello-Silva, Carvalho 907*	–	3 (6)	HUEFS – Herbario da Universidade Estadual de Feira de Santana
Matelea pedalis (E.Fourn.) Fontella & E.A.Schwarz 2	Brazil (Mato Grosso)	*Rapini & al. 1882*	–	3 (6)	HUEFS – Herbario da Universidade Estadual de Feira de Santana
Metastelma northropiae Schltr.	U.S.A. (Florida, Big Pine Key)	*Stern & Brizicki 252*	MADw18240, SJRw51092	6 (18)	U.S. Forest Service Laboratory
Minaria acerosa (Mart.) T.U.P. Konno & Rapini	Brazil (Mato Grosso)	*Rapini A., Ribeiro 1942*	–	8 (12)	HUEFS – Herbario da Universidade Estadual de Feira de Santana
Minaria grazielae (Fontella & Marquete) T.U.P.Konno & Rapini	Brazil (Minas Gerais)	*Rapini A., Ribeiro 1935*	–	3 (5)	HUEFS – Herbario da Universidade Estadual de Feira de Santana

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Appendix 1. Continued.

Accepted species name	Origin	Voucher	Accession number	Radius (Diameter) (mm)	Source institute
Orthosia scoparia (Nutt.) Liede & Meve	Puerto Rico (Route 120)	*Mika & al. 17*	–	2 (4)	University of Bayreuth
Peplonia organensis (E.Fourn.) Fontella & Rapini	Brazil (Minas Gerais)	*Rapini A. 1926*	–	3 (5)	HUEFS – Herbario da Universidade Estadual de Feira de Santana
Pergularia tomentosa L.	Egypt	*Cappers R. & Vermeeren C. s.n.*	L0079626	3 (8)	Naturalis Biodiversity Center
Ruehssia altissima (Jacq.) F.Esp.Santo & Rapini 1	Ecuador	*Matthias M.E. & Taylor D. 5224*	L0085680	3 (10)	Naturalis Biodiversity Center
***Ruehssia altissima* (Jacq.) F.Esp.Santo & Rapini 2**	**Bolivia (Guarayos)**	***Nee M. 38647***	**MADw46593**	**3 (6)**	**U.S. Forest Service Laboratory**
Ruehssia cundurango (Triana) Liede & H.A.Keller	Origin unknown	Collector unknown	Kw13195	7 (20)	Royal Botanic Gardens, Kew
***Ruehssia rubrofusca* (E.Fourn.) F.Esp.Santo & Rapini**	**Venezuela (Amazonas)**	***Maguire B. & al. 43031***	**Tw36820**	**2 (6)**	**Africa museum – Tervuren xylarium**
***Schizostephanus alatus* Hochst. ex K.Schum.**	**Kenya**	***Noltee s.n*.**	**–**	**2 (6)**	**University of Bayreuth**
Secamone afzelii (Roem. & Schult.) K.Schum.	Cameroon (Bertoua)	*Breteler F.J. 1796*	WAG0111827	26	Naturalis Biodiversity Center
Secamone elliptica R.Br.	Philippines	*Ridsdale C.E. 1865*	L0085686	5 (16)	Naturalis Biodiversity Center
Secamone emetica (Retz.) R.Br. ex Sm.	India	*Collector unknown*	Kw24856	20	Royal Botanic Gardens, Kew
Secamone filiformis J.H.Ross	Swaziland (Londosi valley)	*Prior J. 104*	Kw75432	4 (8)	Royal Botanic Gardens, Kew
Secamone racemosa (Benth.) Klack.	Rwanda	*Troupin G. 3*	Tw39299	3 (7)	Africa museum – Tervuren xylarium
Secamone sp.	D.R. Congo (Katanga)	*Blakely P.T. 45*	Tw38863	3 (5)	Africa museum – Tervuren xylarium
Secamone stuhlmannii K.Schum.	Tanzania	*Newman J.L. 83*	Kw13199	9 (18)	Royal Botanic Gardens, Kew
Secamonopsis madagascariensis Jum.	Madagascar	*Collector unknown*	Kw13201	11 (33)	Royal Botanic Gardens, Kew
Stephanotis abyssinica (Hochst.) S.Reuss, Liede & Meve 1	D.R. Congo (Katanga)	*Malaisse F. 1123*	Tw39949	4 (9)	Africa museum – Tervuren xylarium
Stephanotis abyssinica (Hochst.) S.Reuss, Liede & Meve 2	Yemen (Socotra)	Collector unknown	Kw23015	2 (5)	Royal Botanic Gardens, Kew
***Stephanotis floribunda* Jacques**	**Hortus Botanicus Leiden**	***Groningen B.G. 1960***	**L03734**	**2 (5)**	**Hortus Botanicus Leiden**
Stephanotis rubicunda (K.Schum.) S.Reuss, Liede & Meve	Kenya (Kilifi coast)	*Liede & Newton 3199*	–	5 (25)	University of Bayreuth
Stephanotis volubilis (L.f.) S.Reuss, Liede & Meve	Indonesia (Java, Peutjang Island)	*Kostermans 22a*	L0085674	49	Naturalis Biodiversity Center
Tassadia sp.	Surinam	*Lindeman J.C. & Heyde N.M. 1976/267*	U0261426	10 (22)	Naturalis Biodiversity Center
Vincetoxicum anomalum (N.E.Br.) Meve & Liede	Malawi (Chawa forest)	*Liede 2882*	–	2 (6)	University of Bayreuth
Vincetoxicum sylvaticum (Decne.) Kuntze	Senegal (Casamance)	*De Wolf J. s.n.*	Tw51669	5 (9)	Africa museum – Tervuren xylarium

Appendix 2. Summary of number of shifts towards derived woodiness per genus. (sub)Tribe = Marsdenieae (MAR) and Asclepiadeae subtribes Asclepiadinae (Ascl), Astephaninae (Aste), Cynanchinae (Cyna), Diplolepinae (Dipl), Gonolobinae (Gono), Metastelmatinae (Meta), Orthosiinae (Orth), Oxypetalinae (Oxyp), Pentacyphinae (Pent), Tylophorinae (Tylo), – = no subtribe assigned. Dw = number of species with derived woodiness, Spp. = total number of species per genus.

Genus	(sub) Tribe	Dw	Spp.	Shifts	Remarks	References
Asclepias	Ascl	4	100	2	*Asclepias* relationships remain poorly resolved, so interpretation on number of shifts difficult. Sometimes unclear if species is woody enough considering the many herbaceous relatives. Suppl. Fig. S1F.	Goyder & al., 2007; Fishbein & al., 2011, 2018
Barjonia	Meta	1	7	0	*Barjonia* is closely related to the Metastelmatinae core group. Species in suppl. Table S2 not included in published phylogenies so no estimation for shift given.	Liede-Schumann & al., 2014; Fishbein & al., 2018
Blepharodon	Meta	1	15	0	*Blepharodon* is a polyphyletic genus. Suppl. Fig. S1C.	Liede-Schumann & al., 2014; Fishbein & al., 2018
Calciphila	–	1	2	1	Sister to the remaining Asclepiadinae. Genus not included in suppl. Fig. S1. Asclepiadinae ancestor considered woody (shift that includes *Solenostemma*, *Pergularia*, *Calotropis*, and *Kanahia* – suppl. Fig. S1F).	Fishbein & al., 2018
Calotropis	Ascl	2	3	0	*Calotropis* is sister to *Kanahia* (suppl. Fig. S1F).	Fishbein & al., 2018
Cynanchum	Cyna	16	120	7	Probably more shifts towards derived woodiness, but not all species in Khanum & al., 2016 (we treat woodiness much stricter than in Liede & Kunze, 2002). Includes *Graphistemma, Raphistemma, Sichuania*. Suppl. Fig. S1E.	Khanum & al., 2016; Fishbein & al., 2018
Diplolepis	Dipl	5	14	0	See comment *Orthosia*. Suppl. Fig. S1C.	Liede-Schumann & al., 2014; Fishbein & al., 2018
Ditassa	Meta	7	140	1	*Ditassa* is not monophyletic, phylogeny poorly sampled and lacks sufficient resolution to accurately assess the number of transitions towards derived woodiness in the Metastelmatinae core group. All together counted as one shift because closely related species in these genera are scored as basally woody in analysis of Fishbein & al. (2018). Suppl. Fig. S1C.	Liede-Schumann & al., 2014; Fishbein & al., 2018
Hemipogon	Meta	1	8	0	*Hemipogon* is not a monophyletic genus. Suppl. Fig. S1C.	Liede-Schumann & al., 2014; Fishbein & al., 2018
Hoya	MAR	29	250	6	At least six, potentially more because not all woody species have been sequenced. Includes former genus *Clemensiella*. Suppl. Fig. S1B.	Wanntorp & al., 2011
Kanahia	Ascl	2	2	0	Sister to *Calotropis*. Suppl. Fig. S1F.	Fishbein & al., 2018
Matelea	Gono	38	280	2	*Matelea* is not monophyletic, phylogeny poorly sampled and does not include all the woody species; growth form data in species descriptions are controversial amongst authors. Woody *Matelea* species are relatives to basally woody species, which could mean that there is only one shift. Suppl. Fig. S1A.	Liede-Schumann & al., 2014; Mangelsdorff & al., 2016; Fishbein & al., 2018
Microloma	Aste	6	12	0	No phylogeny that includes enough species.	-
Minaria	Meta	15	19	1	Phylogeny lacks resolution. *Minaria* one clear woody group in Fishbein & al. (2018) and Liede-Schumann & al. (2014). Suppl. Fig. S1C.	Liede-Schumann & al., 2014; Fishbein & al., 2018
Nephradenia	Meta	1	5	0	A genus that diverged in the early evolution of Metastelmatinae, probably closely related to *Barjonia*. Not included in suppl. Fig. S1.	Fishbein & al., 2018
Orthosia	Orth	1	25	0	*Orthosia* and *Diplolepis* treated as one shift together with earlier diverging *Pentacyphus* because this grade includes a polytomy (suppl. Fig. S1C).	Fishbein & al., 2018
Oxypetalum	Oxyp	5	125	2	Suppl. Fig. S1C.	Liede-Schumann & al., 2014; Fishbein & al., 2018
Pentacyphus	Pent	1	3	1	Sister to the remaining Metastelmatinae with a lot of growth form variation.	Liede-Schumann & al., 2014; Fishbein & al., 2018
Pergularia	Ascl	1	2	0	Genus diverged in early evolution of subtribe Asclepiadinae. Not included in suppl. Fig. 1.	Fishbein & al., 2018

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Appendix 2. Continued.

Genus	(sub) Tribe	Dw	Spp.	Shifts	Remarks	References
Schizostephanus	Cyna	1	2	1	*Schizostephanus* is sister to *Cynanchum*, but woodiness probably developed several times independently in the latter. Suppl. Fig. S1E.	Fishbein & al., 2018
Solenostemma	–	1	1	0	Genus diverged in early evolution of subtribe Asclepiadinae. Not included in suppl. Fig. S1.	Fishbein & al., 2018
Vincetoxicum	Tylo	29	140	4	Probably more shifts towards derived woodiness, but only a few number of woody species sequenced. Suppl. Fig. S1D.	Liede-Schumann & al., 2016; Fishbein & al., 2018
Total		**168**	**1275**	**28**		