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Anatomical notes on *Marsdenia erecta* (Apocynaceae) Wood: Is it secondarily woody?

Received: 11 January 2011; Accepted: 17 February 2012

Abstract: This study deals with the wood of *Marsdenia erecta* R. Br., which is poorly known from a wood anatomical point of view. *M. erecta*, a woody-based perennial with numerous herbaceous sprawling stems, is distributed in the south and eastern parts of the Balkan Peninsula (incl. Crete) and Asia Minor to Afghanistan. The study aims at describing the wood anatomy of the species in detail based on IAWA list of microscopic features for hardwood identification, and at determining whether it has paedomorphic wood anatomical features. In *M. erecta* wood, the typical decreasing age-on-length graphic for vessel elements and exclusively upright and square ray cells provide strong arguments for paedomorphosis and secondary woodiness. However, to make a precise decision, this result based on wood anatomy should be checked with molecular phylogenetic data of the species investigated.

Additional key words: Asclepiadoideae, paedomorphy, secondary woodiness, wood anatomy

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Introduction

The Apocynaceae includes the five subfamilies: Rauvolfioideae, Apocynoideae, Periplocoideae, Secamonoideae, and Asclepiadoideae (Endress and Bruyns 2000; Endress et al. 2007). Recently many new tribes have been added to the subfamilies based on an updated classification scheme of the Apocynaceae (Endress et al. 2007). However, after the exclusion of the tribes Gonolobeae and Fockeeae based on several taxonomic revisions, the number of tribes of the subfamily Asclepiadoideae is reduced into three: Asclepiadeae (R. Br.) Duby, Ceropegieae Orb., and Marsdenieae Benth. (Endress and Bruyns 2000; Buera et al. 2010).

Marsdenia erecta R. Br. (Syn: *Cionura erecta* (L.) Griseb) is placed in the family Apocynaceae, subfamily Asclepiadoideae, tribe Marsdenieae (Endress and Bruyns 2000; Verhoeven et al. 2003; Wanntorp et al. 2006). This plant is a woody-based perennial with

numerous herbaceous sprawling stems (Browicz 1967), which is distributed in the south and eastern parts of the Balkan Peninsula (incl. Crete) and Asia Minor to Afghanistan (Browicz 1967). In Turkey, it is a widespread species except for Central and North Eastern Anatolia (Yaltirik 1967), and inhabits conglomerate scree, limestone cliffs, fields, river beds, coastal sands and generally unproductive lands (Browicz 1978). Yaltirik (1967) also reports a variety of the plant naturally growing in Turkey, so-called *Marsdenia erecta* R. Br. var. *flava* Yalt.

Paedomorphosis is one of two different results of *heterochrony* defined as a change in the rate or timing of a developmental pathway during an organism's development (Dulin 2008, Dulin and Kirchoff 2010, Box and Glover 2010). Another end result is peramorphosis (Dulin 2008, Dulin and Kirchoff 2010). According to the theory of paedomorphosis applied to the secondary xylem of dicotyledonous plants, in some less woody plants "juvenile characteristics – those

of the primary xylem – have been protracted into the secondary xylem” during development (Carlquist 1962, Dulin 2008). Secondary woodiness is defined as the evolution of secondary xylem in plants whose ancestors are nonwoody (Carlquist 2009, Dulin 2008, Dulin and Kirchoff 2010).

The present study deals with the wood of a woody-based perennial (*M. erecta*) in a group (asclepiods) that is poorly known from a wood anatomical point of view. To our knowledge, wood anatomy of this plant has never been reported earlier. We therefore aim to describe *M. erecta* wood anatomy in detail based on the systems of the IAWA committee (1989), and to determine whether it is secondarily woody based on pedomorphic features of wood anatomy (Carlquist 1962).

Materials and methods

In the field, there were no *M. erecta* individuals having more than the stem diameter of 2.5 cm; therefore, the stems of four different individuals with about 2–2.5 cm diameters (woody-based parts) were taken at the coastal sands of Kizilkum, Bartin, Turkey. The wood samples were split with a knife along the radial and tangential planes so as to make blocks approximately 9 × 9 mm (Schoch et al. 2004), and all the sections were cut with a Euromex sliding microtome at a thickness of about 15 to 20 μm, and stained with a mixed combination of safranin and crystal violette solution. For maceration, a combination of various concentrations of nitric acid with a small quantity of potassium chlorate – Schultze’s method – was used (Han et al. 1999).

All the samples and wood sections have been hold in the wood anatomy laboratory of Bartin Faculty of Forestry. Olympus light microscope (CX-21) was used to take measurements of vessel diameter, vessel element length, fibre length etc and to take counts of vessel frequency and the number of rays per mm, as well as for observation. Measurements from units of ocular micrometer were converted into μm (using the 4× objective, 1 ocular unit = 25 μm; using the 10× objective, 1 ocular unit = 10 μm; using the 40× objective, 1 ocular unit = 2.5 μm; using the 100x objective, 1 ocular unit = 1 μm).

The terminology used in the text and all measurements and counts for the quantitative features of various wood elements follow IAWA List of Microscopic Features for Hardwood Identification (IAWA Committee 1989). Mean vessel element length was determined from macerated wood splinters taken both near to bark and near to pith, and it included vessel element tails. However, for the age-on-length graphic, measurements of vessel element length were compulsorily made using radial sections outwards from the pith. Therefore, the values of vessel element length

on the graphic did not include element tails. In addition, a linear regression line was fitted to the data points of vessel element lengths from the pith to periphery in the stem.

Results and discussion

Anatomical description of the wood: Growth rings distinct. Wood semi-ring-porous (Figs. 1a–d). Vessels mainly in radial and/or diagonal, occasionally in dendritic pattern (Figs. 1b and 1d), radial-oblique multiples of 2–4 as well as many solitary vessels, rounded in transverse section. Vessels 21–(29.5)–35 per mm², tangential diameter 45–(82.3)–190 μm. Vessel element length 90–(214.5)–380 μm near to bark, and 160–(269.9)–440 μm near to pith. Perforations simple in mostly oblique end walls (Fig. 2a). Inter-vessel pits alternate, rounded, 5– (5.8)–7 μm in horizontal diameter, with slit-like apertures (Fig. 2b). Vessel-ray pits with distinct borders; similar to inter-vessel pits. Fibres 340–(473)–570 μm long (The ratio of fibre length to vessel element length: 1.7 – 2.2), thin- to thick-walled; with simple to minutely bordered pits. Axial parenchyma mostly scanty paratracheal, extremely rare. Rays 16–(18.6)–22 per mm, 1–3-seriate, 310–(655.3)–1090 μm high, composed of upright and square cells (Figs. 2c–d). Druses present in pith parenchyma cells (Fig. 2e). According to IAWA List of Microscopic Features for Hardwood Identification (IAWA Committee 1989), the anatomical features of *M. erecta* wood is as shown in Table 1. In addition, the age-on-length graphic for vessel element length based on measuring outwards from the pith in radial wood sections is shown in Fig 3.

There is not much reference data on the wood anatomy of the tribe Marsdenieae. However, the InsideWood database (2009) include wood anatomy of some species from the tribes of the subfamily Asclepiadoideae: *Marsdenia tomentosa* in the tribe Marsdenieae, *Calotropis procera* (Aiton) W.T.Aiton, *Pergularia tomentosa* Span., *Solenostemma argel* Hayne in the tribe Asclepiadeae, and *Leptadenia pyrotechnica* Decne. in the tribe Ceropegieae. However, to our knowledge, wood anatomy of *Marsdenia erecta* has never been reported anywhere. In spite of the similarities in the most of secondary xylem features, *M. erecta* wood is different from *M. tomentosa* wood in terms of some anatomical properties, which are semi-ring porosity, small vessel grouping (2 to 4) as well as many solitary vessels, narrower vessel diameter, wider rays (1 to 3 cells), and lower ray height (< 1mm). Especially based on ray width, *M. erecta* wood can be distinguished from the wood of *M. tomentosa*. Rays in *M. tomentosa* are exclusively uniseriate (InsideWood 2009). Even though there are similar anatomical features, *M. erecta* wood is different from woods of above-mentioned species in the other tribes of the

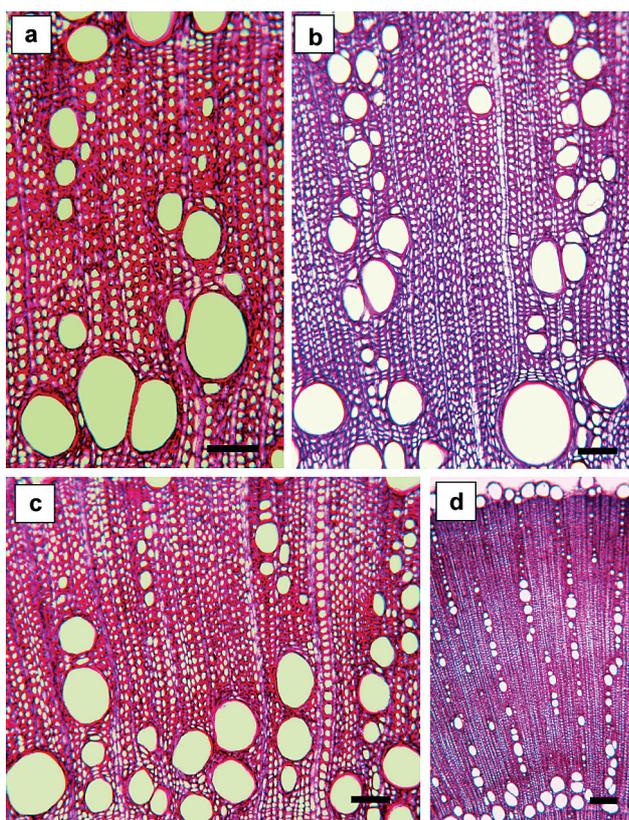


Fig. 1. Transverse sections of *Marsdenia erecta* wood (TS): a–d – semi-ring-porous wood, solitary and grouping vessels; b and d – vessels in distinct radial patterns. Scale bar: 100 μm for a, b & c, 250 μm for d (TS: transverse section)

subfamily Asclepiadoideae (InsideWood 2009). In particular, distinct growth ring boundaries, semi-ring porosity and narrower ray width in *M. erecta* wood are distinctive features in order to distinguish it from *Calotropis procera*, *Pergularia tomentosa* and *Solenostemma argel* in the tribe Asclepiadeae. Moreover, laticifers in *Calotropis procera* (Fahn et al. 1986) and included phloem (concentric and diffuse) in *Solenostemma argel* (InsideWood 2009) don't occur in *M. erecta* wood. Contrary to *M. erecta* wood, prismatic crystals in ray cells, larger rays more than 10-seriate and ray height more than 1 mm, included phloem (diffuse), laticifers and storied structure are present in *Leptadenia pyrotechnica* wood belonging to the tribe Ceropogeeae (InsideWood 2009), which are distinctive properties between these two species.

From the pith to stem periphery, a negatively sloped graphic occurs when a linear regression line is fitted to the data points of vessel element lengths of *M. erecta* (Fig. 3). The squares of coefficient (r^2) is 30.6% ($r = 0.55$). The r^2 value reveals that only 30.6% of the variation in vessel element length (y) is explained by the straight line relationship between vessel element length (y) and the distance from pith.

Carlquist (1962) introduced a theory of pedomorphosis in dicotyledonous woods. According to the the-

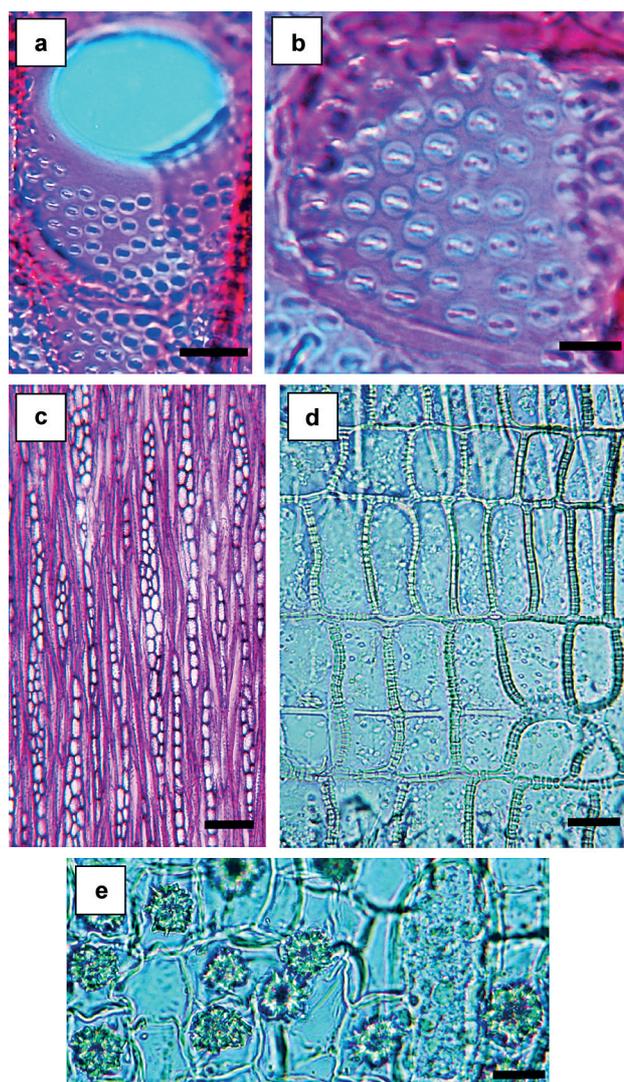


Fig. 2. Sections of *Marsdenia erecta* wood: a – simple perforation plate (RLS); b – intervessel pits (RLS); c – rays with 1–3-seriate (TLS); d – ray structure in RLS; e – druses within pith parenchyma cells (RLS). Scale bar: 20 μm for a, 10 μm for b, 100 μm for c and 25 μm for d & e (RLS: radial longitudinal section. TLS: tangential longitudinal section)

ory, in particular species “juvenile characteristics — those of the primary xylem — have been protracted into the secondary xylem”. Carlquist (1962) indicated that a typical flat or decreasing age-on-length curve for vessel element length is an indication of pedomorphosis. Other indicators are exclusively upright and/or square cells in rays (or the raylessness) and scalariform lateral wall pitting with simple perforation plate in the vessel elements (Carlquist 1970, 2009). In addition, libriform fibres or abundant axial parenchyma are other characteristics common to pedomorphic woods. It is usually considered that many species with pedomorphic wood are derived from herbaceous ancestors, and pedomorphic anatomical properties frequently indicate the possibility of secondary woodiness (Carlquist 1962, 1970, 2009; Lens et al. 2008, 2009). However,

Table 1. IAWA codes and anatomical features of *Marsdenia erecta* wood

Growth rings	
1	Growth ring boundaries distinct
Vessels	
<i>Porosity</i>	
4	Wood semi-ring-porous
<i>Vessel arrangement</i>	
7	Vessels in diagonal and / or radial pattern
8v	Vessels in dendritic pattern
<i>Perforation plates</i>	
13	Simple perforation plates
<i>Intervessel pits: arrangement and size</i>	
22	Intervessel pits alternate
25	Small – 4–7 μm
28	Range of intervessel pit size – 5–7 μm
<i>Vestured pits</i>	
–	Vestures couldn't be observed in light microscopy
<i>Vessel-ray pitting</i>	
30	Vessel-ray pits with distinct borders; similar to intervessel pits in size and shape throughout the ray cell
<i>Tangential diameter of vessel lumina</i>	
41	50–100 μm
44	Mean: 82.3 μm , Standard deviation: \pm 34.1, Range: 45–190 μm , n: 30
<i>Vessels per square millimeter</i>	
48	20–40 vessels per square millimeter
51	Mean: 29.5, Standard deviation: \pm 4.2, Range: 21–35, n: 10
<i>Mean vessel element length</i>	
52	\leq 350 μm
55	Mean: 214.5* μm , Standard deviation: \pm 44.5 μm , Range: 90–380 μm , n: 100
Tracheids and Fibres	
60	Vascular/vasicentric tracheids present
<i>Ground tissue fibres</i>	
61	Fibres with simple to minutely bordered pits
<i>Septate fibres and parenchyma-like fibre bands</i>	
66	Non-septate fibres present
<i>Fibre wall thickness</i>	
69	Fibres thin- to thick-walled
<i>Mean fibre lengths</i>	
71	\leq 900 μm
74	Mean: 473.0 μm , Standard deviation: \pm 53.4 μm , Range: 340–570 μm , n: 30
Axial parenchyma	
75	Axial parenchyma absent or extremely rare
<i>Apotracheal axial parenchyma</i>	
76v	Axial parenchyma diffuse
<i>Paratracheal axial parenchyma</i>	
78	Axial parenchyma scanty paratracheal
<i>Axial parenchyma cell type/strand length</i>	
	Very difficult to find; it couldn't be determined
Rays	
<i>Ray width</i>	
97	Ray width 1 to 3 cells
<i>Rays: cellular composition</i>	
105	All ray cells upright and/or square
<i>Rays per millimeter</i>	
116	\geq 12/mm
164	Europe and Temperate Asia (Brazier and Franklin region 75)
190	Shrub

Anatomical notes: *The mean length of vessel elements determined from the present study data is $214.5 \pm 44.5 \mu\text{m}$ near to bark and $269.9 \pm 58.9 \mu\text{m}$ near to pith. These are much shorter than the mean length for vessel elements in dicotyledonous woods, $649.0 \mu\text{m}$ given in Metcalfe and Chalk (1950). Many parenchyma cells in pith have druses.

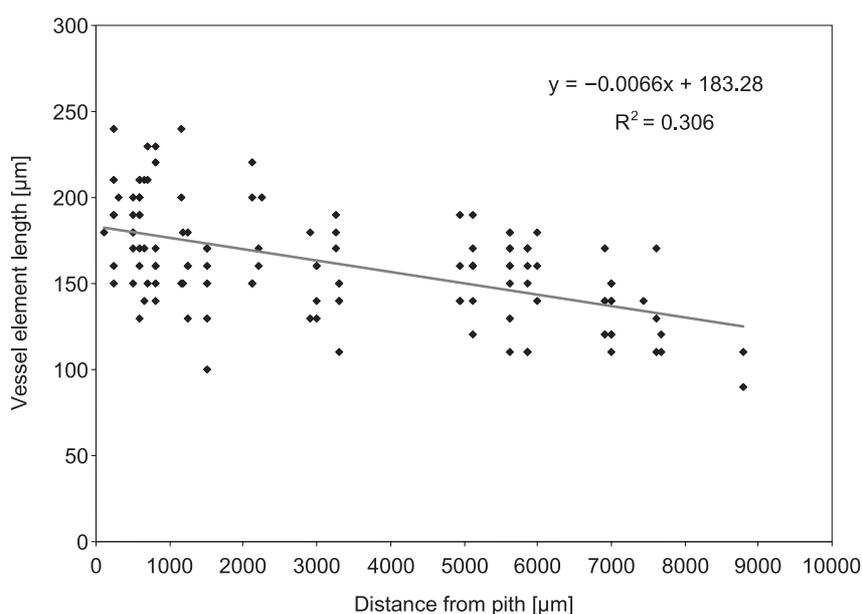


Fig. 3. Age-on-length graphic for vessel element length in *Marsdenia erecta* wood

due to the fact that paedomorphosis can also exist in species with woody ancestors, the presence of paedomorphic properties is insufficient to determine whether a species has secondary woodiness without phylogenetic data (Dulin 2008).

Some of the properties that Carlquist (1962) described as paedomorphic are absent in *M. erecta* wood; in contrast, some anatomical features of the species appear to be highly specialized (e.g. simple perforation plate, alternate inter-vessel pits etc.). However, in the wood of this species, two key features, decreasing age-on-length graphic (Fig. 3) and exclusively upright ray cells mixed with square ones (Figs. 2c and 2d), can provide wood anatomical evidence for paedomorphosis to a degree. The negatively sloped graphic in Fig. 3 is interpreted as a protraction of juvenile characteristics of the primary xylem into the secondary xylem of this plant (permanently juvenile) (Carlquist 1962). Similar graphics are also detected in *Cistanthe guadalupensis* (Dudley) Carolin ex. Hershk. (Carlquist (1962), in *Hibiscus lasiocarpus* A.Gray (Cumbie 1963), in *Delissea undulata* Gaudich. (Carlquist 1975), in *Xanthorhiza simplicissima* Marshall, *Coreopsis gigantea* H.M. Hall and *Mahonia bealei* (Fortune) Carriere (Dulin 2008), in *Martynia annua* L. and *Solanum mitlense* Dunal (Rongen 2009), in *Spermacoce verticillata* Vell. and *Lathraeocarpa acicularis* Bremek (Lens et al. 2009).

From cell shape point of view, *M. erecta* rays are exclusively composed of upright and square cells. Moreover, in *M. tomentosa*, all ray cells are upright and/or square (InsideWood 2009). Even so we have not any knowledge about the type of age-on-length curve for vessel elements of *M. tomentosa*; its characteristic ray feature can be interpreted as an indication of paedomorphosis.

There are some wood features in *M. erecta* that deviate from the typical paedomorphic wood patterns. Nevertheless, according to the study of Carlquist (1962), the decrease of vessel element length outwards from pith on radial wood sections is the decisive factor to infer secondary woodiness in a given species. With respect to the wood anatomy of *M. erecta*, the existence of decreasing age-on-length graphic for vessel elements (Fig. 3) and the occurrence of exclusively upright ray cells mixed with square ones provide strong arguments for secondary woodiness; namely it might be originated from herbaceous ancestors based on Carlquist (1962 and 1992). However, it is suggested that the woodiness should be determined with an integrative approach based on molecular phylogenetic data, woody mutants derived from herbaceous wild plants, and paedomorphic features of wood anatomy (Dulin 2008, Lens et al. 2009). On the basis of a few molecular studies done so far (Wanntorp et al. 2006), it is difficult to make a decision about the phylogenetic position of *M. erecta* within the tribe Marsdenieae. The molecular phylogenetic analyses in the study of Wanntorp et al. (2006) are mainly focused on *Hoya* and its close relatives, and don't really aim to resolve the phylogenetic position of *M. erecta* within the Marsdenieae (L. Wanntorp personal com.). After an extended molecular phylogenetic study of the tribe Marsdenieae including many more taxa, a precise answer might be given to the questions where this plant is in the phlogenetic tree and whether it is secondarily woody.

As a model to explain the genetic basis of both primary and secondary xylem development, *Arabidopsis* has been used recently (Chaffey et al. 2002; Nieminen et al. 2004). Regarding *Arabidopsis thaliana* (L.) Heynh., Mitsuda et al. (2007) showed that NAC tran-

scription factors, NST1 and NST3, regulate the formation of secondary walls. In addition, Melzer et al. (2008) indicated in *Arabidopsis thaliana* that the MADS box proteins (SOC1 and FUL) affect determinacy of all meristems, which have given further understanding of the basis of shifts in growth habit and therefore the related phenomenon of secondary woodiness. This type of the study has allowed us to better understand the molecular basis of secondary woodiness.

Conclusions

Little is known about wood anatomy of *M. erecta*. With this study of the species, both quantitative and qualitative wood anatomical features are identified in detail. *M. erecta* has two important criteria in terms of pedomorphosis, decreasing age-on-length graphic for vessel elements and exclusively upright and square ray cells, which indicate that it might be secondarily woody. However, it is difficult to make a precise decision about *M. erecta*'s ancestral origin without understanding the phylogenetic position of *M. erecta* within the tribe Marsdenieae. Thus, only after an extended molecular phylogenetic study of the tribe Marsdenieae, the comparison of anatomical results with molecular data can provide more thorough conclusions about presence or absence of secondary woodiness in this species.

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