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VEGETATIVE ANATOMY OF THE HAEMODORACEAE AND ITS PHYLOGENETIC SIGNIFICANCE

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Premise of research. Haemodoraceae are a relatively small monocot family consisting of 14 genera and approximately 108 species and are distributed in parts of Australia, southern Africa, South and Central America, and eastern North America. The family is divided into two subfamilies, Haemodoroideae and Conostylidoideae. This research focuses on the vegetative anatomy of the family, with an emphasis on leaf anatomical features. The aims of this project are (1) to acquire new vegetative anatomical data for a large selection of Haemodoraceae and (2) to evaluate these data in the context of both phylogenetic relationships and environmental factors.

Methodology. Cross sections of roots, stems (scapes), or leaves of 60 species and 63 ranked taxa from all 14 genera of the family were prepared and stained using standard histological methods, and SEMs were made of the leaf surface. Line drawings were prepared of leaf cross sections of an exemplar of each genus. Tissues and cells were examined and photographed, and comparisons were made among taxa. For leaf epidermal cells, the ratio of cell wall transectional area : cell transectional area was calculated and plotted. Several discrete anatomical characters and character states were defined and plotted on a recently derived cladogram and examined for phylogenetic signal. Correlation of certain anatomical features with environmental factors was also noted.

Pivotal results. Leaf anatomy provides several phylogenetically informative traits, including bulliform cells, tannin cells, marginal fiber caps, the relative wall transectional area of epidermal cells, the morphology of palisade cells, the distribution of fibers in the vascular bundle, leaf aerenchyma, mucilage cells, and silica bodies. These features generally correlate significantly with the pattern of phylogenetic relationships in the family. Silica cells, tannin cells, and mucilage cells, all of which may function to deter herbivory, are generally restricted to particular clades. The relative epidermal wall thickness of members of the genus *Conostylis* is significantly higher than in other members of the family, a feature that may represent an adaptation to their hot, dry environments.

Conclusions. The systematic and ecological value of studying plant vegetative anatomy is supported by this study. Vegetative anatomical features of the Haemodoraceae show considerable and significant variation. Numerous anatomical features exhibit a high phylogenetic signal and are apomorphic for specific clades. Some anatomical features are possible adaptations to habitat, climate, or herbivory. However, quantifiable ecological data are needed in future studies for assessing the adaptive significance of these anatomical features.

Keywords: anatomy, Haemodoraceae, systematics.

Introduction

Haemodoraceae are a family of monocotyledonous angiosperms, commonly known as bloodworts because of the presence of reddish pigments in roots and rhizomes of many members (Simpson 1998*a*). Haemodoraceae are classified in the order Commelinales and appear to be most closely related to the family Pontederiaceae, based on recent molecular phylogenetic studies (e.g., Chase et al. 2006; Graham et al. 2006; APG III 2009). While no single morphological feature separates Haemodoraceae from other families, all members of the family are perennial herbs with unifacial leaves with linear, plicate, or terete blades. The flowers are bisexual with six tepals; one, three, or six fertile stamens; and a tricarpellate gynoecium developing into a capsular fruit (Simpson 1990, 1998*a*).

Haemodoraceae comprise 14 genera and approximately 108 species (Hopper 1980, 1987*a*, 1987*b*, 1987*c*; Hopper et al. 1987; MacFarlane et al. 1987*a*, 1987*b*, 1987*c*; Goldblatt and Manning 2000; Lyons and Keighery 2006; Barrett et al. 2015;

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the Plant List 2015). The family is divided into the two subfamilies Haemodoroideae and Conostylidoideae (Simpson 1990, 1998*a*; Hopper et al. 1999, 2009). Subfamily Haemodoroideae, with eight genera and 41 species, is distributed in Australia, South Africa, northern South America, tropical Central America, Cuba, and the eastern coastal plain of North America. Members of this subfamily are characterized by red-pigmented roots and rootstocks; one or three stamens; a superior or inferior ovary; nonbranching, usually pilate trichomes; and monosulcate pollen grains. Subfamily Conostylidoideae, with six genera and ca. 67 species, occurs in Australia and Papua New Guinea. Members of this subfamily are characterized by the absence of red-pigmented roots and rootstocks; six stamens; an inferior ovary; mostly branching, usually dendritic trichomes; and porate pollen grains.

Economic uses of Haemodoraceae include edible rootstocks of several Australian species and narcotic effects from the eastern North American *Lachnanthes caroliniana* (Millspaugh 1887). The red pigments of the Australian *Haemodorum corymbosum* are reported to have antitumor (Schwenk 1962) and antibacterial (Narasimhachari et al. 1968) activities. Several Australian species of the family, including species of *Blancoa*, *Conostylis*, *Haemodorum*, *Macropidia*, and *Tribonanthes* and especially *Anigozanthos*, are grown horticulturally. Finally, *L. caroliniana* is listed as an aggressive weed in cranberry bogs (Robertson 1976).

Haemodoraceae have been studied with respect to morphology and taxonomy (Hopper 1987*a*, 1987*b*, 1987*c*; Hopper et al. 1987; MacFarlane 1987*a*, 1987*b*, 1987*c*; MacFarlane et al. 1987; Simpson 1990, 1998*a*; Maas and Maas-van de Kamer 1993; Tillich 1995), chemistry (Ramstad 1953; Cooke and Segal 1955; Edwards et al. 1970; Edwards and Weiss 1974; Harris and Hartley 1980; Cooke and Edwards 1981; Weiss 1984; Hölscher and Schneider 1995, 1997; Opitz et al. 2002, 2003; Otálvaro et al. 2002; Prychid et al. 2003*a*, 2003*b*; Schneider et al. 2005; Dias et al. 2009; Fang et al. 2012), pollen morphology and development (Erdtman 1966; Simpson 1983, 1989, 1990; Rowley and Rowley 1996; Pierce and Simpson 2009), embryology (Stenar 1927, 1938; Dellert 1933; de Vos 1956; Simpson 1988), floral anatomy and development (Simpson and Dickison 1981; Simpson 1990, 1993, 1998*b*), pollination biology (Wilson 1887; Ornduff 1974; Ornduff and Dulberger 1978; Hopper and Burbidge 1978; Buchmann 1980; Simpson 1990; Jesson and Barrett 2002), and molecular phylogenetics (Hopper et al. 1999, 2009).

A limited number of anatomical studies of members of the Haemodoraceae have been published. Tracheary elements have been studied in 22 species from 11 genera (*Anigozanthos*, *Blancoa*, *Conostylis*, *Dilatris*, *Haemodorum*, *Lachnanthes*, *Macropidia*, *Phlebocarya*, *Tribonanthes*, *Wachendorfia*, and *Xiphidium*), as currently defined (Cheadle 1968; Simpson and Dickison 1981; Schneider and Carquist 2005). All investigated members of the family have vessels in the root, with simple perforation plates in all taxa except *Dilatris* (one species studied) and *Tribonanthes* (three species studied), which have scalariform perforation plates. Stem vessels are absent in all Haemodoraceae, with the exception of the monotypic *Lachnanthes*, which has vessels with scalariform perforation plates. Leaves of all family members lack vessels (Schulze 1893; Arber 1925; Stenar 1927, 1938; Green 1959; Cheadle 1968; Simpson and Dickison 1981; Simpson 1990; Helme and Linder 1992; Behnke 2000; Prychid and Rudall 2000; Prychid et al. 2003*a*, 2003*b*). Schneider and Carquist (2005) described the tracheary elements of *Anigozanthos flavidus* and *Wachendorfia thyrsiflora* using SEM. In *A. flavidus*, vessels with mostly simple (but some scalariform) perforation plates are present in the roots. In the underground stems (rhizomes), the tracheary elements are interpreted as nonperforate (and therefore as tracheids). In *W. thyrsiflora*, vessels are present in roots, mostly with simple perforation plates but some with scalariform-like perforations near the cell tip. Stems contain tracheary elements with end walls resembling perforation plates but with narrow bars and threadlike pit membranes, indicative of a vessel-tracheid, possibly transitional between vessels in roots and tracheids in the stem.

Other anatomical studies, while scanty, include comparative studies of the epidermis (number of cell layers, cutinization), trichomes, stomata (presence or absence of subsidiary cells), rhizomes (presence or absence of a sclerenchymatous *mechanischen* cylinder—i.e., a ring of sclerenchymatous tissue surrounding the vascular tissue), and leaves (bundle anatomy; Schulze 1893; Stenar 1927, 1938; Green 1959).

Details of vegetative anatomy are known for *L. caroliniana* of the Haemodoroideae (Simpson and Dickison 1981). In this species, mature roots comprise a uniseriate exodermis, a parenchymatous cortex, a sclerified endodermis and pith, a single-celled continuous pericycle, and a relatively small vascular cylinder. Root vessels have simple perforation plates. The rhizomes and stolons, as well as the aerial stems, have a heavily sclerified cylindrical sheath (the mechanical cylinder of Schulze 1893), within which is a cylinder of numerous vascular strands. Schulze (1893) observed this ring mantle in *Tribonanthes longipetala*, *Blancoa canescens*, *Anigozanthos rufus*, *Anigozanthos preissii*, *Anigozanthos viridis*, *Anigozanthos manglesii*, and *Conostylis candicans*. Leaf trichomes occur on the margins or surfaces in some taxa and are mostly similar to floral trichomes (see also Simpson 1990, 1998*a*). Stomata are paracytic. The vasculature of the blade consists of a ring of alternating large and small collateral bundles, with the xylem oriented toward the leaf center. The veins are surrounded by a sclerenchymatous sheath, which in some taxa forms a continuous band surrounding the entire vasculature.

In *Dilatris*, spherical, epithelial-lined mucilage chambers are found in the leaves (Simpson 1990) and ovary (Simpson 1998*b*). All (and only) members of Conostylidoideae have distinctive tannin cells scattered in various plant organs. These tannin cells are isodiametric to elongate, with a thin, tannin-impregnated wall and numerous minute tanniniferous spherules just interior to the wall (Simpson 1990). Helme and Linder (1992) studied the genus *Wachendorfia* and included descriptions of the leaf anatomy of four species of it as well as of *Dilatris pillansii* and *Dilatris corymbosa*. The authors noted variation in palisade structures, epidermal cuticle thickness, subsidiary cell shape, vascular bundle orientation, and the presence or absence of mucilage canals.

Sieve tube plastid ultrastructure has been investigated in the family for seven species in the five genera *Anigozanthos*, *Conostylis*, *Lachnanthes*, *Wachendorfia*, and *Xiphidium* (Behnke

2000). Plastids in these taxa are of the P2c type, in which cuneate protein bodies are present but starch and filamental protein are not. This form, the most common in the monocots, is also found in all investigated members of the Commelinaceae, Hanguanaceae, Philydraceae, and Pontederiaceae of the Commelinales (Behnke 2000).

Prychid and Rudall (2000) report the presence of calcium oxalate raphides in the Haemodoraceae and styloids in some members of the family. Prychid et al. (2003*a*, 2003*b*) studied the distribution of silica bodies and tapetal raphides in the Haemodoraceae. Silica was observed in leaves in five of the nine genera examined and only in subfamily Conostylidoideae, including the genus *Phlebocarya*, which supports the earlier transfer of this genus from subfamily Haemodoroideae to Conostylidoideae (e.g., Simpson 1990). Tapetal raphides are much less common and were observed only in *Anigozanthos* and *Conostylis* of the Conostylidoideae. However, tapetal raphides were also observed by Simpson (1988) in *Lachnanthes* of the Haemodoroideae.

Brundrett and Abbott (1991) observed that four investigated Australian species of the Haemodoraceae (*A. manglesii*, *Conostylis setosa*, *Haemodorum laxum*, and *Haemodorum spicatum*) all lack any evidence of root mycorrhizal associations. However, Jumpponen and Trappe (1998) cite the colonization of dark septate endophytic fungi in one species (not listed) of the Haemodoraceae. Smith et al. (2011) report the occurrence of sand-binding roots in the Haemodoraceae, in which sand grains are tightly bound to the root surface by persistent root hairs. The majority of genera and species were found to possess sand-binding roots, which are found primarily in semiarid species but also in tropical, subtropical, and wet temperate species. *Conostylis* and *Tribonanthes* have sister taxa with and without this feature. Sand-binding roots were likely to have been present in the ancestor of the family.

The purpose of this study is to describe the vegetative (root, stem, and leaf) tissue and cellular anatomy of the family Haemodoraceae in order to assess intrafamilial variation in these features. This variation, along with some features described in the literature, is used to define discrete characters and character states, which will be examined for phylogenetic signal by plotting on a recent cladogram established for the family. In addition, this character analysis will be used to evaluate correlations with extrinsic factors, such as habitat, climate, and herbivory deterrence.

Material and Methods

Samples

Samples studied are listed in the appendix. Almost all material was fixed in formalin (37%)–glacial acetic acid–water–ethanol (8%-5%-24%-63%) in the field for a minimum of 3 d (followed by permanent storage in 70% ethanol), with a few specimens prepared from dried herbarium material that was rehydrated in 10% Aerosol-OT at 50°C for 12 h. All collections are vouchered and, with a few exceptions, deposited in accredited herbaria (see app.). Descriptions, photographs, and illustrations were made only of selected members of each genus. Information for outgroup taxa, not described or illustrated, is included in tables 1 and 2. A total of 60 species and 63 taxa (including subspecies) were studied: 32 species and 35 taxa of *Conostylis* (the largest genus of the family), five species of *Haemodorum*, four species of *Anigozanthos*, four species of *Dilatris*, three species of *Tribonanthes*, three species of *Wachendorfia*, two species of *Phlebocarya*, and one species each of *Barberetta*, *Blancoa*, *Lachnanthes*, *Pyrrorhiza*, *Schiekia*, *Macropidia*, and *Xiphidium*.

LM

Roots, stems, and leaves were cut to a length of 1–4 mm. One of two methods was used depending on the specimen: paraffin embedding for softer material or resin embedding for hard material. Samples were prepared for paraffin embedding by gradually transferring them from ethanol to 100% tertiary-butyl alcohol (TBA) in steps of 50%:20% to 50%:35% to 45%:50% to 25%:75% ethanol:TBA to three steps of 100% TBA, followed by infusion in a 75°C oven with paraffin (Paraplast), beginning with 50% TBA:50% paraffin, followed by two steps of 100% paraffin. After infusion, the material was embedded in 100% paraffin and sectioned using a rotary microtome at ca. 10 μm with a steel knife, and the ribbons were mounted on slides using Haupt's solution (1% gelatin) as an adhesive and flooded with 4% formalin to expand the material. Slides were placed on a 50°C heating tray for 2–5 min, the excess formalin was drained, and the slides were dried on the heating tray for 5–10 additional minutes. Slides with mounted ribbons were dewaxed in toluene, stained with safranin and fast green in a staining solution series, mounted using Kleermount mounting medium, and hardened in a 55°C oven for 2 d.

Several of the harder, thicker leaves and all of the roots were embedded using Spurr's resin (Spurr 1969). The resin-infiltrated material was polymerized at 70°C for 10 h. The sections were cut on an Ultracut E ultramicrotome at 1–2 μm using glass blades. Sections were stained with 1.0% toluidine blue and mounted with a cover slip in Kleermount mounting medium. Observations were made primarily from transverse (cross) sections of each organ.

SEM

Leaf material was critical-point dried from material fixed in formalin–acetic acid–alcohol. Leaf sections (ca. 5 × 5 mm^2 × 1 mm thick) were dehydrated to 100% ethanol and then placed in a metal capsule and critical-point dried with a Tousimis critical-point dryer using pressurized carbon dioxide as the transition fluid. Once dried, all material was transferred onto a stub covered with double-sided tape, sputter-coated with gold and palladium in a Hummer-4 sputtering apparatus, and photographed on a Hitachi S500 SEM (20 kV).

Observations and Measurements

All photographs were taken using a Nikon Microphot-FX microscope and a Nikon CoolPix 990 camera. Line drawings of leaves were prepared by tracing composite photographs using a graphics program. Measurements from photographs

Table 1

Root Anatomical Characters in the Haemodoraceae

Taxon	Cortex	Inner cortical cell shape	Endodermis orientation	Endodermis cell wall	Xylem poles
Haemodoroideae:					
Barberetta aurea	Radially aligned/10 layered	Tangentially oblong (2:1)	Isodiametric/no orientation	Unequally thickened, outer tangential thin	5 arch
Dilatris viscosa	Radially aligned/12 layered	Tangentially oblong (2:1)	Isodiametric/no orientation	Unequally thickened, outer tangential thin	13 arch
Haemodorum venosum	Radially aligned/10 layered, aerenchymatous	Tangentially rectangular (3:1)	Isodiametric/no orientation	Uniformly thickened	12 arch
Lachnanthes caroliniana	Radially aligned/11 or 12 layered, aerenchymatous	Tangentially oblong (1.5:1)	Isodiametric/no orientation	Uniformly thickened	6 or 7 arch
Pyrrorhiza neblinae	Radially aligned/10–12 layered, aerenchymatous	Tangentially oblong (1.5–2:1)	Isodiametric/no orientation	Unequally thickened, outer tangential thin	11 arch
Schiekia orinocensis	Radially aligned/eight layered	Tangentially oblong (2:1)	Isodiametric/no orientation	Unequally thickened, outer tangential thin	6 arch
Wachendorfia brachyandra	Radially aligned/eight layered	Tangentially oblong (2:1)	Isodiametric/no orientation	Unequally thickened, outer tangential thin	9 arch
Xiphidium caeruleum	Radially aligned/four layered	Tangentially oblong (2:1)	Isodiametric/no orientation	Unequally thickened, outer tangential thin	8 arch
Conostylidoideae:					
Anigozanthos flavidus	Radially aligned near endodermis only/16–20 layered	Tangentially oblong (2:1)	Tangentially elongate (2–3:1)	Thin walled	23–26 arch
Anigozanthos rufus	Not radially aligned/18–20 layered	Isodiametric to tangentially oblong (2:1)	Isodiametric/no orientation	Unequally thickened, outer tangential thin to uniformly thickened	20 arch
Blancoa canescens	Not radially aligned/20 layered	Tangentially rectangular (3:1)	Rectangular/radially oriented (3:1)	Very thick, uniformly thickened	16 arch
Conostylis aculeata subsp. *spinuligera*	Not radially aligned/ 12 or 13 layered	Tangentially rectangular (2–3:1)	Rectangular/radially oriented (2:1)	Not thickened in immature roots	20 arch
Conostylis juncea	Not radially aligned/12 layered	Tangentially rectangular (5:1)	Rectangular/radially oriented	Very thick, uniformly thickened	10 arch
Conostylis neocymosa	Not radially aligned/10 layered	Tangentially rectangular (4:1)	Rectangular/radially oriented	Moderately thick, uniformly thickened	30 arch
Conostylis pauciflora subsp. *euryhipis*	Not radially aligned/18 layered	Tangentially rectangular (3:1)	Rectangular/radially oriented	Very thick, uniformly thickened	30 arch
Conostylis petrophiloides	Not radially aligned/13 layered	Tangentially rectangular (3:1)	Rectangular/radially oriented	Very thick, uniformly thickened	12 arch
Conostylis seminuda	Not radially aligned/13 layered	Tangentially rectangular (4:1)	Rectangular/radially oriented	Moderately thick, uniformly thickened	
Conostylis setigera subsp. *dasys*	Not radially aligned/17–20 layered	Tangentially elongate (3:1)	Isodiametric/no orientation	Unequally thickened, outer tangential thin to uniformly thickened	8 arch
Conostylis vaginata	Not radially aligned/10 layered	Tangentially elongate (4:1)	Rectangular/radially oriented	Very thick, uniformly thickened	23 arch
Conostylis wongonensis	Not radially aligned/10 layered	Tangentially elongate (4:1)	Rectangular/radially oriented	Moderately thick, uniformly thickened	12 arch
Macropidia fuliginosa	Not radially aligned/17–20 layered	Tangentially elongate (2–3:1)	Rectangular/radially oriented	Very thick, uniformly thickened	13 arch
Phlebocarya ciliata	Not radially aligned/16 or 17 layered	Narrowly rectangular (3–4:1)	Slightly rectangular/ radially oriented	Uniformly thickened	10 arch
Phlebocarya pilosissima	Not radially aligned/12 or 13 layered	Tangentially elongate (3–4:1)	Slightly rectangular/ radially oriented	Uniformly thickened	24 arch
Tribonanthes australis	Not radially aligned/10 layered	Isodiametric to tangentially elongate (2:1)	Isodiametric/no orientation	Not thickened	2 arch

Table 2

Leaf Anatomical Characters and Character States for the Haemodoraceae

Taxon	Bulliform cells	Leaves fistulose	Leaf aerenchyma	Leaf tannin cells	Mucilage cells
Haemodoroideae:					
Barberetta aurea	Present	Absent	Absent	None observed	Absent
Dilatris corymbosa	Absent	Absent	Absent	None observed	Present
Dilatris ixioides	Absent	Absent	Absent	None observed	Present
Dilatris pillansii	Absent	Absent	Absent	None observed	?
Dilatris viscosa	Absent	Absent	Absent	None observed	Present
Haemodorum laxum	Absent	Absent	Absent	None observed	Absent
Haemodorum loratum	Absent	Absent	Absent	None observed	Absent
Haemodorum simplex	Absent	Absent	Absent	None observed	Absent
Haemodorum simulans	Absent	Absent	Absent	None observed	Absent
Haemodorum spicatum	Absent	Absent	Absent	None observed	Absent
Haemodorum venosum	Absent	Absent	Absent	None observed	Absent
Lachnanthes caroliniana	Absent	Absent	Present	None observed	Absent
Pyrrorhiza neblinae	Absent	Absent	Absent	None observed	Absent
Schiekia orinocensis	Absent	Absent	Absent	None observed	Absent
Wachendorfia brachyandra	Present	Absent	Absent	None observed	Absent
Wachendorfia paniculata	Present	Absent	Absent	None observed	Absent
Wachendorfia thyrsiflora	Present	Absent	Absent	None observed	Absent
Xiphidium caeruleum	Absent	Absent	Absent	None observed	Absent
Conostylidoideae:					
Anigozanthos flavidus	Absent	Absent	Absent	Present	Absent
Anigozanthos humilis	Absent	Absent	Absent	Present	Absent
Anigozanthos preissii	Absent	Absent	Absent	?	Absent
Anigozanthos rufus	Absent	Absent	Absent	Present	Absent
Blancoa canescens	Absent	Absent	Absent	Present	Absent
Conostylis aculeata subsp. *bromelioides*	Absent	Absent	Absent	Present	Absent
C. aculeata subsp. *spinuligera*	Absent	Absent	Absent	Present	Absent
Conostylis androstemma	Absent	Absent	Absent	Present	Absent
Conostylis angustifolia	Absent	Absent	Absent	Present	Absent
Conostylis aurea	Absent	Absent	Absent	?	Absent
Conostylis bracteata	Absent	Absent	Absent	?	Absent
Conostylis candicans	Absent	Absent	Absent	?	Absent
Conostylis canteriata	Absent	Absent	Absent	Present	Absent
Conostylis caricina subsp. *caricina*	Absent	Absent	Absent	Present	Absent
C. caricina subsp. *elachys*	Absent	Absent	Absent	Present	Absent
Conostylis crassinerva subsp. *absens*	Absent	Absent	Absent	?	Absent
Conostylis dielsii subsp. *teres*	Absent	?	?	?	Absent
Conostylis festucacea subsp. *filifolia*	Absent	?	Absent	Present	Absent
Conostylis juncea	Absent	Absent	Absent	?	Absent
Conostylis latens	Absent	Absent	Absent	?	Absent
Conostylis micrantha	Absent	Absent	Absent	?	Absent
Conostylis misera	Absent	Absent	Absent	?	Absent
Conostylis neocymosa	Absent	Absent	Absent	Present	Absent
Conostylis pauciflora	Absent	Absent	Absent	Present	Absent
Conostylis petrophiloides	Absent	Absent	Absent	Present	Absent
Conostylis prolifera	Absent	Absent	Absent	Present	Absent
Conostylis pusilla	Absent	Absent	Absent	Present	Absent
Conostylis resinosa	Absent	Absent	Absent	?	Absent
Conostylis robusta	Absent	Absent	Absent	Present	Absent
Conostylis seminuda	Absent	Absent	Absent	?	Absent
Conostylis setigera	Absent	Absent	Absent	?	Absent
C. setigera subsp. *dasys*	Absent	Absent	Absent	Present	Absent
Conostylis setosa	Absent	Absent	Absent	Present	Absent
Conostylis stylidioides	Absent	Absent	Absent	Present	Absent
Conostylis teretifolia subsp. *planesens*	Absent	Absent	Absent	Present	Absent
C. teretifolia subsp. *teretifolia*	Absent	Absent	Absent	Present	Absent
Conostylis teretiuscula	Absent	Absent	Absent	Present	Absent
Conostylis tomentosa	Absent	Absent	Absent	Present	Absent
Conostylis vaginata	Absent	Present	Absent	Present	Absent
Conostylis villosa	Absent	Absent	Absent	Present	Absent
Conostylis wonganensis	Absent	Absent	Absent	Present	Absent

Table 2 (*Continued*)

Taxon	Bulliform cells	Leaves fistulose	Leaf aerenchyma	Leaf tannin cells	Mucilage cells
Macropidia fuliginosa	Absent	Absent	Absent	Present	Absent
Phlebocarya ciliata	Absent	Absent	Absent	Present	Absent
Phlebocarya pilosissima	Absent	Absent	Absent	Present	Absent
Tribonanthes australis	Absent	Present	Present	Present	Absent
Tribonanthes brachypetala	Absent	Present	Present	Present	Absent
Tribonanthes longipetala (*T. uniflora*)	Absent	?	Present	Present	Absent
	Epidermal wall relative area	Epidermal wall uniformity	Epidermal cell layer no.	Epidermal surface shape	Marginal fibers
Haemodoroideae:					
B. aurea	Thin	Uniform	1	Flat	Absent
D. corymbosa	Thin	Uniform	1	Flat	Absent
D. ixioides	Thin	Uniform	1	Undulate	Absent
D. pillansii	Thin	Uniform	1	Flat	Absent
D. viscosa	Thin	Uniform	1	Flat	Absent
H. laxum	Thin	Not uniform	1	Flat	Absent
H. loratum	Thin	Uniform	1	Flat	Absent
H. simplex	Thin	Uniform	1	Flat	Absent
H. simulans	Thin	Uniform	1	Flat	Absent
H. spicatum	Thin	Uniform	1	Invaginate	Absent
H. venosum	Thin	Uniform	1	Flat	Absent
L. caroliniana	Thin	Uniform	1	Flat	Absent
P. neblinae	Thin	Uniform	1	Invaginate	Absent
S. orinocensis	Thin	Uniform	1	Flat	Absent
W. brachyandra	Thin	Uniform	1	Flat	Absent
W. paniculata	Thin	Uniform	1	Flat	Absent
W. thyrsiflora	Thin	Uniform	1	Flat	Absent
X. caeruleum	Thin	Uniform	1	Flat	Absent
Conostylidoideae:					
A. flavidus	Thin	Not uniform	1	Flat	Absent
A. humilis	Thin	Uniform	1	Flat	Absent
A. preissii	Thin	Uniform	1	Flat	Absent
A. rufus	Thin	Uniform	1	Flat	Absent
B. canescens	Thick	Uniform	1	Invaginate	Present
C. aculeata subsp. *bromelioides*	Thick	Uniform	1	Invaginate	Present
C. aculeata subsp. *spinuligera*	Thick	Uniform	1	?	Present
C. androstemma	Thick	Uniform	1	Invaginate	Absent
C. angustifolia	Thick	Uniform	1	Invaginate	Absent
C. aurea	Thick	Uniform	1	Undulate/invaginate	Absent
C. bracteata	Thick	Uniform	1	Undulate	Present
C. candicans	Thick	Uniform	1	Undulate	Absent
C. canteriata	Thick	Uniform	1	Invaginate	Present
C. caricina subsp. *caricina*	Thick	Uniform	1	Undulate	Present
C. caricina subsp. *elachys*	Thick	Uniform	1	Undulate	Present
C. crassinerva subsp. *absens*	Thick	Uniform	1	Undulate	Present
C. dielsii subsp. *teres*	Thick	Uniform	2 or more	Invaginate	?
C. festucacea subsp. *filifolia*	Thick	Uniform	2 or more	Invaginate	?
C. juncea	Thick	Uniform	1	Undulate	Absent
C. latens	Thick	Uniform	2 at margin	Flat	Absent
C. micrantha	Thick	Uniform	1	Flat/undulate	Absent
C. misera	Thick	Not uniform	2 at margin	Flat	?
C. neocymosa	Thick	Uniform	2 at margin	Undulate	Present
C. pauciflora	Thick	Uniform/outer	1	Flat	Present
C. petrophiloides	Thick	Uniform	1	Flat	Present
C. prolifera	Thick	?	2 or more	Invaginate	Absent
C. pusilla	Thick	?	1	Undulate	Absent
C. resinosa	Thick	?	1	Flat	Absent
C. robusta	Thick	Uniform	2 at margin	Flat	Present
C. seminuda	Thick	Uniform	2 or more	Invaginate	Absent
C. setigera	Thick	Uniform	2 at margin	Flat	Absent
C. setigera subsp. *dasys*	Thick	Uniform	1	Flat	Absent
C. setosa	Thick	Uniform	1	Flat	Absent
C. stylidioides	Thick	Uniform	1	Flat	Absent

Table 2 (*Continued*)

Taxon	Epidermal wall relative area	Epidermal wall uniformity	Epidermal cell layer no.	Epidermal surface shape	Marginal fibers
C. teretifolia subsp. *planesens*	Thick	Uniform	1	Flat	Absent
C. teretifolia subsp. *teretifolia*	Thick	Uniform	1	Flat	Absent
C. teretiuscula	Thick	Uniform	1	Undulate	Present
C. tomentosa	Thick	Uniform	1	Undulate	Absent
C. vaginata	Thick	Uniform	1	Undulate	Absent
C. villosa	Thick	Uniform	1	Undulate	Absent
C. wonganensis	Thick	Uniform	1	Flat	Absent
M. fuliginosa	Thin	Not uniform	1	Flat	Absent
P. ciliata	Thin	Not uniform	1	Flat	Absent
P. pilosissima	Thin	Uniform	1	Flat	Absent
T. australis	Thin	Uniform	1	Flat	Absent
T. brachypetala	Thin	Uniform	1	Flat	Absent
T. longipetala [*T. uniflora*]	Thin	Uniform?	1	Flat	Absent
	Palisade cell morphology	Vascular contact with epidermis	Fibers in vascular bundle	Raphide crystals	Silica bodies
Haemodoroideae:					
B. aurea	Absent	Absent	Absent	None observed	None observed
D. corymbosa	1 layer	Absent	Partial	Present	None observed
D. ixioides	2 layers	Absent	Partial	Present	None observed
D. pillansii	?	Absent	Partial	Present	None observed
D. viscosa	1 layer	Absent	Partial	Present	None observed
H. laxum	?	Present	Complete	Present	None observed
H. loratum	?	Present/absent	Partial	Present	None observed
H. simplex	Absent	Present	Partial	Present	None observed
H. simulans	More than 2	Present	Complete	Present	None observed
H. spicatum	More than 2	Present	Partial	Present	None observed
H. venosum	?	Present	Complete	Present	None observed
L. caroliniana	Absent	Present/absent	Partial	Present	None observed
P. neblinae	?	Present/absent	Partial	Present	None observed
S. orinocensis	Absent	Present	Partial	Present	None observed
W. brachyandra	Absent	Absent	Partial	Present	None observed
W. paniculata	?	Absent	Partial	Present	None observed
W. thyrsiflora	Absent	Absent	Partial to none	Present	None observed
X. caeruleum	Absent	Present/absent	Partial to none	Present	None observed
Conostylidoideae:					
A. flavidus	2 layers	Present/absent	Partial	Present	None observed
A. humilis	1 layer	Absent	Partial	Present	None observed
A. preissii	1 layer	Absent	Partial	Present	Present
A. rufus	2 layers	Absent	Partial	Present	None observed
B. canescens	2 layers	Present	Partial/complete	Present	Present
C. aculeata subsp. *bromelioides*	2 layers	Present	Complete	Present	Present
C. aculeata subsp. *spinuligera*	2 layers	Present	Complete	Present	Present
C. androstemma	2 layers	Present	Complete	?	None observed
C. angustifolia	More than 2	Present	Partial	Present	None observed
C. aurea	2 layers	Absent	Complete	Present	None observed
C. bracteata	2 layers	Present	Complete	Present	None observed
C. candicans	2 layers	Present	Partial	Present	?
C. canteriata	2 layers	Absent	Complete	Present	?
C. caricina subsp. *caricina*	1 layer	Present	Complete	?	?
C. caricina subsp. *elachys*	?	Present	Complete	None observed	?
C. crassinerva subsp. *absens*	?	Absent	Partial	None observed	None observed
C. dielsii subsp. *teres*	2 layers	Absent	Complete	None observed	None observed
C. festucacea subsp. *filifolia*	More than 2	Present	Complete	Present	Present
C. juncea	2 layers	Present	Complete	Present	Present
C. latens	2 layers	Absent	Complete	Present	Present
C. micrantha	2 layers	Absent	Complete	None observed	None observed
C. misera	?	Absent	Partial	Present	Present
C. neocymosa	?	Present	Partial	Present	?
C. pauciflora	2 layers	Present	Complete	Present	None observed
C. petrophiloides	2 layers	Present	Partial/complete	Present	None observed
C. prolifera	2 layers	Absent	Partial/complete	Present	None observed

Table 2 (*Continued*)

Taxon	Palisade cell morphology	Vascular contact with epidermis	Fibers in vascular bundle	Raphide crystals	Silica bodies
C. pusilla	2 layers	Absent	Complete	Present	Present
C. resinosa	?	Absent	Partial	Present	Present
C. robusta	More than 2	Present/absent	Partial	Present	None observed
C. seminuda	?	Absent	Complete	Present	None observed
C. setigera	2 layers	Absent	Complete	None observed	Present
C. setigera subsp. *dasys*	2 layers	Absent	Complete	None observed	?
C. setosa	2 layers	Absent	Partial	None observed	Present
C. stylidioides	2 layers	Absent	Partial	None observed	None observed
C. teretifolia subsp. *planesens*	2 layers	Absent	Complete	None observed	Present
C. teretifolia subsp. *teretifolia*	2 layers	Absent	Complete	Present	Present
C. teretiuscula	2 layers	Present	Complete	None observed	Present
C. tomentosa	?	Absent	Partial	None observed	Present
C. vaginata	2 layers	Absent	Complete	None observed	None observed
C. villosa	2 layers	Absent	Complete	None observed	Present
C. wonganensis	2 layers	Absent	?	None observed	Present
M. fuliginosa	2 layers	Present/absent	Partial	Present	?
P. ciliata	Absent	Present	Complete	Present	Present
P. pilosissima	2 layers	Absent	Partial	None observed	Present
T. australis	2 layers	Absent	Absent	Present	None observed
T. brachypetala	Absent	Absent	Absent	Present	None observed
T. longipetala [*T. uniflora*]	Absent	Absent	Absent	None observed	None observed

Note. A question mark indicates that the character state could not be determined.

were taken using ImageJ software (Abramoff et al. 2004; Rasband 2007). The area of the epidermal cell in transection and that of its cell wall in transection were measured, and the transectional wall area : cell area ratio (termed throughout as "epidermal cell wall relative area") was calculated and plotted.

Analysis of Phylogenetic Signal and Habitat Adaptations

Eight vegetative anatomical characters and character states (see table 2) were tabulated and plotted using parsimony optimization on the cladogram generated by Hopper et al. (2009) from a molecular phylogenetic study, using the program MacClade (Maddison and Maddison 2005) with parsimony optimization only. Only those taxa for which anatomical data were available were coded; all character states were coded as unordered. The characters and character states plotted are leaf bulliform cells (absent: 0; present: 1), leaf tannin cell presence (absent: 0; present: 1), marginal fiber cap (absent: 0; present: 1), epidermal cell wall relative area (thick [relative cell wall layer >50%]: 0; thin [relative cell wall layer <50%]: 1), palisade cell (absent: 0; one layer: 1; two layers: 2; more than two layers: 3), fiber distribution in vascular bundles (absent: 0; partial: 1; complete: 2), leaf aerenchyma (absent: 0; present: 1), and mucilage cells (absent: 0; present: 1).

An additional five anatomical characters (table 2) were described but not plotted: relative epidermal wall uniformity (uniform: 0; not uniform: 1), epidermal cell layer number (one layer: 0; two or more layers: 1; two layers at margin only: 2), epidermal surface shape (flat: 0; undulate: 1; invaginate: 2), vascular bundle contact with the epidermis (absent: 0; present: 1), and silica body presence (absent: 0; present: 1).

Correlations of specific character states with clades were noted. In addition, correlations of certain anatomical features with extrinsic features were described, and their evolution as a possible adaptive feature was discussed.

Results

Root Anatomy Descriptions

In the subfamily Haemodoroideae (fig. 1; examined for eight species in all eight genera; see table 1; app. for a list of taxa), the epidermis is uniseriate and thin walled (fig. 1*F*). Cortical cells are thin walled, and those near the endodermis are tangentially oblong in cross-sectional shape (tangential : radial ratio = 1.5–3 : 1) and mostly radially aligned (at least the inner layers near the endodermis; e.g., fig. 1*A*) in four to 12 layers. The cortical region is aerenchymatous in *Haemodorum venosum* (fig. 1*D*), *Lachnanthes caroliniana* (not shown, but seen in Simpson and Dickison 1981) and *Pyrrorhiza neblinae* (fig. 1*F*). The endodermis is uniseriate (fig. 1*A*–1*J*), with lamellate secondary cell walls that are unequally thickened, with inner tangential and radial walls thick and the outer wall thin in all taxa (fig. 1*A*–1*C*, 1*F*–1*J*) except *H. venosum* (fig. 1*D*) and *L. caroliniana* (fig. 1*E*), in which the secondary cell wall is uniformly thick. Endodermal cells are isodiametric (fig. 1*G*, 1*H*) to slightly elliptic-rectangular and tangentially elongate in cross section (fig. 1*E*, 1*I*). The pericycle is mostly uniseriate (one- or two-seriate in *Dilatris viscosa* [fig. 1*C*] and *H. venosum* [fig. 1*D*]), with thin or moderately thick cell walls. The ground tissue cells of the central vascular cylinder have thick to moderately thick secondary cell walls (fig. 1*A*, 1*C*), except in *P. neblinae*, in which the peripheral cells have thin second-

ary walls (fig. 1*G*), and in *Wachendorfia brachyandra*, in which all cells are irregular and thin walled (fig. 1*I*). The number of xylem poles ranges from five to 13 (fig. 1*A*, 1*C*, 1*F*), with each group generally having one large vessel arranged in a circle and one to three smaller vessels. Phloem occurs in seven to 16 groups of cells that are mostly alternate with (but occasionally opposite) the xylem (fig. 1*A*), with each group having one to four sieve tube members and two to 10 companion cells (fig. 1*B*, 1*G*, 1*H*; table 1).

In the subfamily Conostylidoideae (examined for 16 species in all six genera; see table 1; app. for a list of taxa), the epidermis is uniseriate and the cells are isodiametric, rarely tangentially elongate, and thin walled (inner and outer tangential walls are thick in *Phlebocarya ciliata*; not illustrated). The cortex consists of ca. 10–20 layers of isometric (rarely irregularly shaped), thin-walled cells in most taxa. Those near the periphery are isodiametric and polygonal in cross section with unlignified (rarely lignified) walls (figs. 2*A*, 3*G*), those in the middle region are often large and irregularly shaped with large intercellular spaces (fig. 2*A*), and those near the endodermis are tangentially oblong in cross section; the cells are not radially aligned (figs. 2*D*, 2*F*, 3*D*–3*G*) except in *Anigozanthos rufus*. Cortical cells of *A. rufus* (fig. 2*A*) and *Blancoa canescens* have homogeneous, tanniniferous contents. Endodermal cells are mostly rectangular (rarely isodiametric) and mostly radially oriented in cross section (figs. 2*D*–2*F*, 2*H*–2*J*, 3*B*–3*F*). The endodermal cell walls are uniformly thick, rarely unequally thickened (outer tangential wall is thin; e.g., *A. rufus*; fig. 2*B*), and not thickened in *Conostylis aculeata* and *Tribonanthes australis* (fig. 3*H*, 3*I*). The pericycle is uniseriate (where apparent), and the cells are isodiametric and thin to moderately thick walled (figs. 2*I*, 3*D*). Ground tissue of the central vascular cylinder consists of small isodiametric cells, having thin to moderately thick cell walls, with peripheral globular, tanniniferous deposits present in *P. ciliata* (fig. 3*D*, 3*E*), *Phlebocarya pilosissima*, and *T. australis*. The number of xylem poles is variable. *Tribonanthes australis* is unique in having two xylem poles (i.e., a diarch; fig. 3*H*, 3*I*); all others range from eight to 26 (figs. 2*A*, 2*I*, 3*A*–3*C*). In many taxa (*Anigozanthos flavidus*, *A. rufus*, *B. canescens*, *C. aculeata*, *Conostylis juncea*, *Conostylis setigera*, *Conostylis vaginata*, *Conostylis wongonensis*, *Macropidia fuliginosa*), four to 17 large vessels encircle the vascular cylinder center, with smaller vessel groups to the periphery (fig. 2*A*). In other taxa (*Conostylis neocymosa*, *Conostylis pauciflora*, *Conostylis petrophiloides*, *Conostylis seminuda*, *P. ciliata*, *P. pilosissima*), there are eight to 20 larger vessels throughout the central region of the vascular tissue and smaller vessels to the periphery (fig. 3*A*). *Tribonanthes australis* has a single large vessel in the center, flanked by 10–12 small vessels on either side (fig. 3*H*, 3*I*). In all species of *Anigozanthos*, *Blancoa*, *Conostylis*, and *Macropidia*, phloem occurs in 10–30 groups alternating with the xylem poles of the central vascular cylinder, with these groups usually consisting of a single sieve tube member flanked by one to seven companion cells (figs. 2*G*, 2*J*, 3*C*). *Tribonanthes australis* is unique again in having only two groups of phloem alternating between the xylem arches (fig. 3*H*, 3*I*). The two species of *Phlebocarya* differ in having 20–24 groups of sieve tube members that are apparently randomly dispersed at the periphery of the vascular cylinder (fig. 3*D*).

Scape Anatomy Descriptions

In the subfamily Haemodoroideae (fig. 4; examined for five species in five genera; see app. for a list of taxa), the scape is usually circular to oval in cross section (fig. 4*A*, 4*B*), sometimes with small protrusions of the epidermis, trichomes, or other epidermal appendages (fig. 4*C*). The cortex is variable. Some taxa show no clear difference of cells between the cortex and inner layers of the scape (fig. 4*A*, 4*D*). Others have a cortex consisting of several layers of cells (fig. 4*B*, 4*C*, 4*E*). Cortical vascular bundles are present in some taxa (fig. 4*B*). A sclerenchyma cylinder is present in most taxa, being distinct from cortex cells and inner parenchyma cells, with the cylinder relatively uniform in thickness and about four cell layers thick where present (fig. 4*B*, 4*C*, 4*E*). Vascular bundles are present mainly in parenchymatous tissue or within (or occasionally embedded in) the sclerenchyma cylinder. Bundles are arranged randomly throughout, extending to the axis center (fig. 4*A*, 4*B*). There is no obvious pattern for bundle orientation.

In the subfamily Conostylidoideae (fig. 5; examined for six species in six genera; see app. for a list of taxa), the scape is usually circular to oval in cross section (fig. 5*A*, 5*C*). The cortex is two to several cell layers thick, with some taxa having irregularly shaped cortical cells (fig. 5*E*, 5*F*), while others have round cells of varying size (fig. 5*A*–5*D*). A sclerenchyma cylinder is present, variable in thickness (two to several cell layers thick; fig. 5*A*), and distinct from adjacent cortical cells at the outer edge, grading with parenchyma cells at the inner edge. Vascular bundles are occasionally present in the cortical tissue (fig. 5*B*) but are generally inside the sclerenchyma cylinder (fig. 5*A*–5*F*). Bundles appear randomly arranged throughout, extending toward the center but not found in it. There is no obvious pattern for bundle orientation. Bundles are often surrounded by sclerenchyma. Tannin cells were observed throughout the axis in some taxa (fig. 5*A*, 5*D*).

Leaf Anatomy Descriptions

In the subfamily Haemodoroideae (examined for 10 species in all eight genera; see app. for a list of taxa), the leaves are unifacial and flattened in all taxa examined (fig. 6) except for *Haemodorum simplex*, which is semiterete (fig. 6*D*); *Haemodorum spicatum*, which is terete (fig. 6*E*); and *Barberetta aurea* (fig. 6*A*) and *Wachendorfia paniculata* (fig. 6*I*), which have plicate leaves. Leaf cross-sectional outlines of other taxa are mostly narrowly elliptic (e.g., *D. viscosa*; fig. 6*B*) to linear (e.g., *Xiphidium caeruleum*; fig. 6*J*). The plicate-leaved taxa, *B. aurea* and *W. paniculata*, have prominent ridges at outer leaf bends (fig. 6*A* and 6*I*, respectively), while some taxa have several surface invaginations corresponding to longitudinal grooves (e.g., *H. spicatum* [fig. 6*E*] and *P. neblinae* [fig. 6*G*]). Epidermal cell shape (in face view) ranges from roughly isodiametric (e.g., *D. viscosa*; fig. 7*A*) to axially elongate (e.g., *W. paniculata*; fig. 7*E*). The cell body is generally raised relative to the junction with adjacent cells (fig. 7). Epidermal papillae are absent except in *Schiekia orinocensis* (fig. 7*D*), in which they occur in two or three rows. Wax deposits were not definitively observed. Hairs are absent on the leaf surface. Surface invaginations are absent. The epidermis is uniform and uni-

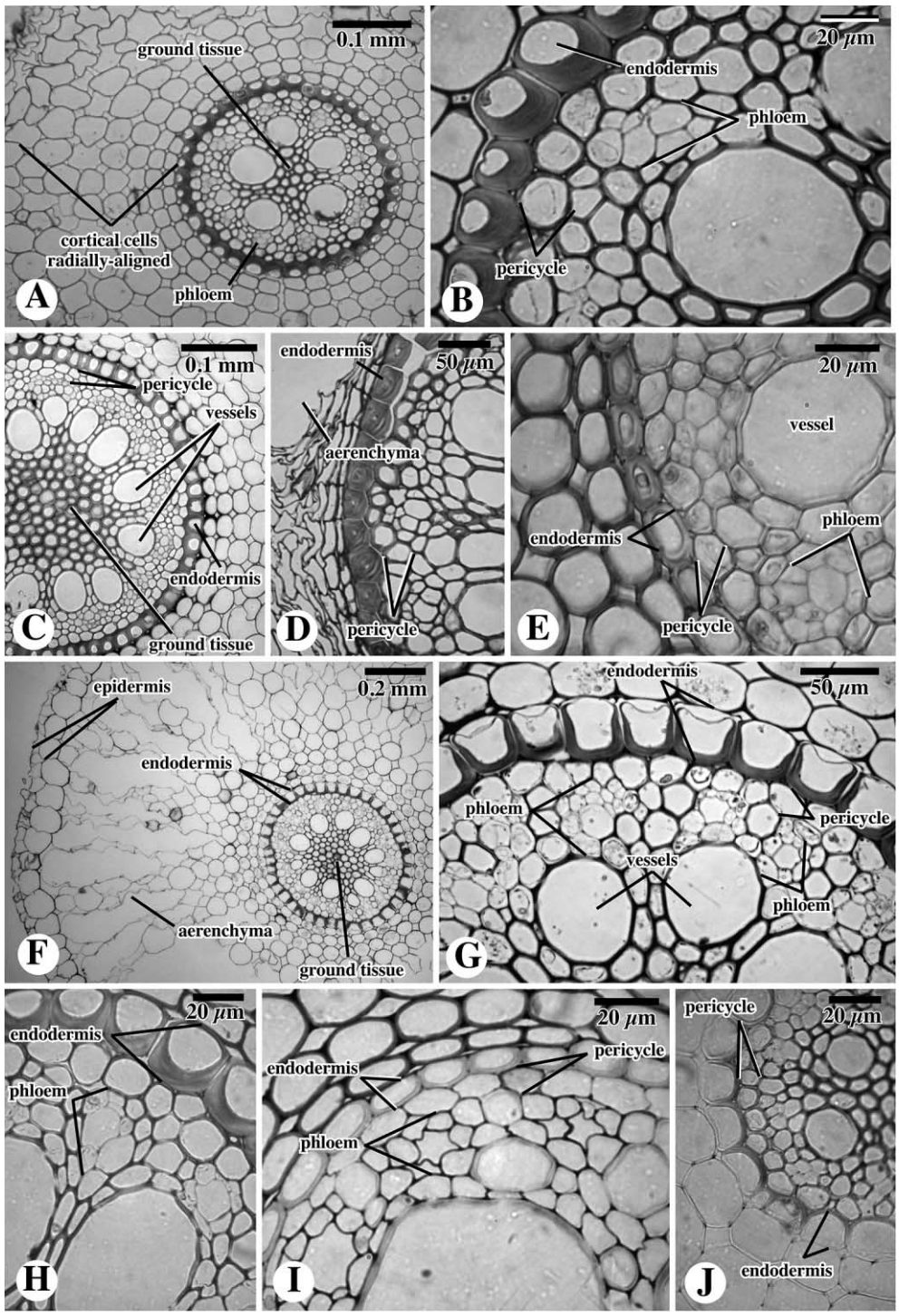

Fig. 1 Root cross sections, subfamily Haemodoroideae. *A*, *B*, *Barberetta aurea*. *C*, *Dilatris viscosa*. *D*, *Haemodorum venosum*. *E*, *Lachnanthes caroliniana*. *F*, *G*, *Pyrrorhiza neblinae*. *H*, *Schiekia orinocensis*. *I*, *Wachendorfia brachyandra*. *J*, *Xiphidium caeruleum*.

seriate, with relatively thin epidermal walls (figs. 8*B*, 9*E*, 9*G*). In cross section, epidermal cells are more or less isodiametric to tangentially elongate, having a thin cuticle (figs. 8*C*, 9*E*–9*H*). In *B. aurea* and *W. paniculata*, the epidermal cells on the sides opposite the projecting ridges of the leaf plications are enlarged and often irregularly shaped and bulliform (fig. 8*F*). Stomata are dispersed over the entire leaf surface (fig. 7). Stomata in all taxa have two paracytic subsidiary cells (e.g., figs. 7, 8*C*, 9*H*). Stomatal cavities range from ca. 15 to 150 μm deep (figs. 8*C*, 9*B*, 9*G*, 9*H*); stomatiferous grooves

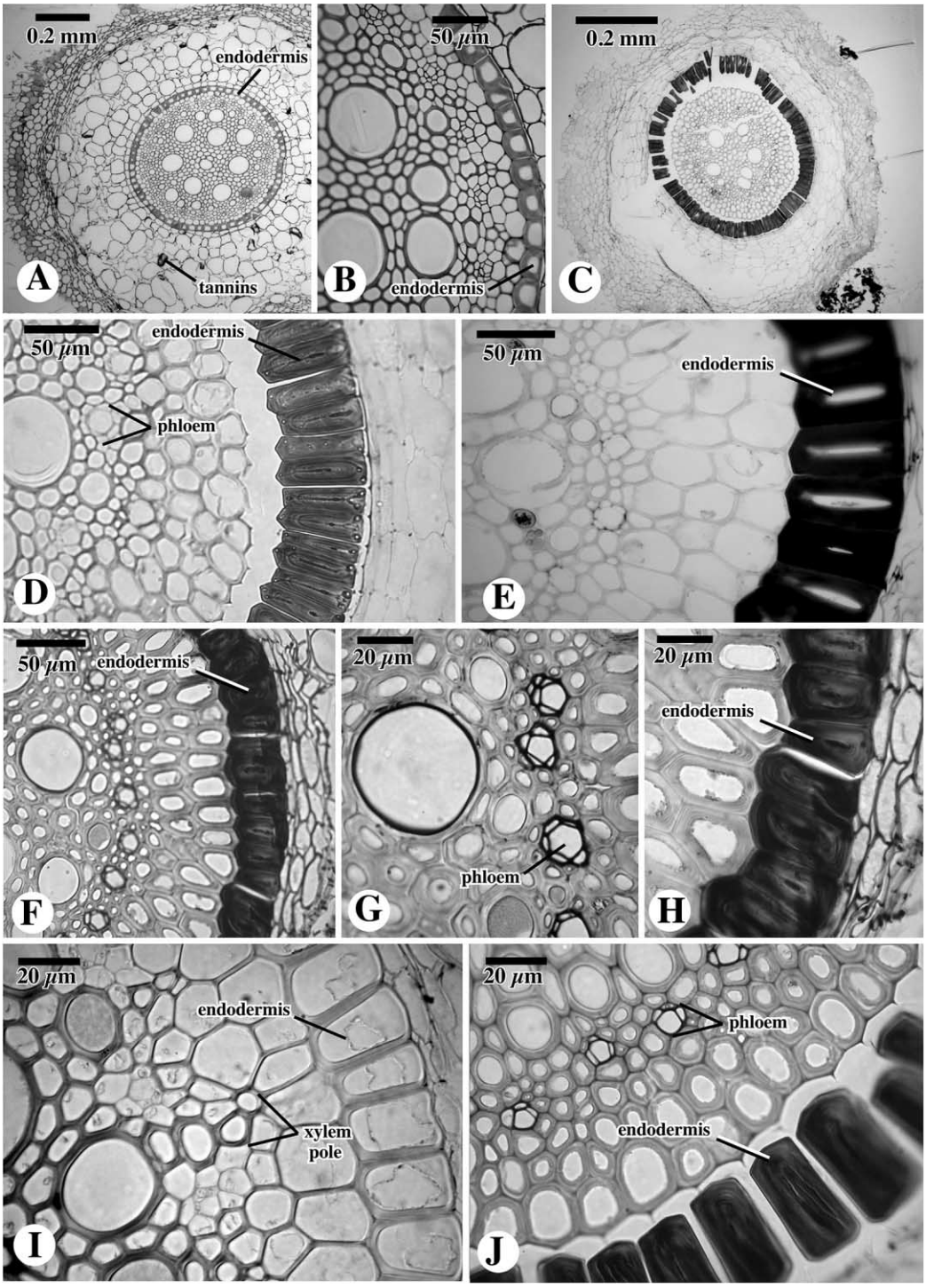

Fig. 2 Root cross sections, subfamily Conostylidoideae. *A*, *B*, *Anigozanthos rufus*. *C*, *D*, *Blancoa canescens*. *E*, *Conostylis juncea*. *F–H*, *Conostylis pauciflora* subsp. *euryhipis*. *I*, *Conostylis seminuda*. *J*, *Conostylis vaginata*.

are absent. The mesophyll of most taxa is chlorophyllous throughout, consisting of one to 10 layers of palisade-like cells (e.g., fig. 9*F*, 9*G*). *Barberetta aurea* (fig. 8*A*) and *X. caeruleum* (fig. 9*H*) lack distinct palisade layers. Many taxa also have inner layers consisting of larger, achlorophyllous spongy cells (e.g., *H. spicatum*; fig. 8*G*). The vascular bundles are found mainly along the leaf perimeter (fig. 6), sometimes contacting the epidermis (figs. 8*E*, 9*C*). Bundles are consistently collateral and generally occur in two rows, one on each side of the leaf, with the xylem toward the center, usually al-

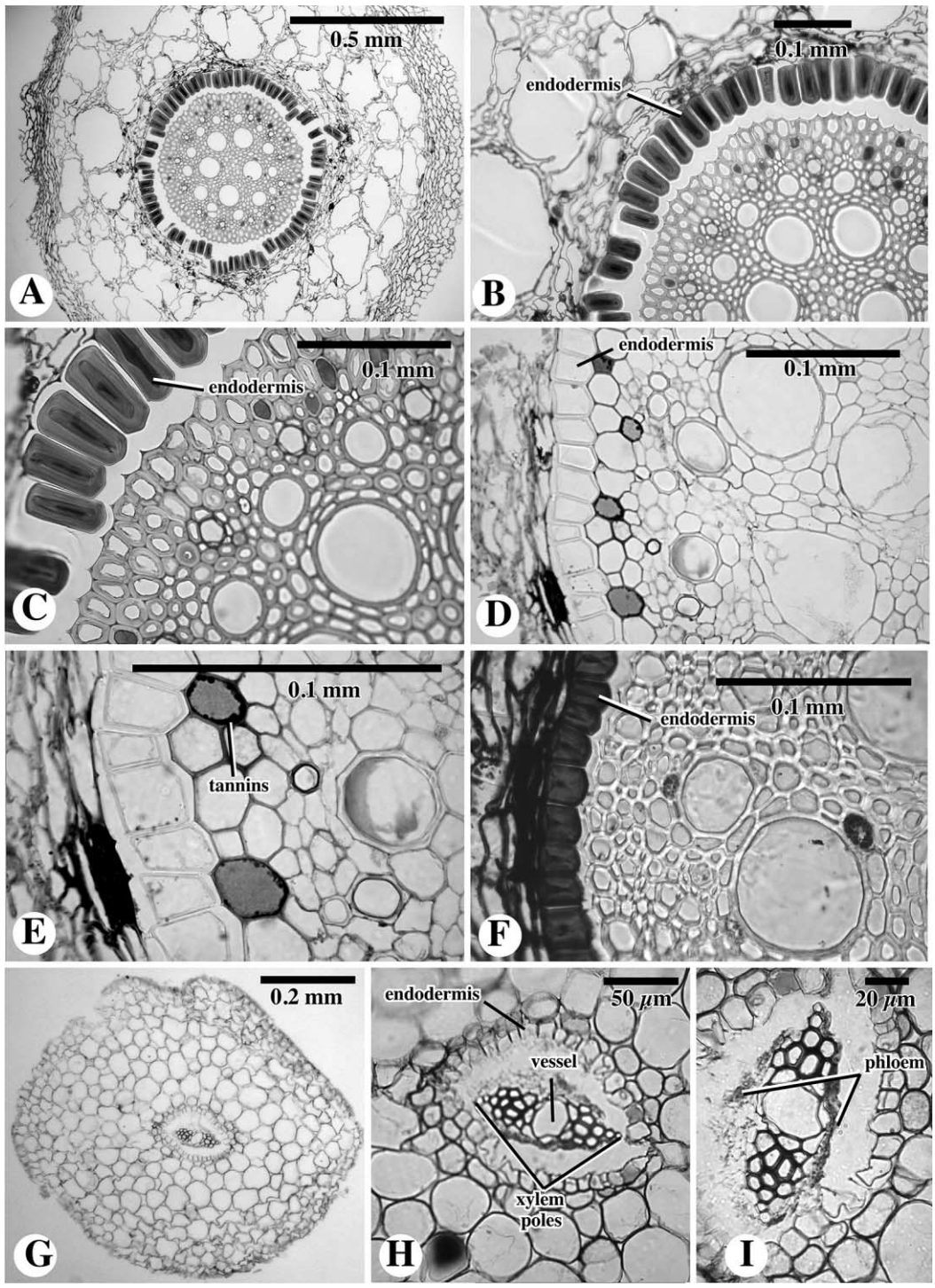

Fig. 3 Root cross sections, subfamily Conostylidoideae. *A–C*, *Macropidia fuliginosa*. *D–F*, *Phlebocarya ciliata*. *G–I*, *Tribonanthes australis*.

ternating between large and small along a leaf side (fig. 6). In thin leaves or leaves with thin margins, only one row of vascular bundles is present, with these usually alternating in orientation (e.g., *X. caeruleum*; fig. 6*J*). In species that have plicate leaves (*W. paniculata* and *B. aurea*), large bundles are located in the projecting ridges of the plications and small vascular bundles are scattered in between (fig. 6*A*, 6*I*). In *H. simplex* and *H. spicatum*, the vascular bundles encircle the leaf center (fig. 6*D*, 6*E*). Vascular bundles are generally opposite one another in xylem orientation among taxa that have only one row (fig. 6*A*, 6*I*, 6*J*). Most bundles in cross section are elliptic or orbicular to oblong in cross section (figs. 6, 8*E*, 9*D*).

Within the vascular bundles, the xylem consists of one to 10 layers of vessels, while the phloem usually consists of one or two to four or five layers of sieve tube members (figs. 8*A*, 8*E*, 9*D*). Bundle sheaths are present in all taxa and contain an outer single layer of mostly achlorophyllous, thin-walled cells (figs. 8*E*, 9*D*, 9*F*, 9*G*). Inside the bundle sheath are layers of thick-walled sclerenchyma fibers concentrated at the phloem end of bundles (figs. 8*E*, 9*D*), occasionally extending to the xylem end (fig. 9*F*) or completely encircling the bundle (e.g., fig. 8*E*). *Barberetta aurea* (fig. 8*A*) is the exception, having a bundle sheath consisting of a single layer of chlorophyllous thin-walled cells with chloroplasts concentrated on the outer perimeter of the sheath; sclerenchyma fibers are absent. Marginal epidermal cells of some taxa have cell walls similar to those of the rest of the leaf (fig. 9*E*); those of other taxa have thicker outer tangential walls (fig. 9*B*). Several taxa contain a marginal vascular bundle oriented at a right angle or slightly oblique to other vascular bundles (fig. 9*E*, 9*G*). *Haemodorum simulans* has a mass of sclerenchyma at the leaf margin (fig. 8*F*). *Haemodorum simplex* (fig. 8*D*) has leaf margins with no associated sclerenchyma, marginal vein, or epidermal cell wall thickenings. All taxa examined possess raphide crystals found within the mesophyll (fig. 9*H*, 9*I*), with the exception of *B. aurea*, which showed none. In *L. caroliniana*, raphide crystals were not observed, but this species does contain safranin-staining bodies in the mesophyll (fig. 9*B*). All four examined species of *Dilatris* possess leaf mucilage cavities (fig. 8*B*). Leaf tannin cells or silica bodies were not observed in any members of the Haemodoroideae.

In the subfamily Conostylidoideae (examined for 12 species in all six genera; see app. for a list of taxa), the leaves are unifacial and flattened, varying from linear to elliptic or fusiform in cross section (fig. 10*A*–10*E*, 10*H*, 10*J*–10*L*), or are terete and orbicular in cross section (fig. 10*G*, 10*I*, 10*K*). Some taxa have leaves with outer longitudinal grooves (fig. 10*D*, 10*F*, 10*H*). Epidermal cells are axially elongate, in some taxa being much longer than they are wide (fig. 11*C*–11*F*). The cell body is generally raised relative to the junction with adjacent cells (fig. 11). Epidermal papillae are absent. In taxa with outer, longitudinal grooves or invaginations, trichomes are typically present along the inner surfaces of the groove (fig. 11*B*, 11*E*). Stomata are dispersed across the leaf surface, with each stomate having two paracytic subsidiary cells (figs. 11, 12*E*) and with cavities ranging from 15 to 65 μm deep (figs. 12*F*, 13*B*, 13*E*). In taxa with numerous outer, longitudinal grooves or invaginations in the leaves, stomata are common within the grooves, anatomically corresponding to what in cross-sectional view we term a stomatiferous groove (figs. 12*E*, 13*B*). *Conostylis vaginata*, which has only two opposing longitudinal grooves (fig. 10*I*), is different in lacking stomates within the grooves. In cross section, epidermal cells are mostly isodiametric, with radial and inner tangential walls thin and outer tangential walls thicker but with a thin, outer cuticle (figs. 12*A*, 12*B*, 13*C*, 13*G*). Variation in epidermal cell cross-sectional outline includes cells that are irregular in shape and size, tangentially elongate, or radially elongate (fig. 13*E*). Epidermal cell wall thickness varies greatly from thin (fig. 12*A*) to very thick (fig. 13*C*). In *Conostylis prolifera*, the epidermal cells within stomatiferous grooves differ from those outside the groove (fig. 13*B*). Mesophyll in most taxa consists of one to three layers of radially elongate, chlorophyllous palisade-like cells and numerous inner layers of irregularly shaped achlorophyllous spongy cells (fig. 12*A*), with the exceptions being *C. prolifera* and *M. fuliginosa*, which have chlorophyllous cells throughout. *Anigozanthos humilis*, *Conostylis tomentosa*, and *P. ciliata* lack a discrete palisade layer. Vascular bundles are positioned in two rows in most taxa, usually alternating between large and small in size (fig. 10). In *Conostylis teretifolia* subsp. *teretifolia* and *T. australis*, the bundles are arranged in a ring as viewed in cross section (fig. 10*G*, 10*K*). *Macropidia fuliginosa* is unique in that the bundles are in more or less one row (fig. 10*L*). In the majority of the taxa examined, vascular bundles are directly beneath the surface, making contact with the surface in some taxa (e.g., *C. petrophiloides*; fig. 13*A*). In *C. teretifolia* subsp. *teretifolia* and *M. fuliginosa*, some bundles are confluent (fig. 15*F*). Most bundles in cross section are radially elliptic and collateral (fig. 12*D*), although some smaller bundles are more orbicular in shape (fig. 10). Within vascular bundles, the xylem consists of two to 10 layers of vessels and phloem consists of two to eight layers of sieve tube members (figs. 12*D*, 14*A*). Bundle sheathes were observed in all taxa except *C. vaginata*, in which the outer, achlorophyllous cells are absent or replaced by tannin cells. In most taxa, the outer sheath consists of a single layer of usually clear, achlorophyllous, thin-walled cells, with silica deposits present in some (figs. 12*E*, 12*F*, 13*A*, 13*C*, 13*D*). Bundle sheaths are usually complete but in some taxa are present only at the xylem end of the bundle, especially when in contact with the epidermis (e.g., fig. 13*A*). The inner sheath consists of several layers of thick-walled sclerenchyma cells concentrated at the phloem end of the bundle (figs. 12*D*, 13*D*) and sometimes extending to the epidermis, occasionally extending to the xylem, especially in larger bundles (fig. 13*D*). Sclerenchyma is lacking in some of the smaller bundles in some taxa. Leaf margins are of two general types, with some variation (fig. 16): (1) marginal epidermal cells with markedly thickened walls (e.g., *P. ciliata*; fig. 16*F*) or (2) subepidermal marginal sclerenchyma fibers (sclerenchyma cap) present and marginal vein oriented at right angle or slightly oblique to other vascular bundles (e.g., various species of *Conostylis*; fig. 16*C*). Tannin cells were observed in all taxa except *C. teretifolia* subsp. *teretifolia*. Tannin cells are usually found in the mesophyll (figs. 12*A*, 12*B*, 13*F*, 14*B*, 14*C*) although are sometimes present in bundle sheath cells (fig. 13*F*). Raphide crystals were also observed in numerous taxa, usually within pockets in the mesophyll (fig. 12*C*). Granular silica crystals were found in a large number of taxa (figs. 12*E*, 12*F*, 13*C*, 13*D*; see table 2).

Systematic Correlations

Many of the anatomical character and character states evaluated here correlate well with the pattern of phylogenetic relationships (after Hopper et al. 2009). The following summarizes that concordance and evaluates possible adaptive significance.

Leaf bulliform cells. *Barberetta* and *Wachendorfia* (fig. 9*F*) possess enlarged epidermal bulliform cells in the regions opposite the tissue ridges of their plicate leaves. The presence of bulliform cells constitutes a clear apomorphy for these two genera (fig. 18*A*) and is evidently associated with the plicate condition and thus not an independent apomorphy.

Leaf tannin cells. Tannin cells are identified as cells that stain a deep red with safranin stain, either uniformly homogenous or with granular-like accretions that are not bire-

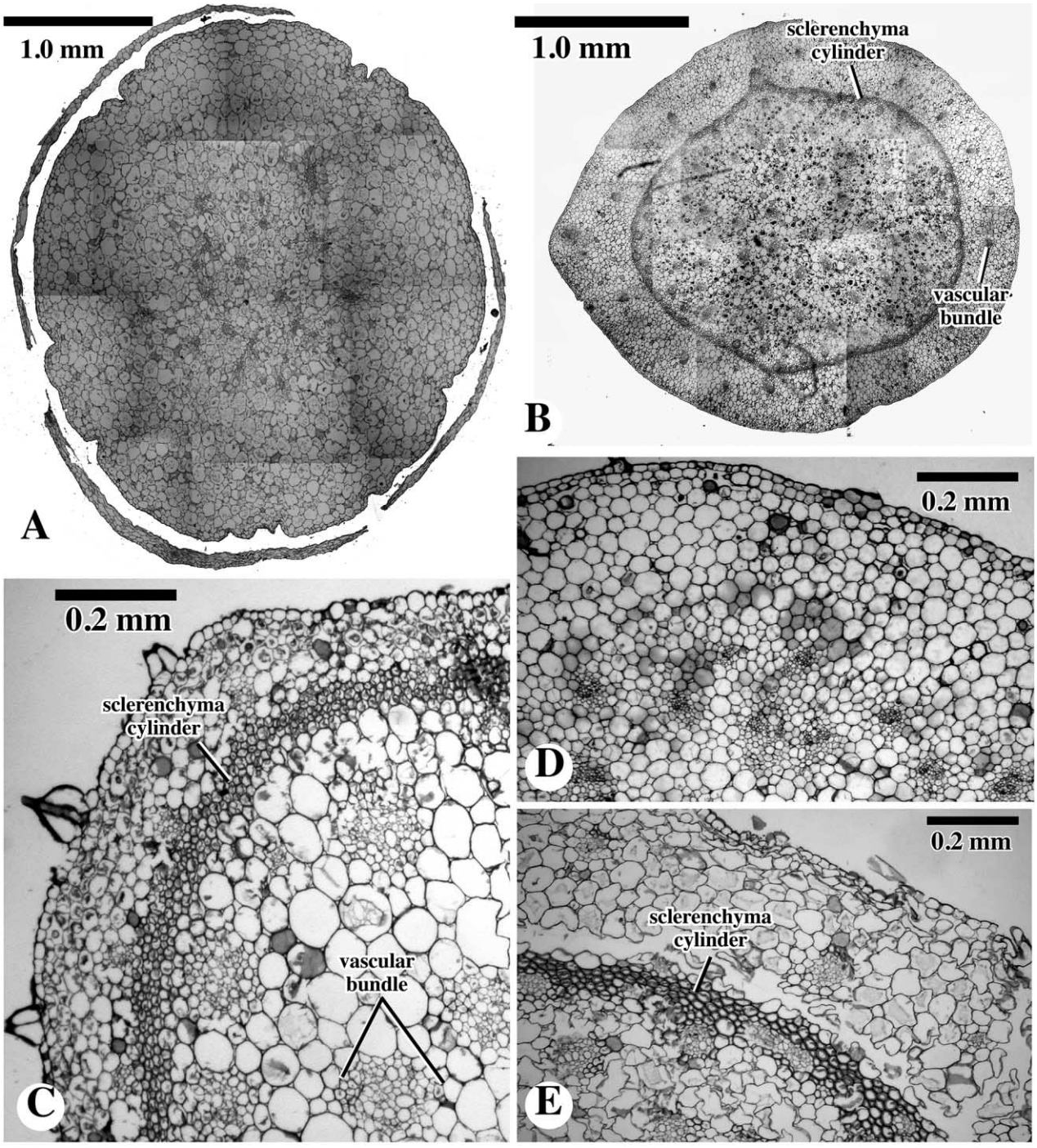

Fig. 4 Scape cross sections, subfamily Haemodoroideae. *A*, *Barberetta aurea*. *B*, *Xiphidium caeruleum*. *C*, *Wachendorfia brachyandra*. *D*, *Dilatris viscosa*. *E*, *Schiekia orinocensis*.

fringent under polarized light (figs. 13*F*, 14*B*, 14*C*, 16*A*, 16*E*, 16*F*). Because of intergradations between the two forms of tannin cells, only the absence or presence of these cells was coded. All examined members of the subfamily Haemodoroideae lack leaf tannin cells. In contrast, leaf tannin cells are present in all members of the Conostylidoideae examined and are an apparent apomorphy for this subfamily (fig. 18*B*).

Marginal fiber cap. In *B. canescens* and in some species of *Conostylis*, thickened fibers are clustered at the margins of the leaf, forming a cap. Leaf marginal fiber caps appear to have evolved at least three times in the Conostylidoideae within the *Blancoa-Conostylis* clade (fig. 19*A*).

Epidermal cell wall relative area. There is pronounced variation in the relative cell wall thickness of the epidermis in the

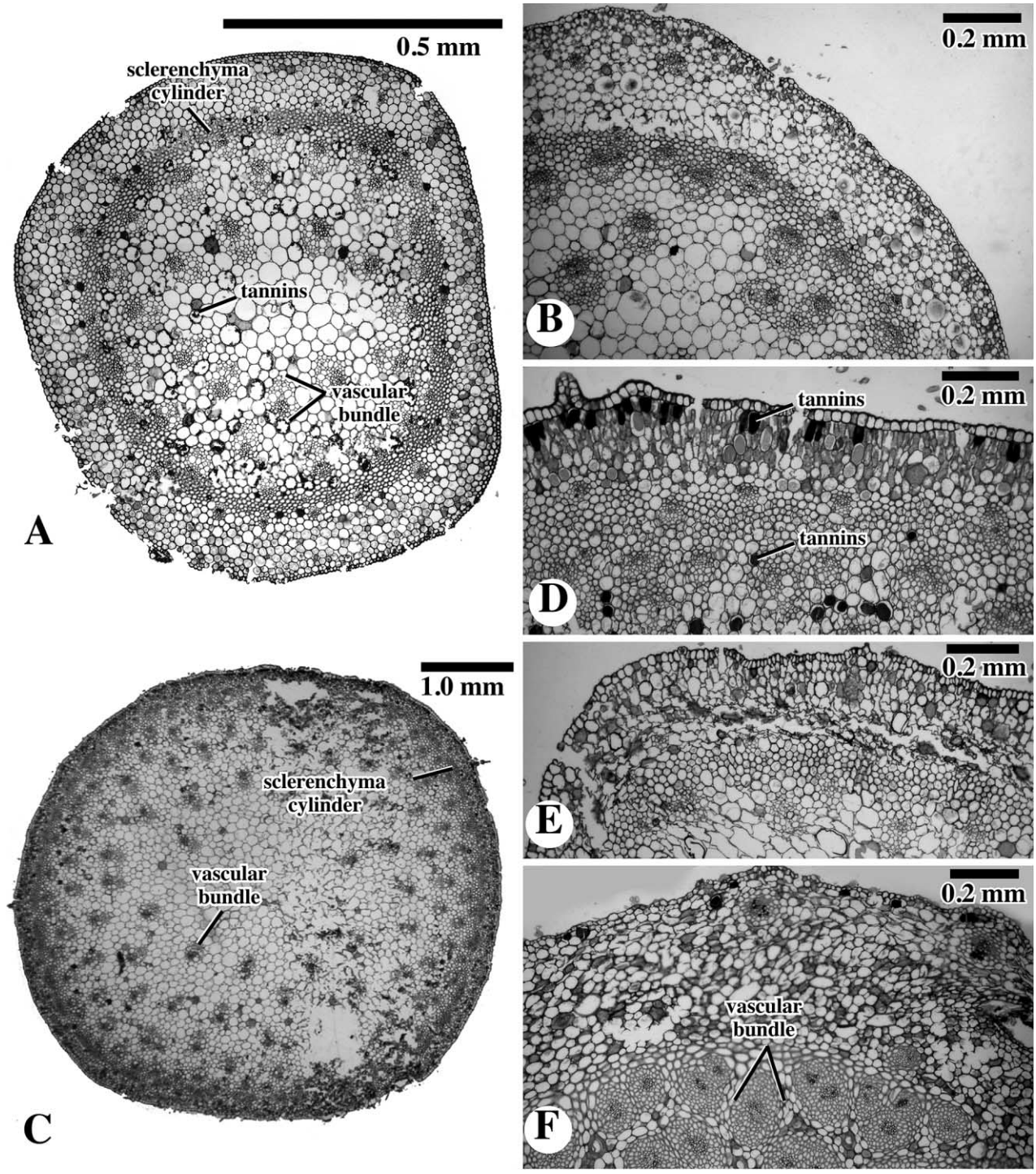

Fig. 5 Scape cross sections, subfamily Conostylidoideae. *A*, *Phlebocarya ciliata*. *B*, *Conostylis bracteata*. *C*, *Macropidia fuliginosa*. *D*, *Anigozanthos rufus*. *E*, *Tribonanthes australis*. *F*, *Blancoa canescens*.

Haemodoraceae (see fig. 17). *Blancoa* and all the members of *Conostylis* (the *Blancoa-Conostylis* clade) are characterized by a transectional cell wall area (relative to that of the entire cell) that is above 50%, an apomorphy for this clade; all other members of the Haemodoraceae have a relative cell wall area of less than 50% (fig. 19*B*).

Palisade cell morphology. The number of layers of palisade cells varies in the Haemodoraceae from absent to more than two layers. All members of the Haemodoroideae lack palisade cells except for *Dilatris*, which may have either one or two layers of these cells. All examined members of the Conostylidoideae have palisade cells except for *Tribonanthes*

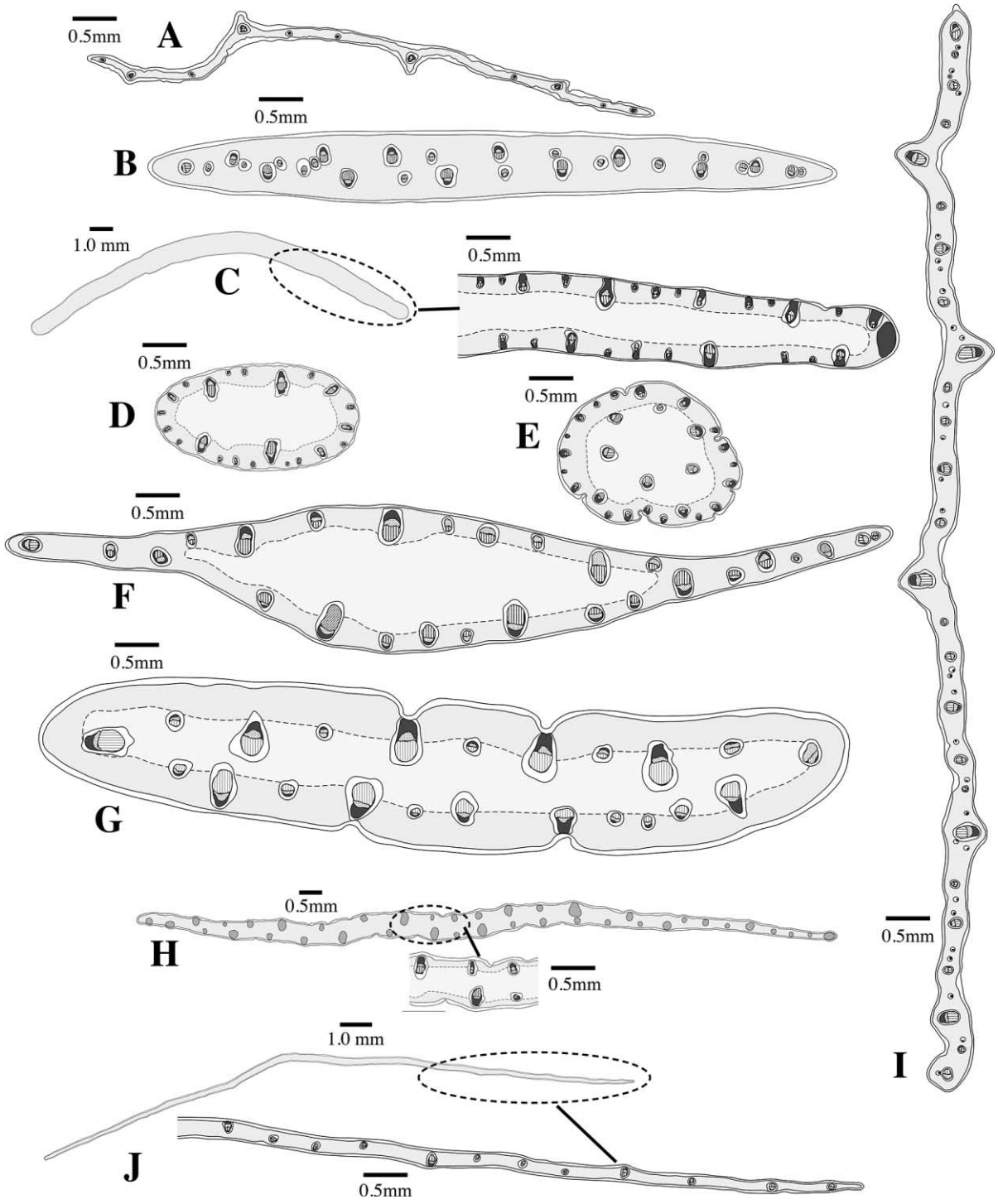

Fig. 6 Subfamily Haemodoroideae. Leaf cross-sectional outlines. *A*, *Barberetta aurea*. *B*, *Dilatris viscosa*. *C*, *Haemodorum simulans*. *D*, *Haemodorum simplex*. *E*, *Haemodorum spicatum*. *F*, *Lachnanthes caroliniana*. *G*, *Pyrrorhiza neblinae*. *H*, *Schiekia orinocensis*. *I*, *Wachendorfia paniculata*. *J*, *Xiphidium caeruleum*. Shading: white within a vascular bundle = outer bundle sheaths; black = fibers; hatch marks = xylem; dotted regions = phloem; inner dashed lines within a leaf in some species = junction of outer chlorenchyma and inner aerenchyma or achlorophyllous spongy cells.

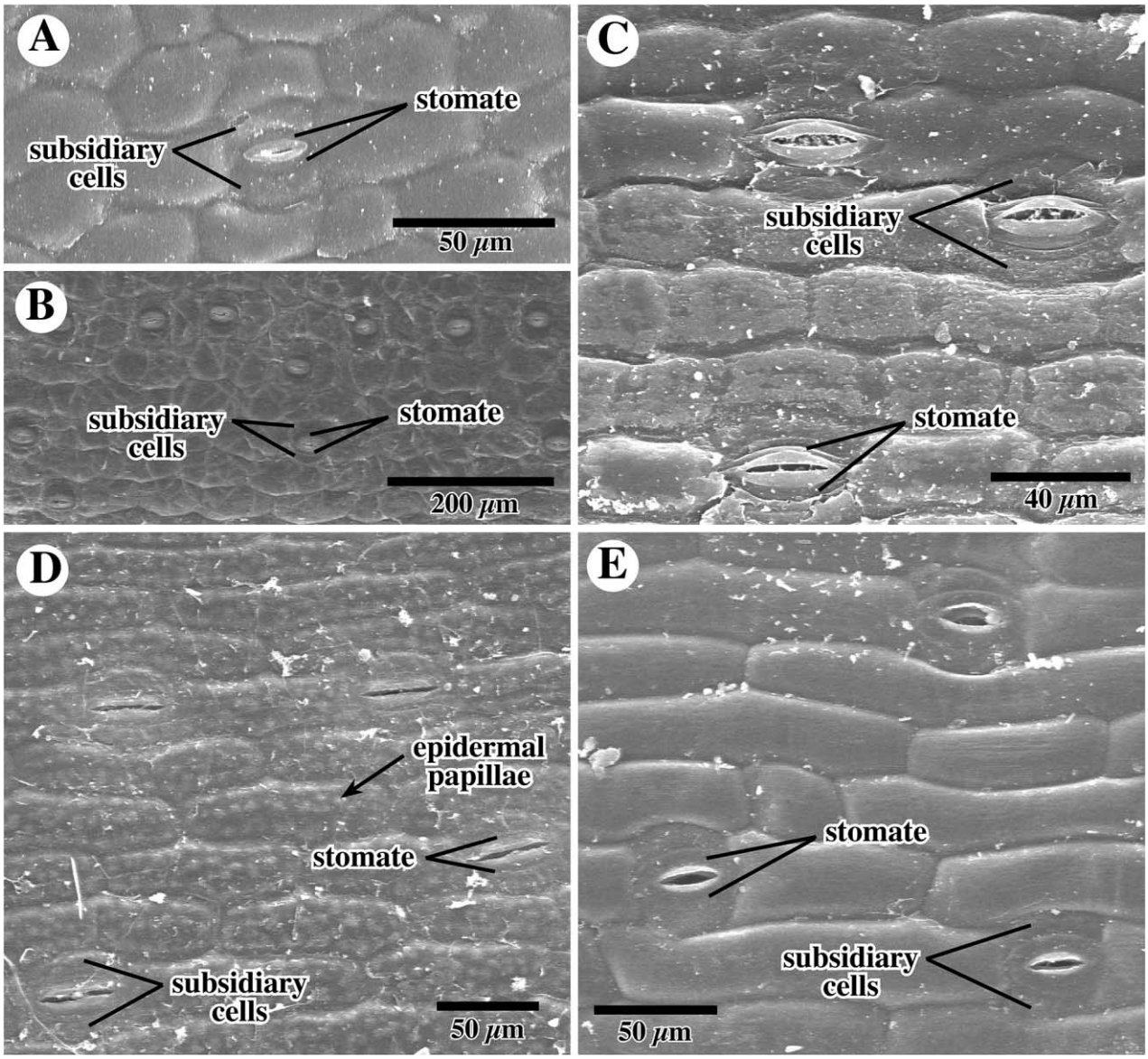

Fig. 7 Subfamily Haemodoroideae. Leaf surface, SEMs, and the longitudinal axis of the leaf from left to right. *A*, *Dilatris viscosa*. *B*, *Pyrrorhiza neblinae*. *C*, *Haemodorum spicatum*. *D*, *Schiekia orinocensis*. *E*, *Wachendorfia paniculata*. All taxa have paracytic subsidiary cells. See the main text for a discussion of the variation of epidermal cells.

and *Phlebocarya* (fig. 20*A*). Two species of *Anigozanthos*, *B. canescens*, most *Conostylis* species, and *M. fuliginosa* have two layers of palisade cells. Two other species of *Anigozanthos*—*A. humilis* and *A. preissii*—have one layer of palisade cells, and one species of *Conostylis* has three layers of palisade cells (fig. 20*A*).

Fiber distribution in vascular bundles. The fibers that are associated with the vascular bundle contain lignin, which is birefringent when viewed under polarized light. The presence of fibers associated with the vascular bundle and the amount and distribution of these fibers were studied. Three character states were defined: (1) fibers absent, (2) fibers present and partially enveloping the vascular bundles (usually a cap at the phloem end of the bundle), and (3) fibers present and completely enveloping the vascular bundles. The presence of fibers that partially envelop the vascular bundles is an apomorphy for the Haemodoraceae as a whole (fig. 20*B*). *Barberetta aurea* of the Haemodoroideae and one examined species of *Tribonanthes* of the Conostylidoideae have lost vascular bundle fibers (fig. 20*B*). The absence of bundle fibers in these taxa may be correlated with their environment (*Barberetta* in a mesic, forest floor habitat and *Tribonanthes* in periodically wet vernal pools).

Leaf aerenchyma. All the species of *Tribonanthes* (Conostylidoideae) studied contain aerenchyma distributed along the center of the leaves. *Lachnanthes* (Haemodoroideae) also contains aerenchyma, but the tissue is restricted to the bulging center

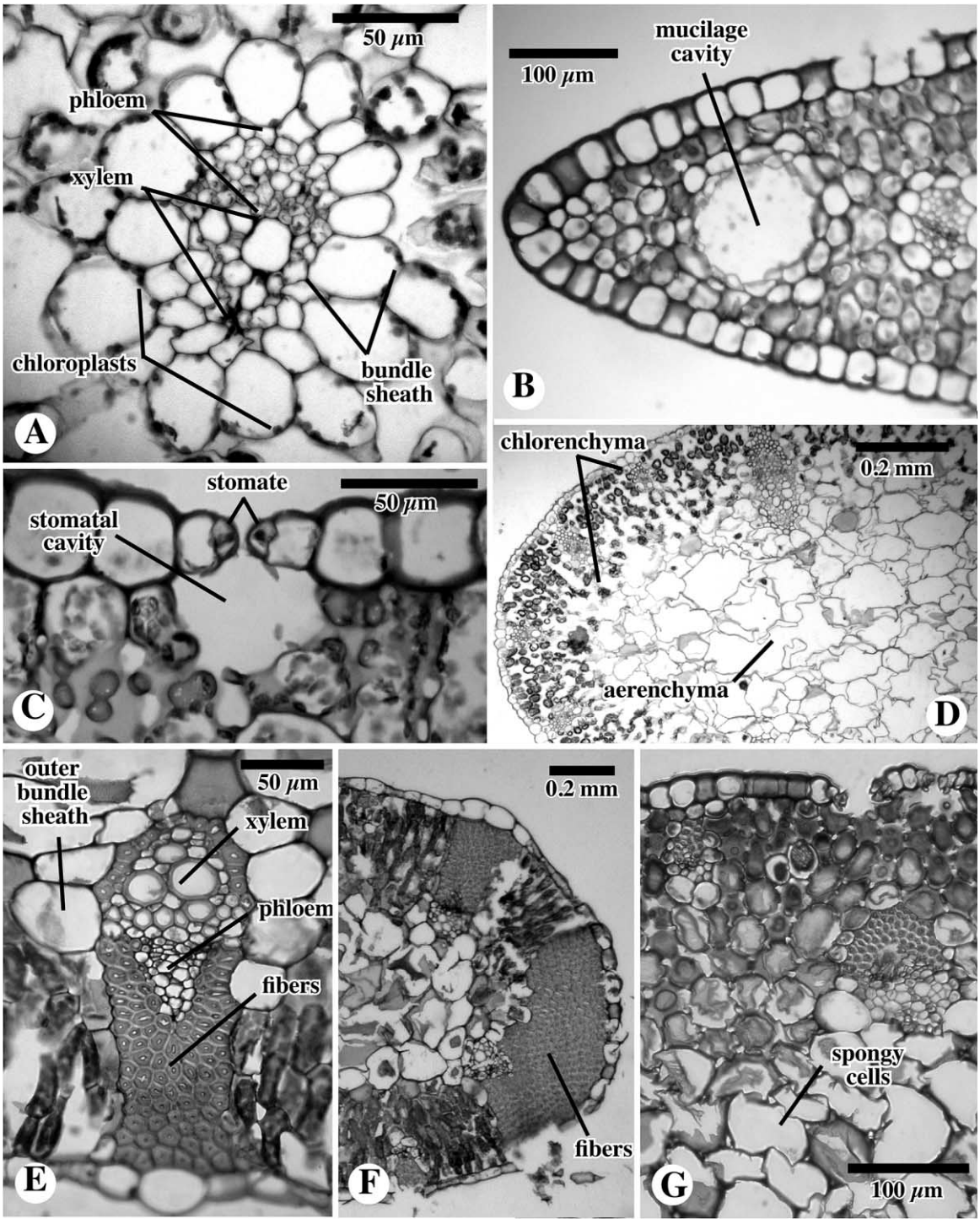

Fig. 8 Subfamily Haemodoroideae. Leaf cross sections. *A*, *Barberetta aurea*, showing a large vascular bundle; note the lack of sclerenchyma and the outer bundle sheath surrounding the bundle with chloroplasts. *B*, *C*, *Dilatris viscosa*. *B*, Section showing the margin; note the mucilage chamber. *C*, Close-up of the epidermal section with stomata. *D*, *Haemodorum simplex*. Leaf margin; note the achlorophyllous central region. *E*, *F*, *Haemodorum simulans*. *E*, Section with a large vascular bundle; note the numerous layers of sclerenchyma at the phloem end. *F*, Leaf margin with several layers of sclerenchyma; note the achlorophyllous central region. *G*, *Haemodorum spicatum*. Section showing the leaf edge extending toward the interior; note the achlorophyllous cells in the interior region.

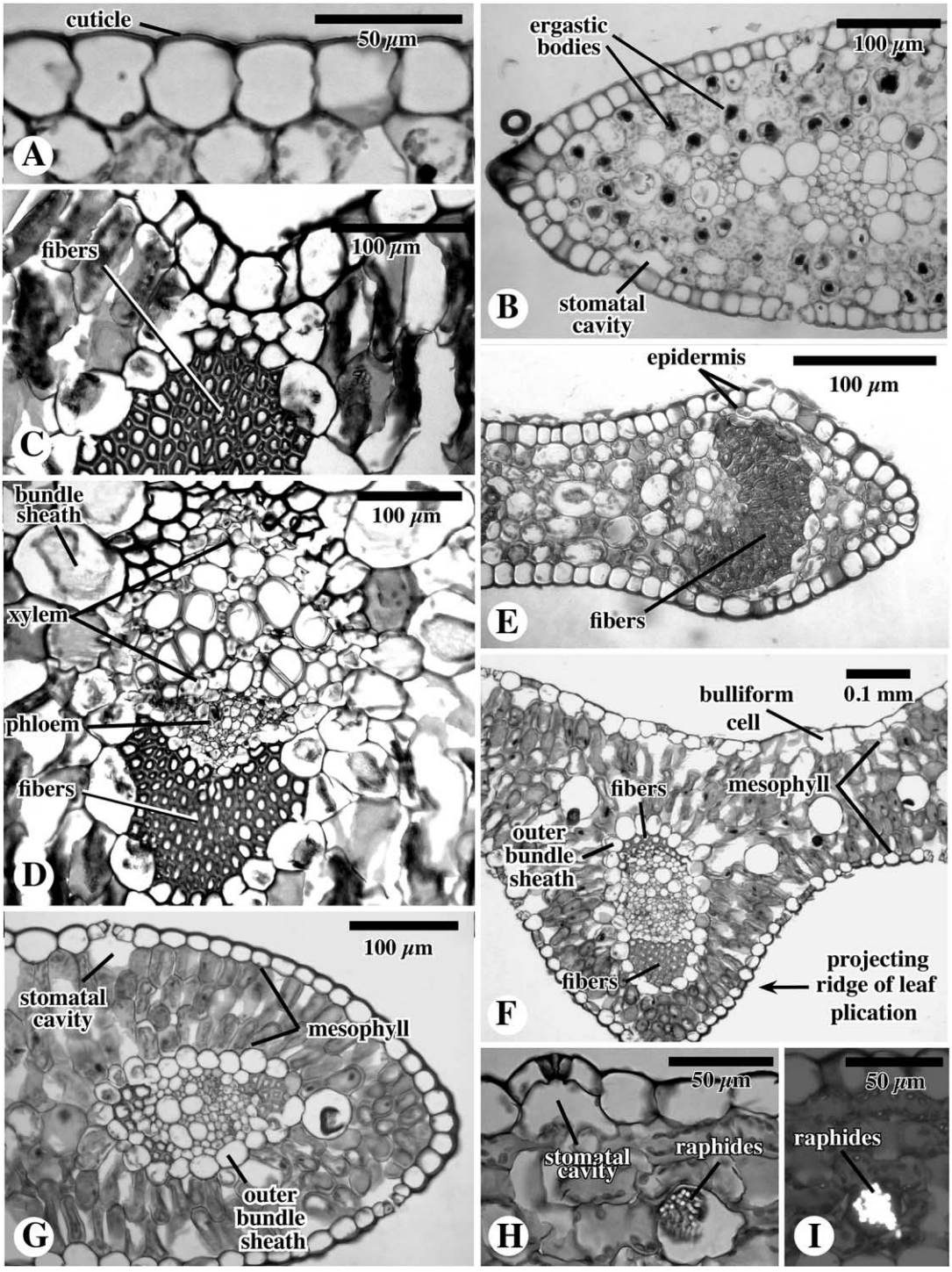

Fig. 9 Subfamily Haemodoroideae. Leaf cross sections. *A*, *B*, *Lachnanthes caroliniana*. *A*, Epidermis close-up, showing cuticle. *B*, Leaf margin; note the marginal vascular bundle and the thickened epidermal layer. *C*, *D*, *Pyrrorhiza neblinae*. *C*, Section showing invaginations where the vascular bundle makes contact with the epidermal layer. *D*, Section with a vascular bundle; note the large vessels of xylem and sclerenchyma cap at the phloem end of the bundle. *E*, *Schiekia orinocensis*. Leaf margin with an enlarged bundle. *F*, *G*, *Wachendorfia paniculata*. *F*, Section showing plication; note the bulliform cells opposite the ridge. *G*, Leaf margin; note the marginal vascular bundle and thickened epidermal layer. *H*, *I*, *Xiphidium caeruleum*. *H*, Close-up of the epidermal section with stomate; note the raphide crystals. *I*, Close-up of the raphide crystals under polarized light.

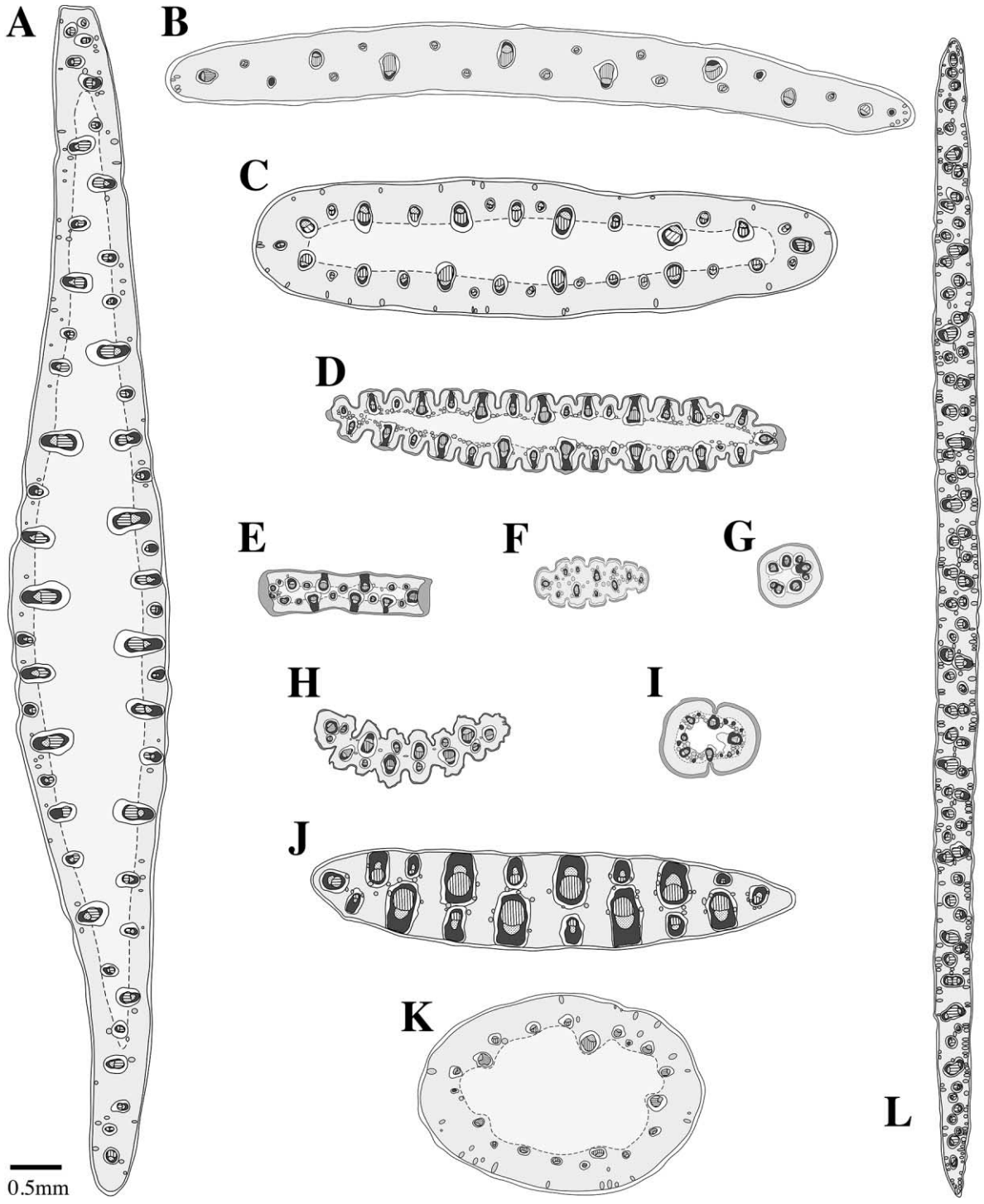

Fig. 10 Subfamily Conostylidoideae. Leaf cross sections and line outlines. *A*, *Anigozanthos flavidus*. *B*, *Anigozanthos humilis*. *C*, *Anigozanthos preissii*. *D*, *Blancoa canescens*. *E*, *Conostylis petrophiloides*. *F*, *Conostylis prolifera*. *G*, *Conostylis teretifolia*. *H*, *Conostylis tomentosa*. *I*, *Conostylis vaginata*. *J*, *Phlebocarya ciliata*. *K*, *Tribonanthes australis*. *L*, *Macropidia fuliginosa*. Shading definitions are the same as given in fig. 6.

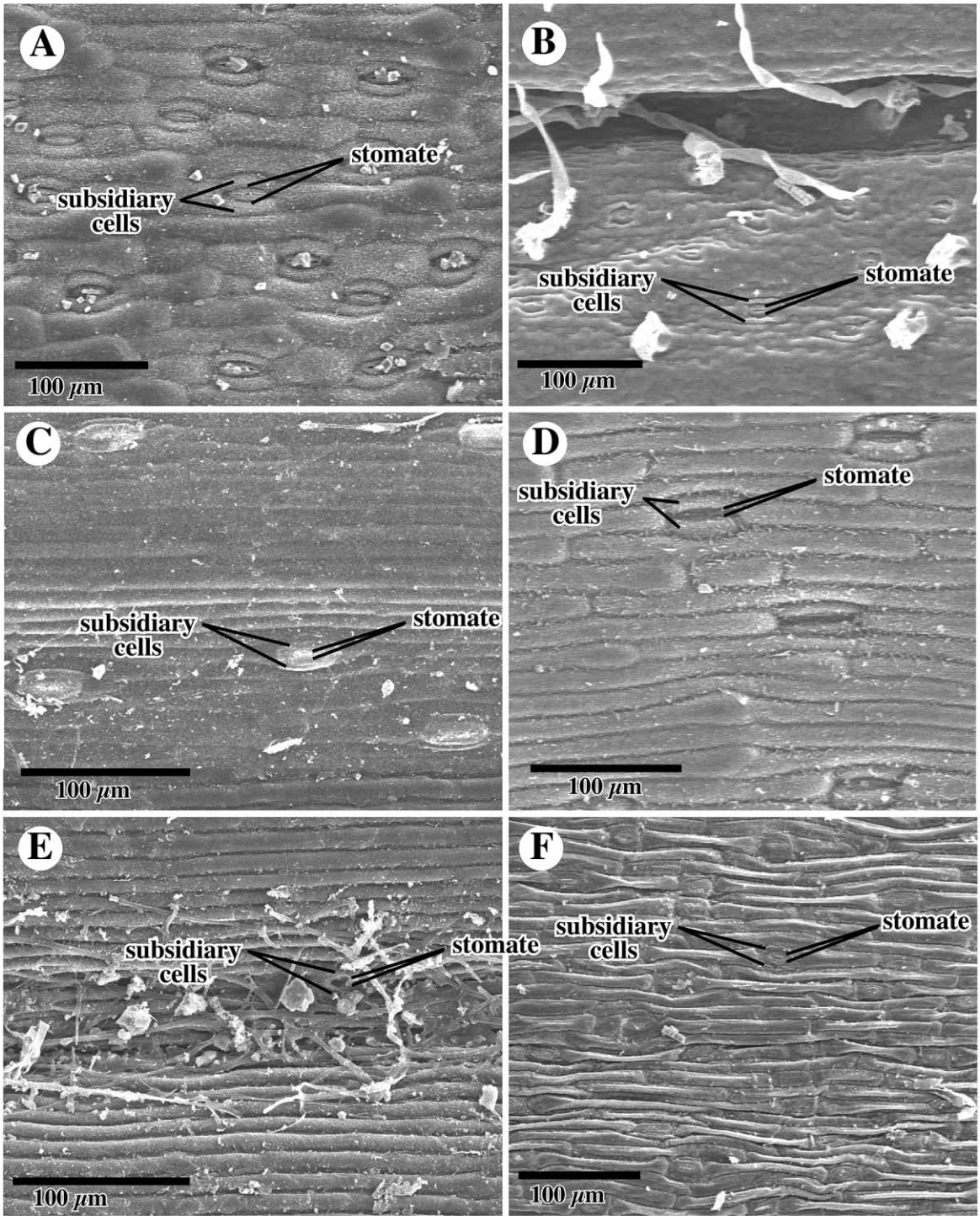

Fig. 11 Subfamily Conostylidoideae. Leaf surface, SEMs, and the longitudinal axis of the leaf from left to right. *A*, *Anigozanthos preissii*. *B*, *Blancoa canescens*. *C*, *Conostylis prolifera*. *D*, *Macropidia fuliginosa*. *E*, *Phlebocarya ciliata*. *F*, *Tribonanthes australis*. Note the paracytic subsidiary cells.

of the leaf. Parsimony optimization (fig. 21*A*) clearly shows that aerenchyma evolved independently in these genera; this is supported by their different anatomy.

Mucilage cells. Mucilage cavities are spherical to elongate bodies that appear to contain an amorphous substance. *Dilatris* is the only taxon in which large mucilage cells (fig. 8*B*) are present, an apomorphy for the genus (fig. 21*B*).

Relative epidermal wall uniformity (not plotted). Epidermal wall uniformity denotes whether the thickness of the walls of the epidermal cells is more or less consistent along the radial

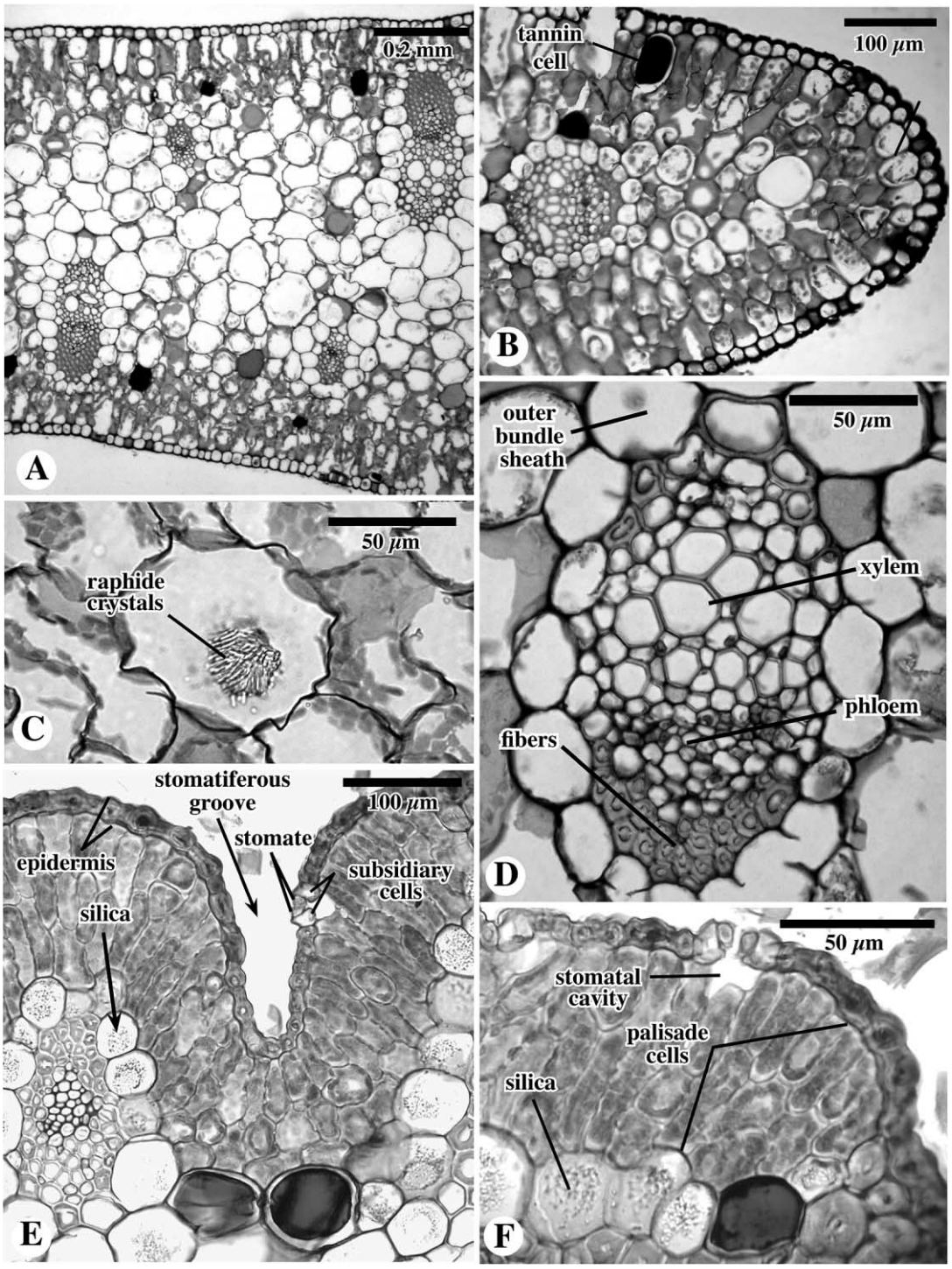

Fig. 12 Subfamily Conostylidoideae. Leaf cross sections. *A*, *B*, *Anigozanthos flavidus*. *A*, Expanded leaf cross section showing vascular bundle orientation; note the achlorophyllous tissue in the center. *B*, Leaf margin; note the vascular bundle orientation. *C*, *Anigozanthos humilis*. Section with raphide crystals. *D*, *Anigozanthos preissii*. Section showing vascular bundle; note the sclerenchyma at the phloem end. *E*, *F*, *Blancoa canescens*. *E*, Section with deep invagination; note the thick epidermal cells and the location of the stomata within the stomatiferous groove. *F*, Stomata with a small stomatal cavity; note the two or three layers of palisade cells.

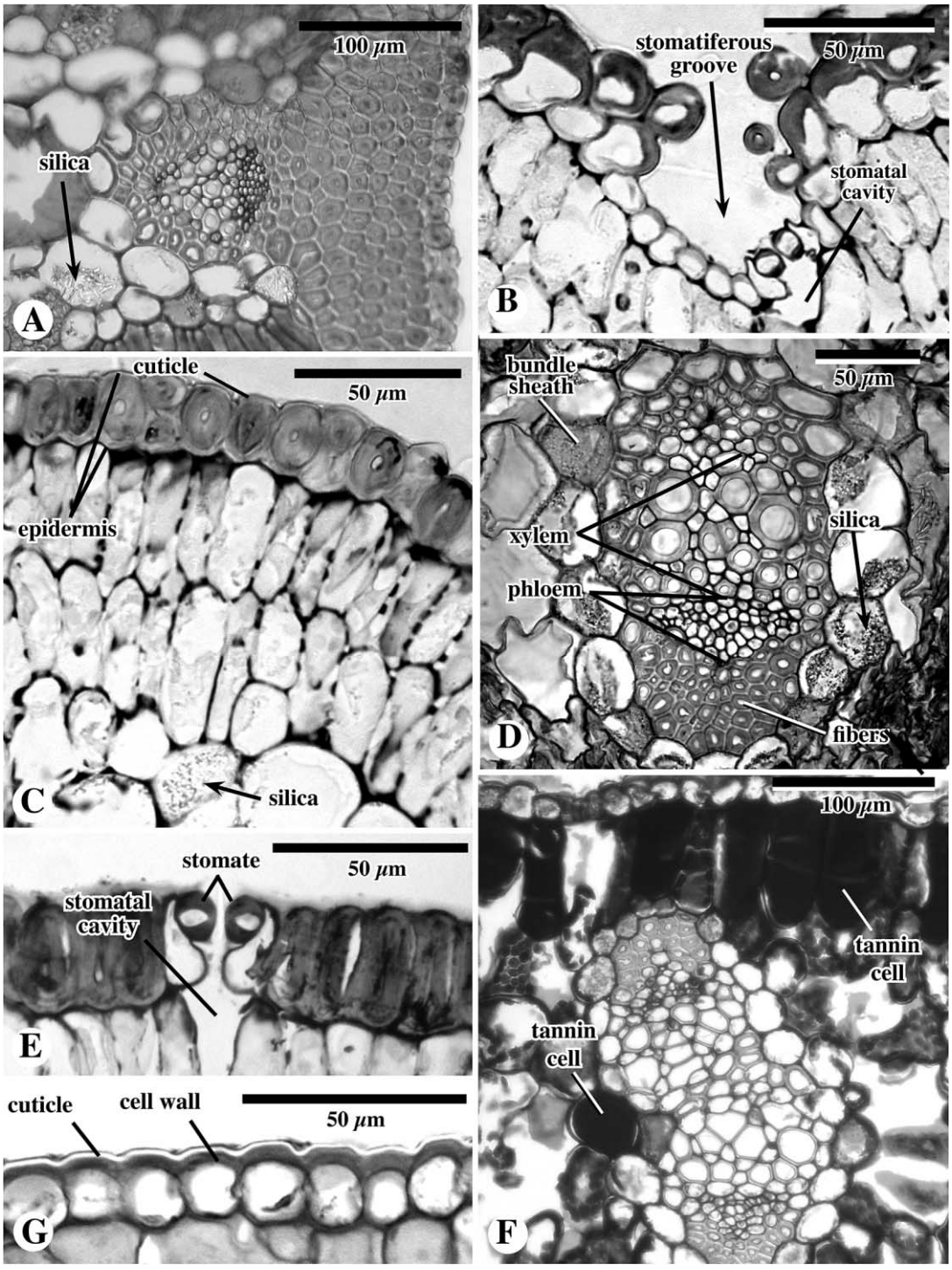

Fig. 13 Subfamily Conostylidoideae. Leaf cross sections. *A*, *Conostylis petrophiloides*. Close-up of the leaf margin; note the numerous layers of sclerenchyma and the bundle orientation. *B*, *Conostylis prolifera*. Section with a stomatiferous groove and stomata with a small cavity; note the uniseriate layer of the epidermis within the stomatiferous groove. *C*, *Conostylis teretifolia*. Close-up of the outer region showing epidermal cells with thick walls and an outer cuticle; note the two layers of palisade cells. *D*, *Conostylis tomentosa*. Section with a vascular bundle; note the vascular tissue surrounded by sclerenchyma and the bundle sheath cells with silica deposits. *E*, *Conostylis vaginata*. Close-up of the epidermal section with stomata and the stomatal cavity. *F*, *Macropidia fuliginosa*. Section showing two confluent vascular bundles; note the tannin cells. *G*, *Phlebocarya ciliata*. Epidermis close-up, showing the cuticle and the thick outer tangential cell wall.

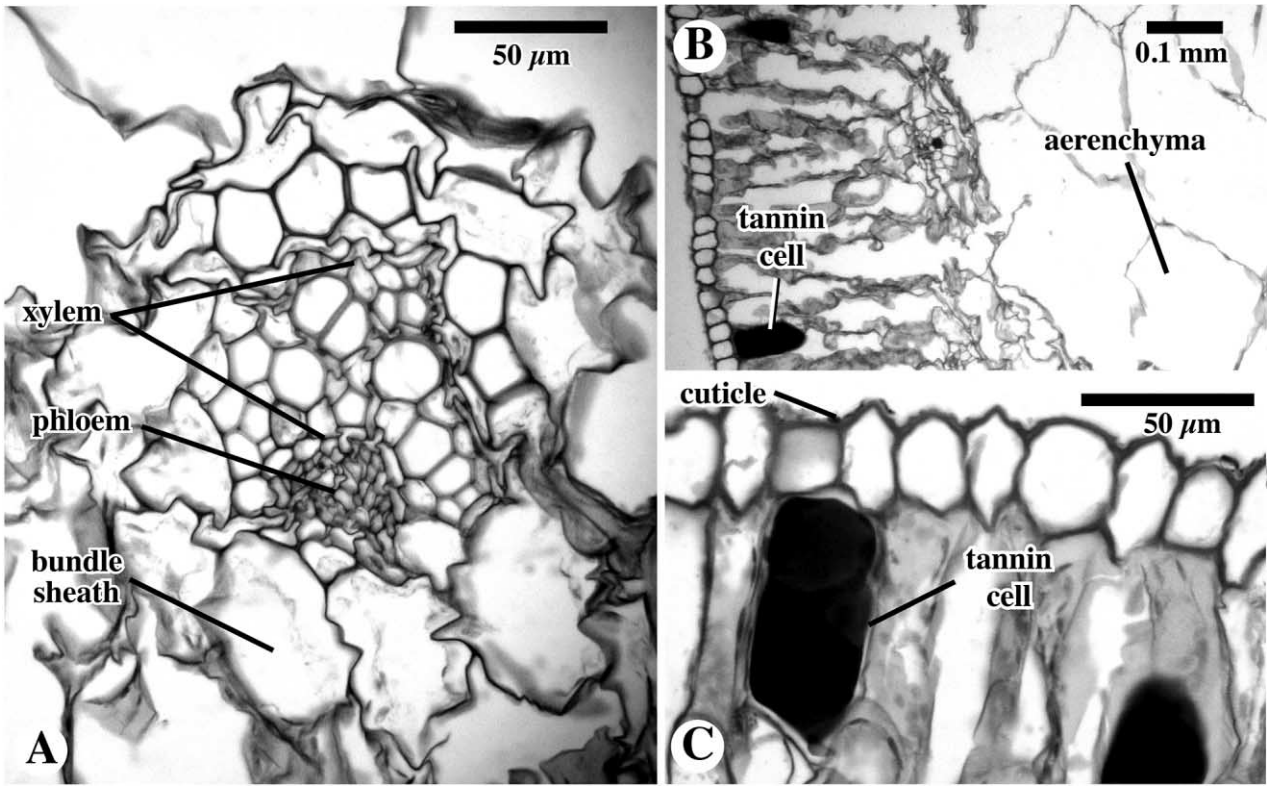

Fig. 14 Subfamily Conostylidoideae. Leaf cross sections. *A*–*C*, *Tribonanthes australis*. *A*, Section with a vascular bundle; note the lack of sclerenchyma and the irregularly shaped bundle sheath cells. *B*, Section showing the palisade region and the aerenchymous leaf center. *C*, Epidermal region, showing the epidermal cells and the presence of tannin cells.

and tangential wall regions. Nonuniform epidermal cells are found in one species in the Haemodoroideae, *Haemodorum laxum*, while the rest have uniform cell walls. In the Conostylidoideae, *Macropidia*, one species of *Anigozanthos*, several species of *Conostylis*, and one species of *Phlebocarya* have nonuniform epidermal cell walls, showing no clear phylogenetic trends. Only two species of *Conostylis*—*Conostylis misera* and *C. pauciflora*—do not have uniform epidermal cell walls (table 2).

Epidermal cell layer number (not plotted). A single epidermal cell layer is the most common condition in the Haemodoraceae and is found in all members of the Haemodoroideae. The number of epidermal cell layers varies only in the genus *Conostylis*. Within *Conostylis*, some species have two or more epidermal layers around the entire perimeter of the leaf, while other species have two layers present only at the leaf margins (table 2).

Epidermal surface shape (not plotted). Most of the taxa in the study have a relatively planar epidermal surface, and these are coded as flat. Other taxa have slight depressions that deviate from planar, and these are coded as undulate. Finally, taxa coded as invaginate have very pronounced, uniform, and discrete invaginations in the leaf surface, which is the result of longitudinal grooves. Epidermal surface deviations from flat are found only in a few members of the Haemodoraceae. Within the Haemodoroideae, three taxa—*Dilatris ixioides* (not shown), *H. spicatum*, and *P. neblinae*—have undulate or grooved/invaginate leaves (fig. 6*E*, 6*G*), with these constituting independent evolutionary events. Within the Conostylidoideae, it appears that a leaf epidermal surface with longitudinal grooves or epidermal invaginations may constitute an evolutionary event that unites *Blancoa* and *Conostylis*, as the former and some members of the latter are the only members of the subfamily with that feature (fig. 10*D*, 10*F*, 10*H*, 10*I*; table 2).

Vascular bundle contact with epidermis (not plotted). In some species, the vascular bundle or bundle fibers are in direct contact with the epidermal wall, with no palisade tissue between the two structures. In the Haemodoroideae, all members of *Haemodorum* examined (e.g., fig. 8*E*) show vascular bundle contact with the epidermis. *Schiekia* was the only other genus in the subfamily to consistently display this character, although *L. caroliniana*, *P. neblinae*, and *X. caeruleum* are polymorphic, having some bundles that make contact. In the Conostylidoideae, vascular bundle contact with the epidermis occurs in *B. canescens* (fig. 15*D*) and *P. ciliata* and is polymorphic for other taxa (e.g., *C. petrophiloides* [fig. 13*A*] and *M. fuliginosa* [fig. 15*F*]; table 2).

Silica body presence (not plotted). We found silica bodies to be present only in members of the Conostylidoideae, an apparent apomorphy for the subfamily; however, not all members of this subfamily were observed to possess them (table 2).

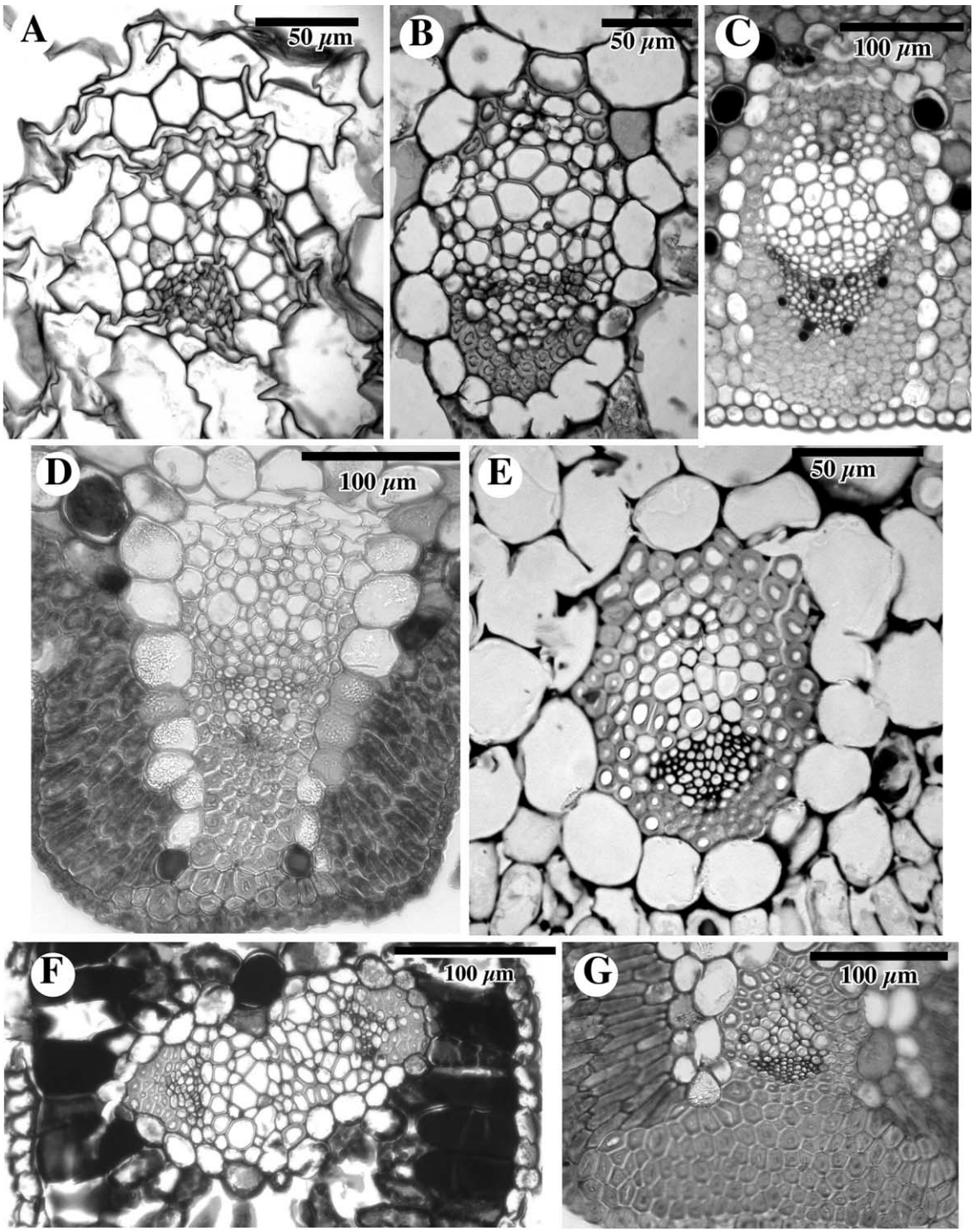

Fig. 15 Vascular bundles of subfamily Conostylidoideae (xylem poles uppermost). *A*, *Tribonanthes australis*; note the lack of sclerenchyma and the irregularly shaped bundle sheath cells. *B*, *Anigozanthos preissii*, with sclerenchyma at the phloem end. *C*, *Phlebocarya ciliata*, with vascular tissue surrounded by sclerenchyma and clear bundle sheath cells. *D*, *Blancoa canescens*, with a vascular bundle between the stomatiferous grooves; note the vascular tissue surrounded by sclerenchyma. *E*, *Conostylis teretifolia*, with vascular tissue surrounded by sclerenchyma and clear bundle sheath cells. *F*, *Macropidia fuliginosa*, with two confluent vascular bundles. *G*, *Conostylis petrophiloides*, with a marginal vascular bundle with numerous layers of sclerenchyma and a bundle orientation perpendicular to the rest of the bundles in leaf section.

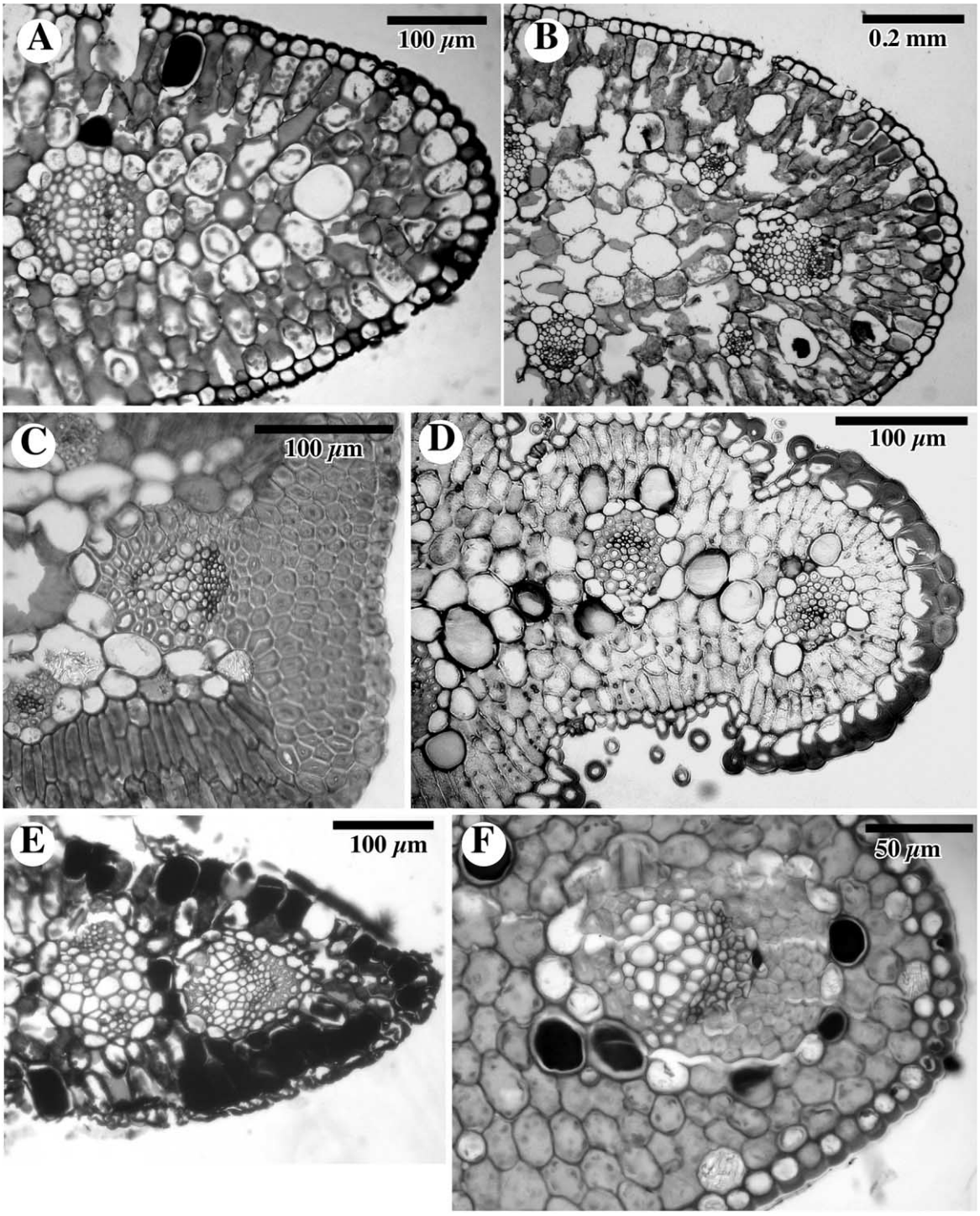

Fig. 16 Leaf margins of subfamily Conostylidoideae. *A*, *Anigozanthos flavidus*; note the vascular bundle orientation. *B*, *Anigozanthos preissii*; note the vascular bundle orientation. *C*, *Conostylis petrophiloides*; note the numerous layers of sclerenchyma and the bundle orientation. *D*, *Conostylis prolifera*, with a two-layered epidermis and a vascular bundle; note the broad, shallow stomatiferous groove near the margin. *E*, *Macropidia fuliginosa*; note the orientation of the marginal vascular bundle and numerous tannins. *F*, *Phlebocarya ciliata*, with a marginal vascular bundle; note the tannin cells.

Discussion

Root Anatomy

Root anatomy (figs. 1–3) in the Haemodoraceae is fairly uniform, with the exception of a few taxa. A few root features distinguish the two subfamilies, and a few features characterize specific members of each subfamily (table 1).

Within the Haemodoroideae, all examined taxa have a uniseriate epidermis with thin cell walls (see table 1). The cortical cells are generally precisely radially aligned, consisting of four (*Xiphidium caeruleum*), eight (*Schiekia* and *Wachendorfia*), or 10–12 (all other taxa) layers of cells. Prominent intercellular spaces are usually present, with a well-developed aerenchyma in *Haemodorum*, *Lachnanthes*, and *Pyrrorhiza*. The inner cortical cells are tangentially oriented and rectangular to oblong, with a length : width ratio (in cross section) of 1.5–2(3) : 1 (table 1). The endodermal cells are roughly isodiametric, with no evident orientation in cross-sectional view. The endodermal cell walls are unevenly thickened, being thicker in the radial and inner tangential planes, in *Barberetta*, *Dilatris*, *Pyrrorhiza*, *Schiekia*, *Wachendorfia*, and *Xiphidium*. In contrast, *Haemodorum* and *Lachnanthes* are distinctive in having uniformly thickened endodermal cell walls (table 1). This may constitute a derived feature for these two taxa. However, developmental stages were not observed; the differences between the two groups could possibly be a factor of root age, as endodermal cell walls commonly become more lignified with time. The number of xylem poles varies from five to 13.

Within the Conostylidoideae, all examined taxa also have a uniseriate epidermis with thin cell walls (see table 1). The cortical cells are generally not radially aligned (radially aligned near the endodermis only in *Anigozanthos flavidus*) and consist of from 10 to 20 layers of cells. Prominent intercellular spaces are usually present but with no aerenchyma. The inner cortical cells are tangentially oriented and rectangular to oblong (some being isodiametric in *Tribonanthes*), with a length : width ratio (in cross section) of 2–5 : 1 (table 1). The endodermal cells are mostly rectangular and radially oriented, being isodiametric with no relative orientation in *Anigozanthos rufus*, *Conostylis setigera* subsp. *dasys*, and *Tribonanthes australis*. Endodermal cells are either thin or thick walled, with the outer tangential wall occasionally thin in *A. rufus* and *C. setigera* subsp. *dasys*. The number of xylem poles varies from two in *Tribonanthes* to eight to 30 in all other taxa.

In conclusion, root anatomy shows anatomical variation in the Haemodoraceae mainly with respect to the cortical cell orientation and endodermal cell cross-sectional shape, orientation, and relative cell wall thickness. All members of the Haemodoroideae have radially aligned cortical cells, and all have isodiametric endodermal cells, most with the cell wall unequally thickened and the outer tangential wall relatively

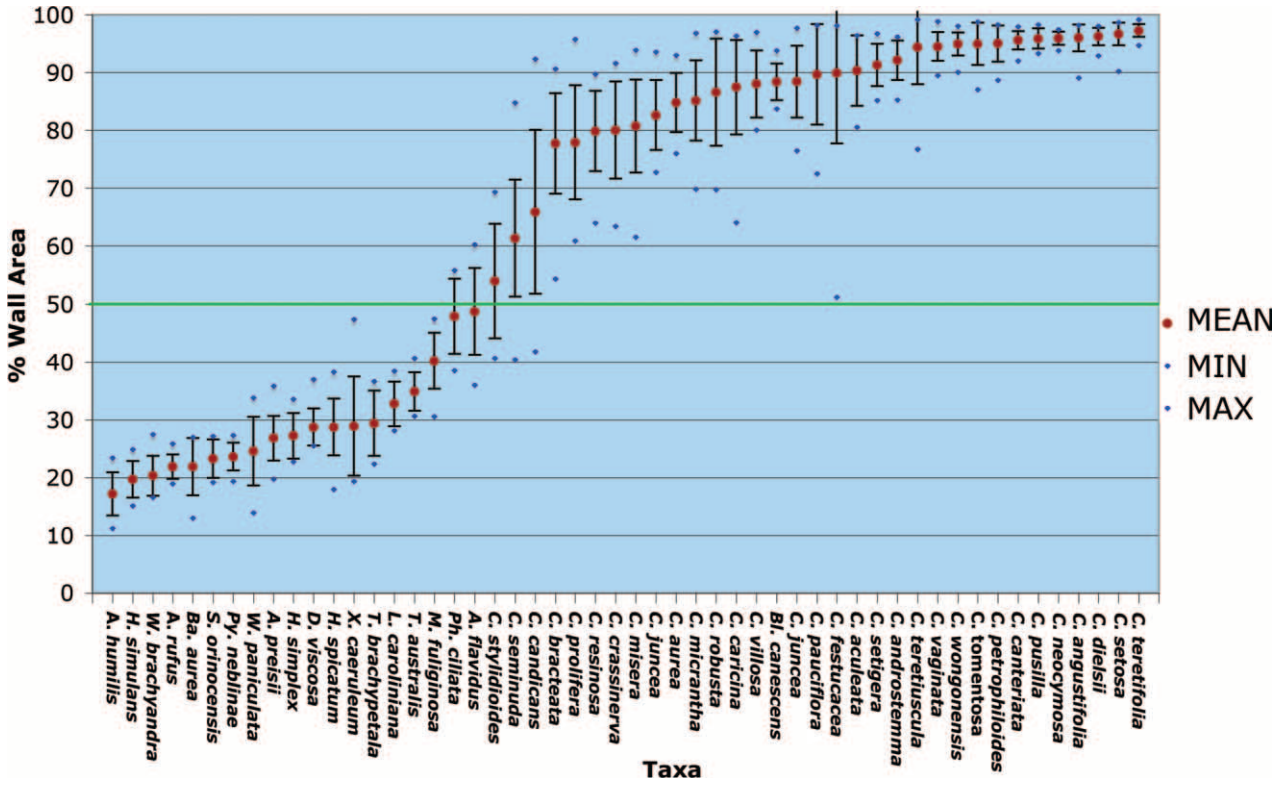

Fig. 17 Graph of the epidermal wall relative area. Bars above and below the mean = 1 SD. *Blancoa* and all *Conostylis* have an epidermal wall area greater than 50%. All other taxa have less than 50%. *A.* = *Anigozanthos*; *Ba.* = *Barberetta*; *Bl.* = *Blancoa*; *C.* = *Conostylis*; *D.* = *Dilatris*; *H.* = *Haemodorum*; *L.* = *Lachnanthes*; *M.* = *Macropidia*; *Ph.* = *Phlebocarya*; *Py.* = *Pyrrorhiza*; *S.* = *Schiekia*; *T.* = *Tribonanthes*; *W.* = *Wachendorfia*; *X.* = *Xiphidium*.

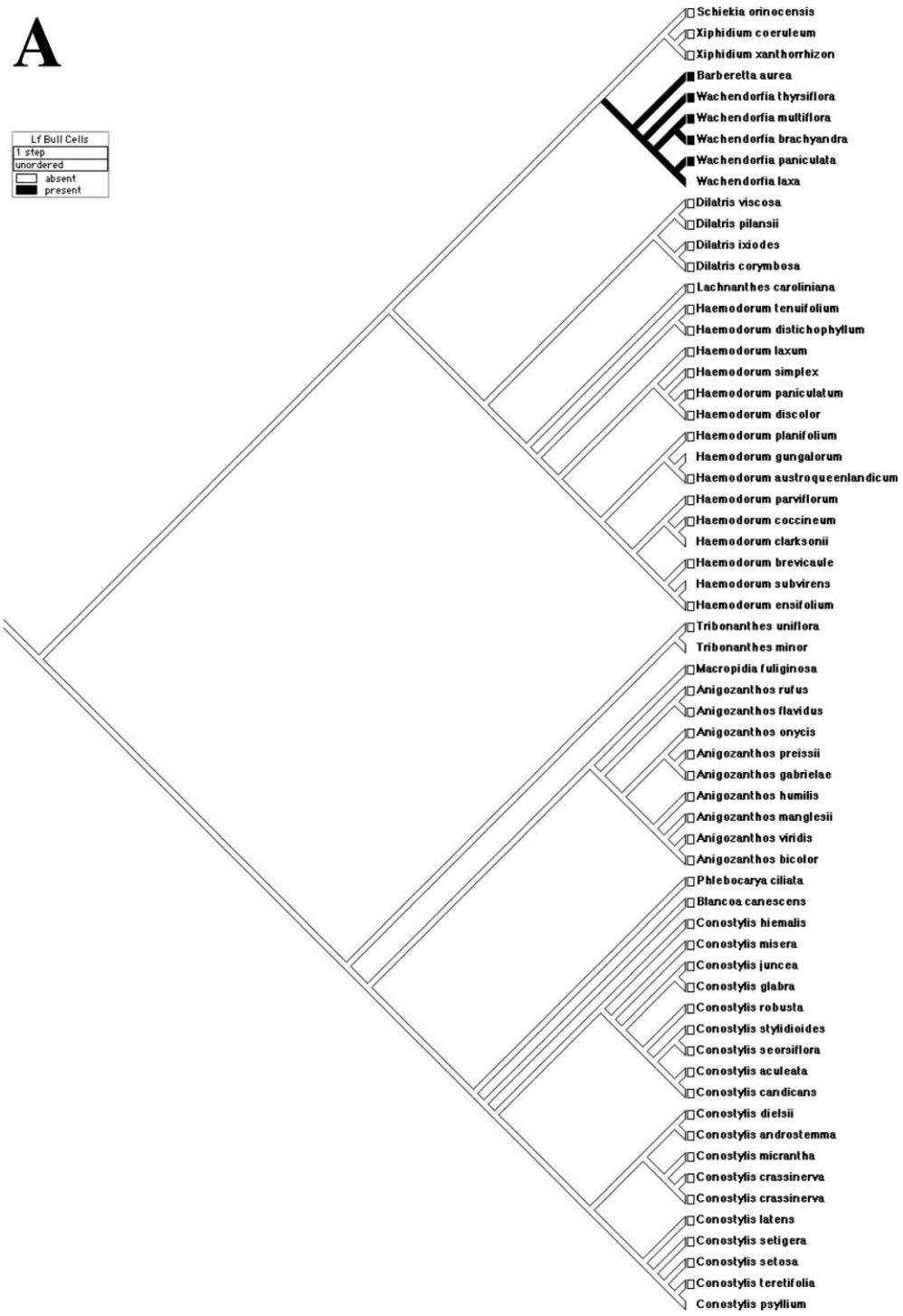

Fig. 18 Evolution of leaf anatomical characters, traced on a phylogeny of the Haemodoraceae (after Hopper et al. 2009). *A*, Bulliform cells. Open = absent; filled = present. The presence of bulliform cells is an apomorphy for *Barberetta* and *Wachendorfia*. *B*, Tannin cells. Open = absent; filled = present. The presence of leaf tannin cells unites members of the subfamily Conostylidoideae.

thin. In contrast, all but one member of the Conostylidoideae have cortical cells that are not radially aligned and have rectangular, radially oriented endodermal cells, with the cell wall uniformly thickened.

Scape Anatomy

The anatomy of the aerial scape of family members (figs. 4, 5) showed the least variation. Several species in both the

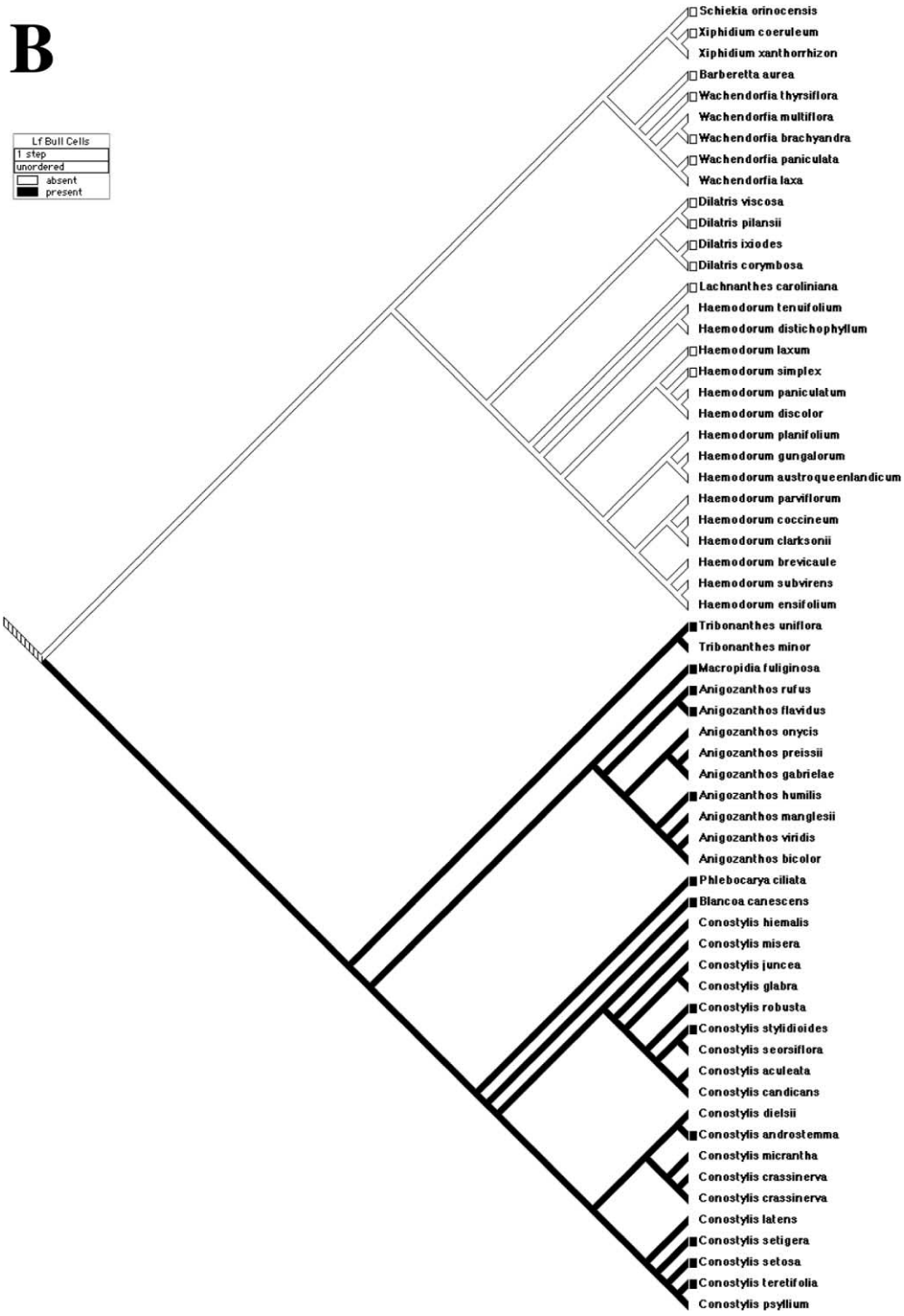

Fig. 18 (*Continued*)

Haemodoroideae and Conostylidoideae contained an outer cortex distinct from the inner parenchyma cells. Most of these have a sclerenchyma cylinder separating the inner and outer regions. This is similar to the findings of Shulze (1893), who described a "*mechanische* ring."

Leaf Anatomy

Leaf anatomy (figs. 6–17) shows the greatest amount of variation among the three major vegetative organs. Fifteen discrete anatomical characters were established (table 2), some of

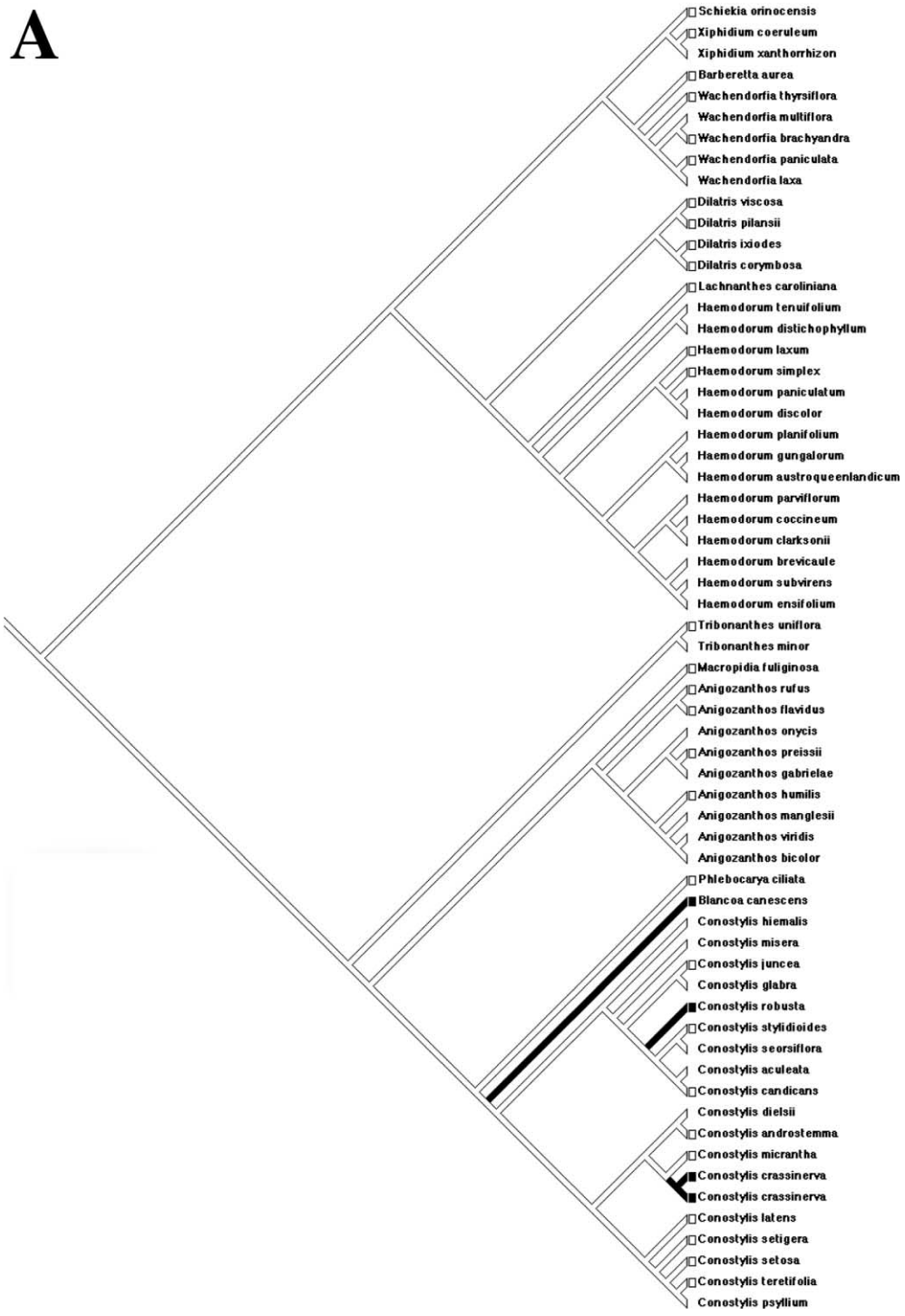

Fig. 19 Evolution of leaf anatomical characters, traced on a phylogeny of the Haemodoraceae (after Hopper et al. 2009). *A*, Marginal fiber caps. Open = absent; filled = present. *Blancoa* and select members of *Conostylis* are the only taxa to contain marginal fiber caps. *B*, Epidermal wall thickness. Open = absent; filled = present. *Blancoa* and *Conostylis* are united as a result of thick epidermal cell walls.

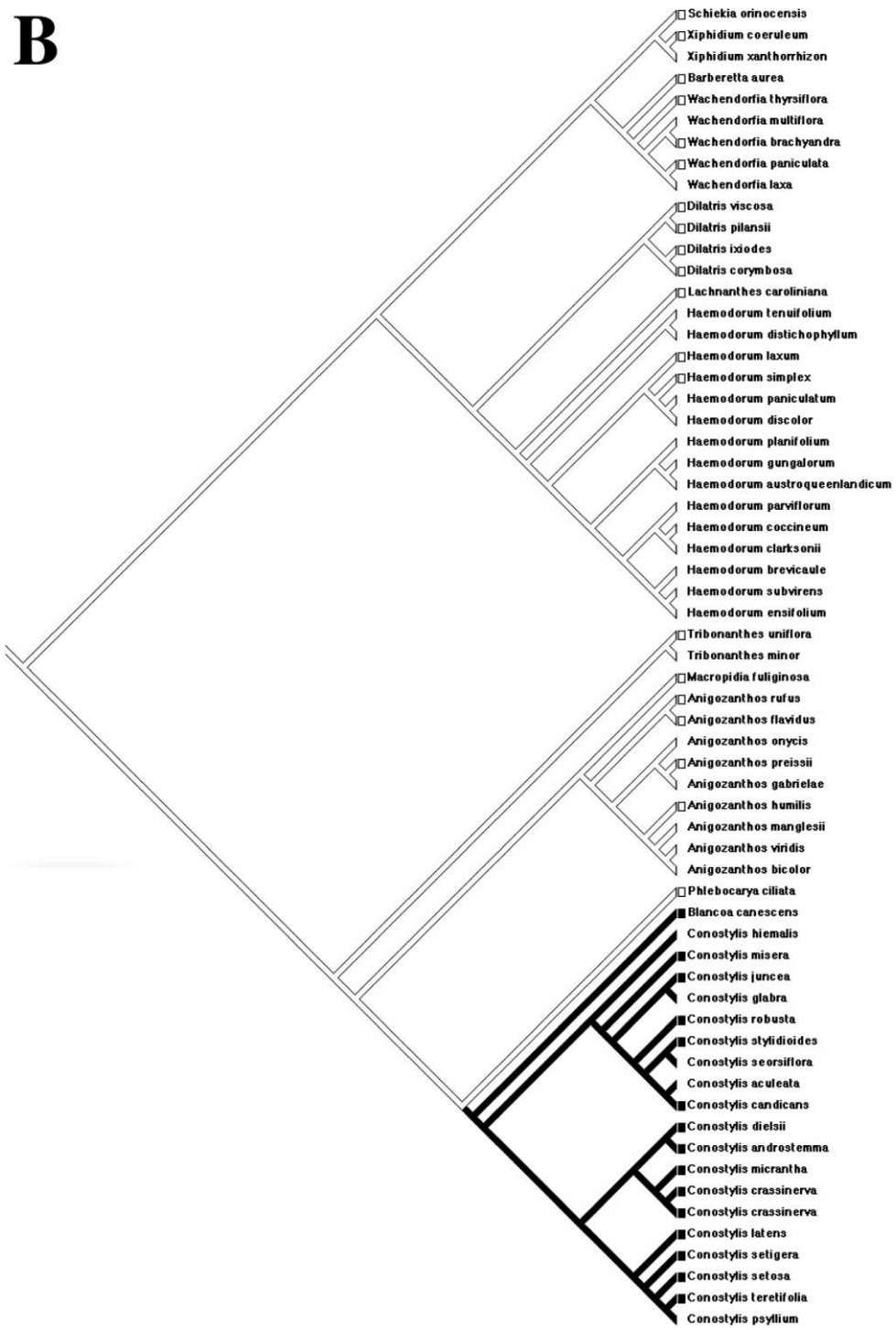

Fig. 19 (*Continued*)

which are evaluated phylogenetically (see "Systematic Correlations"). The following briefly explains each character and its associated states.

Bulliform cells are enlarged epidermal cells with thin anticlinal walls (Esau 1977). *Barberetta* (fig. 6*A*) and *Wachendorfia* (figs. 6*I*, 9*F*) are the only two taxa to have bulliform cells present. The fact that these bulliform cells are correlated with the plicate leaves of these two genera may indicate that they function in leaf development or as a means of altering leaf conformation with changes in water availability. This anatomical feature corroborates previous studies (Simpson 1990; Hopper et al. 2009) that place these two as sister taxa, with

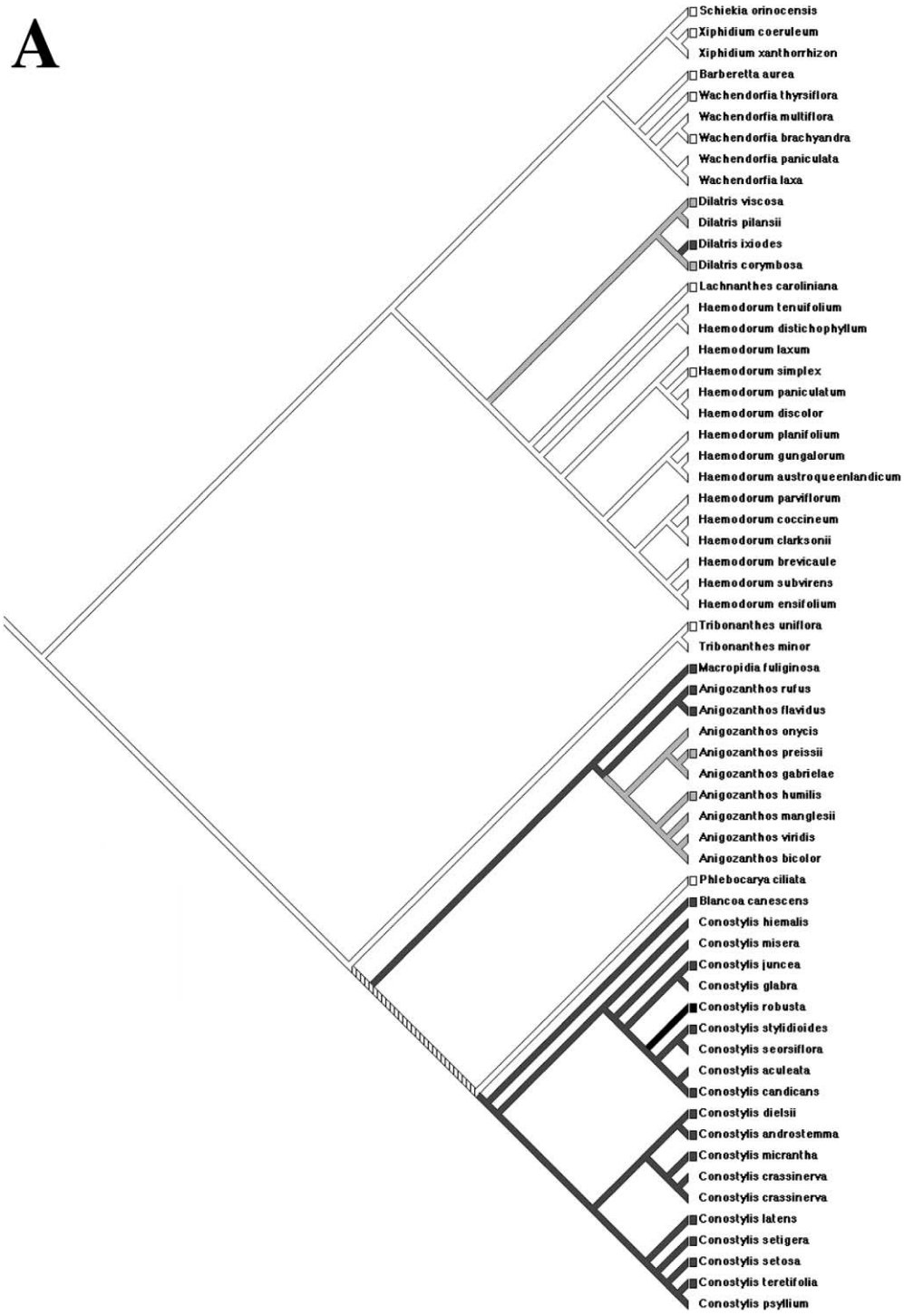

Fig. 20 Evolution of leaf anatomical characters, traced on a phylogeny of the Haemodoraceae (after Hopper et al. 2009). *A*, Palisade cell morphology. Open = absent; light gray shading = one layer; dark gray shading = two layers; filled = two or more layers. Most taxa in Conostylidoideae have two layers of palisade cells. *Tribonanthes* and *Phlebocarya* lack typical palisade cell layers. In the Haemodoroideae, variation is seen only in *Dilatris*. *B*, Vascular bundle fibers. Open = absent; gray shading = partially enclosing bundles; filled = completely enclosing bundles. Most species of *Conostylis* have fibers completely enclosing the vascular bundle.

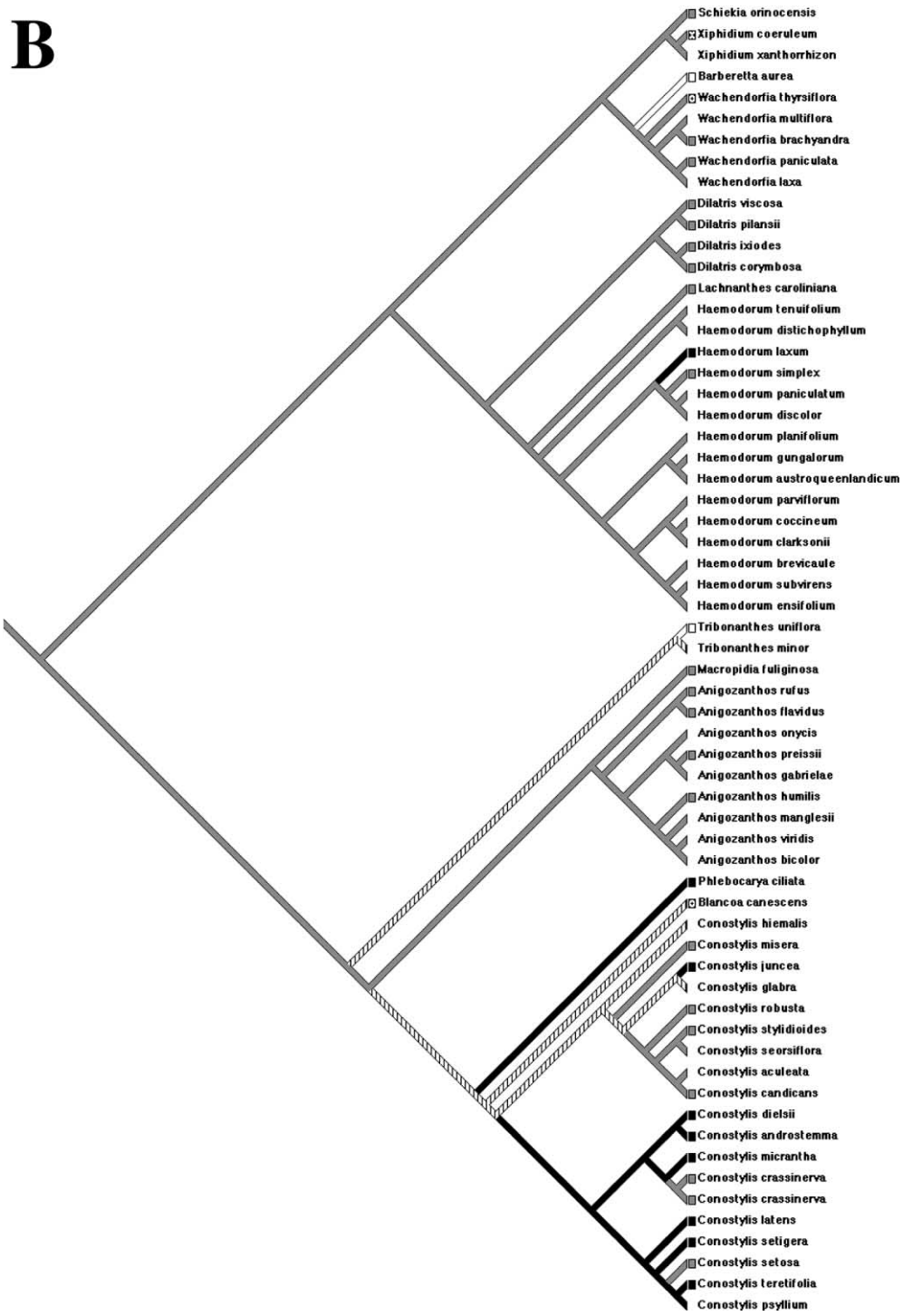

Fig. 20 (*Continued*)

the correlated presence of plicate leaves being a likely apomorphy for the clade (Simpson 1990).

Fistulose leaves are those that have a hollow pith region. Taxa of the Haemodoraceae having fistulose leaves include all species of *Tribonanthes* (fig. 10*K*) and one species of *Conostylis*, *Conostylis vaginata* (fig. 10*I*). All three species of *Tribonanthes* observed in this study are terete in shape and have an aerenchymous leaf center (fig. 14*B*). This is the only genus observed having a combination of fistulose leaves and an aerenchymous center. Only one other species, *Lachnanthes caroliniana* of the Haemodoroideae, contains aerenchyma. *Lachnanthes caroliniana* is linear in leaf cross-sectional shape,

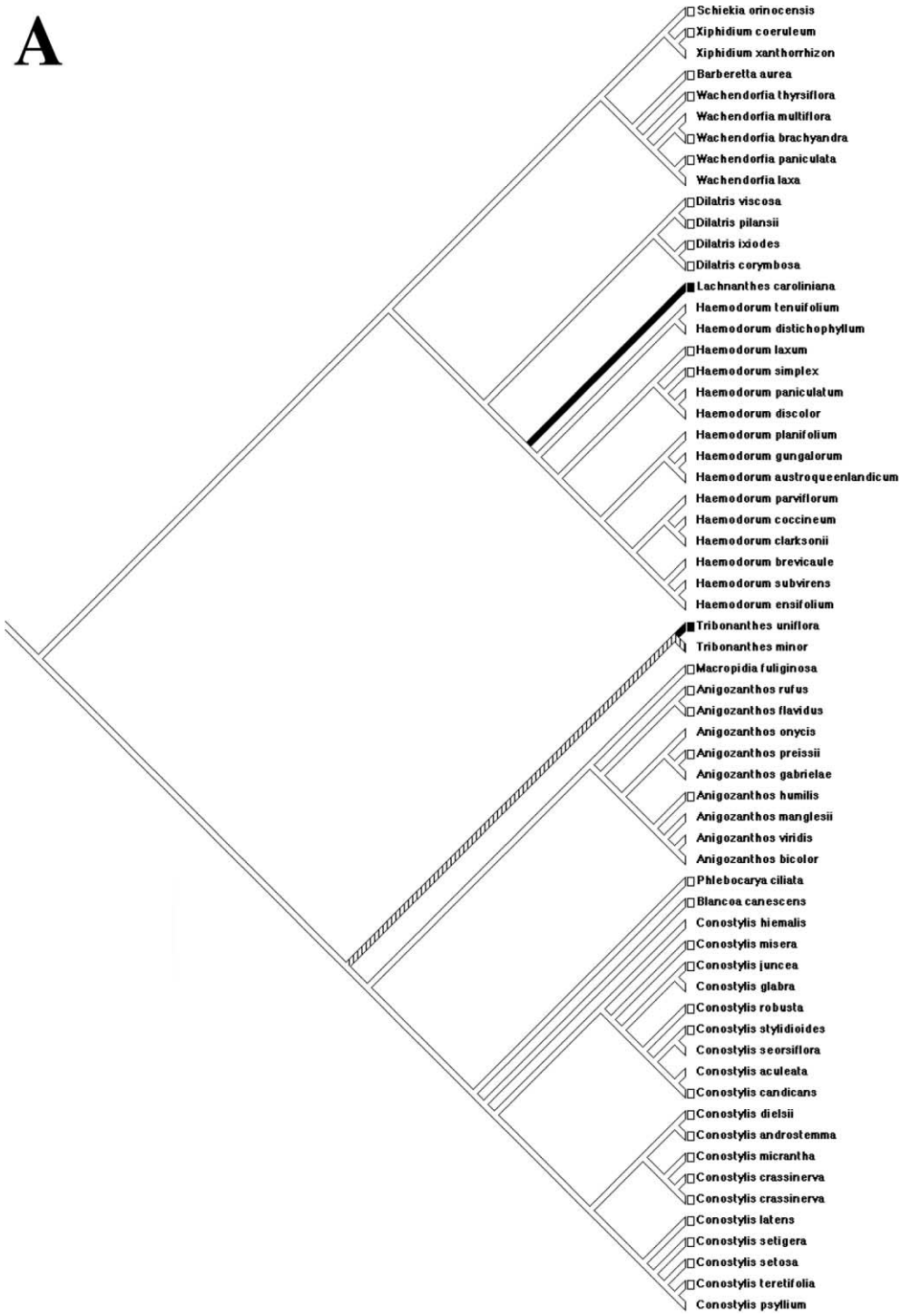

Fig. 21 Evolution of leaf anatomical characters, traced on a phylogeny of the Haemodoraceae (after Hopper et al. 2009). *A*, Aerenchyma. Open = absent; filled = present. *Lachnanthes* and investigated members of *Tribonanthes* possess aerenchyma. *B*, Mucilage cells. Open = absent; filled = present. *Dilatris* is the only genus possessing mucilage cells.

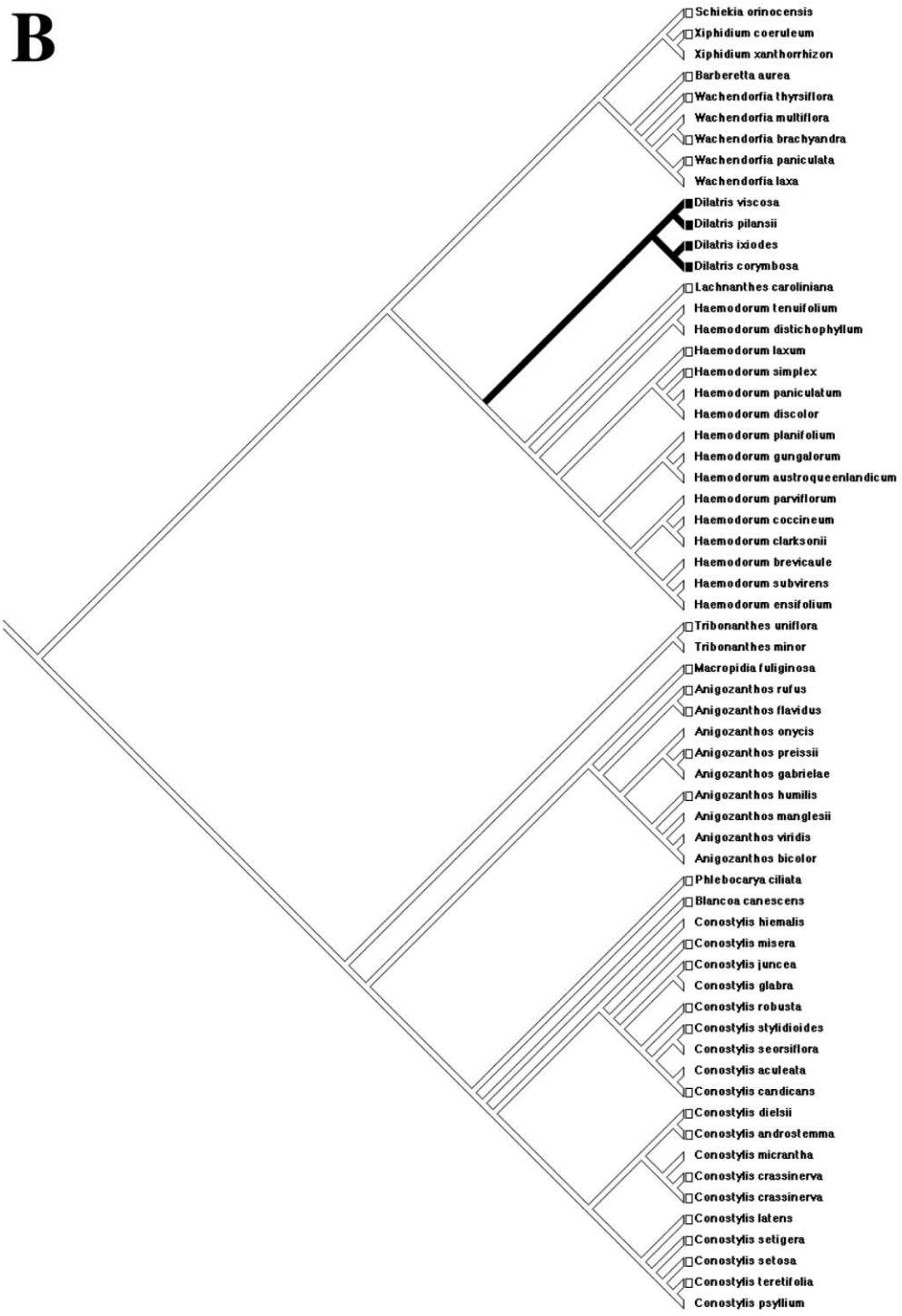

Fig. 21 (*Continued*)

with a swollen leaf center where the aerenchymous tissue is present (fig. 6*F*; see Simpson and Dickison 1981).

Tannin is a common ergastic substance in parenchyma cells. The tannin compounds in tanniniferous cells are oxidized to a brown or reddish-brown color (Esau 1977). Tannin cells in the leaves and flowers are found only in the Conostylidoideae (figs. 13*F*, 14*C*, 16*E*, 16*F*), although these were not observed in all members of this subfamily.

What are described as mucilage cells are cells containing an amorphous mucilage-like deposit. Of all the taxa observed, only the leaves of *Dilatris* have mucilage cells (fig. 8*B*). These cells were observed in three of the four examined species, *Di-*

latris pillansii being uncertain with regard to their presence. In a study by Helme and Linder (1992), the mucilage cell (canal) was detected in *D. pillansii*.

An important character discovered in our study is the presence of thick epidermal cells. Two genera, *Conostylis* and *Blancoa*, are united by both having thick epidermal cells. The amount of variation in epidermal wall thickness is evident in the graph in figure 17. *Blancoa* and most of the members of *Conostylis* have over 80% relative epidermal wall relative area (figs. 12*E*, 12*F*, 13*C*, 13*E*). In table 2, cells with epidermal wall relative area less than 50% are categorized as thin walled and those with over 50% are categorized as thick walled.

Another character is relative epidermal wall uniformity. The majority of taxa in the Haemodoraceae have epidermal cell walls that are uniformly thick around the perimeter (i.e., in both radial and tangential wall regions). However, a few taxa (table 2) have a thickened outer tangential wall (e.g., figs. 12*B*, 13*G*, 16*F*) and are categorized as not uniform.

Epidermal cell layer number is a measure of the number of layers of epidermal cells present. There is variation in the epidermal cell layer number within the Conostylidoideae. All members of the Haemodoroideae have the typical structure, with one layer of epidermal cells around the perimeter of the leaf (figs. 8*B*, 9*B*, 9*G*). The majority of the Conostylidoideae also have one layer of epidermal cells (e.g., figs. 12*A*, 12*B*, 13*G*, 14*C*). However, some members of the genus *Conostylis* have two or more layers of epidermal cells around the leaf perimeter (figs. 13*B*, 16*D*; table 2). Other members of *Conostylis* were observed with two layers of epidermal cells only at the leaf margins. These are coded as "2 at margin" in table 2 to distinguish from those that have two or more layers around the entire perimeter.

Epidermal surface shape can vary from flat to undulate to invaginate. An invaginate epidermal surface is present in a few Haemodoroideae but is much more common in *Blancoa* and species of *Conostylis* in the Conostylidoideae (fig. 10*D*, 10*F*, 10*H*, 10*I*). Stomata are typically common within the groove of this invagination, termed a stomatiferous groove (Esau 1977), but in at least one taxon (*C. vaginata*), they are absent.

Marginal fibers are a conglomeration of sclerenchyma concentrated at the leaf margins. Marginal fibers are completely absent in the Haemodoroideae. The sclerenchyma concentrated at the margin of the leaf is found only in *Blancoa* and numerous members of the genus *Conostylis* (e.g., figs. 13*A*, 15*G*, 16*C*). Marginal fibers are not to be confused with taxa that have more than one layer of epidermal cells at the margin.

Palisade cell morphology is variable in both subfamilies. Two members of the genus *Tribonanthes* in Conostylidoideae and several taxa in Haemodoroideae lack any cells showing resemblance to a typical palisade cell structure (e.g., fig. 9*B*; "absent" in table 2). The rest of the taxa have one, two (fig. 12*E*, 12*F*), or more than two (fig. 8*E*, 8*F*) layers of palisade cells. Helme and Linder (1992) examined the palisade layer in *Dilatris* and *Wachendorfia* and observed two layers of palisade cells in *D. pillansii*. In our study, palisade layer number varies within *Dilatris* (see table 2). Helme and Linder (1992) observed variation in palisade cell layer number in *Wachendorfia*.

Vascular bundle orientation and arrangement showed much variation in the Haemodoraceae. Two general patterns in vascular bundle anatomy were noted. First, there is variation in the contact of the vascular bundles with the epidermis, being either present (e.g., figs. 10*J*, 15*C*) or absent (fig. 10*B*, 10*C*; see table 2). Second, there is variation in the amount and distribution of sclerenchyma fibers in the vascular bundle. Some taxa lack fibers in the vascular bundles (fig. 15*A*). Fibers at one end of the bundle, only partially encircling the vascular tissue, are most common (fig. 15*B*). Lastly, some taxa have thick-walled fibers completely encircling the xylem and phloem tissue (fig. 15*C*).

Raphide crystals have been reported to be present throughout the family (Prychid et al. 2003*a*, 2003*b*). In our study, raphide crystals were observed in at least one species of every genus, with the exception of *Barberetta*, in which they were not observed.

Finally, corroborating the results of Prychid et al. (2003*a*, 2003*b*), only members of the Conostylidoideae were observed to have silica bodies (e.g., figs. 12*E*, 12*F*, 13*A*, 13*C*, 13*D*), but these were not observed in all members of that subfamily, especially from those samples that were resin embedded (table 2).

Systematic and Adaptive Significance

Our results demonstrate the generally strong concordance of numerous anatomical characters with monophyletic groups within the family Haemodoraceae. Some of these may serve as nonmolecular apomorphies for delimiting groups within the family. We speculate here on the possible adaptive significance of some of these anatomical features.

The presence of leaf bulliform cells (fig. 18*A*) as an anatomical apomorphy for *Barberetta* and *Wachendorfia* corroborates past assessments of the close relationship of these two taxa (Simpson 1990; Helme and Linder 1992; Hopper et al. 1999, 2006). Bulliform cells are correlated with and likely function in the folding of the plicate leaf morphology found only in these two genera, with plication being a recognized morphological apomorphy for the two genera (Simpson 1990). However, the adaptive function of this and other types of leaf plication is uncertain (Dahlgren and Clifford 1982) and is possibly involved in leaf development.

The presence of leaf tannin cells is identified as a clear apomorphy of subfamily Conostylidoideae (fig. 18*B*). The presence of leaf tannin cells is undoubtedly correlated with the presence of tannin idioblasts in other organs—e.g., floral placental cells, also restricted to the Conostylidoideae (Simpson 1990). Their function and chemistry have not been studied. They may possibly be an adaptation to deter herbivory.

Leaf marginal fiber caps, found only in *Blancoa canescens* and in some species of *Conostylis* (apomorphic for three clades; fig. 19*A*) may function, as fibers generally do, to physically strengthen the leaf. This feature may constitute an adaptation for maintaining leaf integrity in these perennial species that must withstand periodically high temperatures in the Australian heathland. It could also function to mechanically deter herbivory. However, both of these hypotheses are speculative.

The epidermal cell wall relative area is thick (>50%; see fig. 19*B*) only in the clade containing *Blancoa* and all the

members of *Conostylis*. It is possible that the thickened epidermal cell walls in leaves of these taxa function to inhibit water loss, a possible adaptation to the more xeric environments of southwestern Australia where these taxa are found. Alternatively, as with leaf marginal fiber caps, this feature may function to provide added structural support or to deter herbivory. However, again, these hypotheses are speculative and may require ecological studies for confirmation.

The presence of increased numbers of palisade cell layers (fig. 20*A*) on both sides of the leaf in *Anigozanthos*, *Blancoa*, *Conostylis*, and *Macropidia* of the Conostylidoideae may have evolved as a response to more xeric environmental conditions (Esau 1977) in the Australian heathland where these taxa occur. Their absence in the Haemodoroideae is unclear. Although some members of the latter subfamily occur in more mesic habitats, others are found in habitats similar to those cited above—e.g., *Dilatris* and *Wachendorfia* found in South African fynbos.

The presence of fibers that at least partially envelop the vascular bundles is an apomorphy for the Haemodoraceae as a whole (fig. 20*B*). This feature may function in augmenting leaf physical integrity. The loss of this feature in *Barberetta aurea* of the Haemodoroideae and one examined species of *Tribonanthes* of the Conostylidoideae may possibly be related to their more mesic (or semiaquatic in *Tribonanthes*) habitats. The increased fiber envelopment in the vascular bundles of *Haemodorum*, *Phlebocarya*, and several *Conostylis* species may also be an adaptation to a more xeric environment than these taxa generally occupy, but this is speculative without precise ecological data.

Leaf aerenchyma (fig. 21*A*) is found only in *Tribonanthes* of the Conostylidoideae and *Lachnanthes* of the Haemodoroideae, having evolved separately in the two genera. Leaf aerenchyma is correlated with wet habitat, and both of these taxa occur in habitats that are at least periodically wet. However, other members of the Haemodoroideae that lack aerenchyma occur in similar habitats (e.g., *Wachendorfia brachyandra* and *Wachendorfia thyrsiflora*).

Mucilage cells (fig. 21*B*) are found only in *Dilatris* of the Haemodoroideae. Generally, mucilage compounds swell in water and increase the capacity of cells to absorb and retain water (Esau 1977). It is unclear what the adaptive significance of these cells, if any, is in *Dilatris*.

The relative epidermal wall uniformity shows no clear phylogenetic trends in the Haemodoraceae. However, there appears to be a correlation between the presence of thickened epidermal walls and a uniform distribution of the cell wall around the lumen.

The epidermal cell layer number is ancestrally uniseriate for all members of the Haemodoraceae. Species of *Conostylis* are the only family members that possess more than one epidermal cell layer. These extra epidermal layers may be an adaptation to inhibit water loss, as most members of *Conostylis* are found in xeric habitats.

Epidermal surface shape shows some phylogenetic signal, with an undulate or invaginate cell shape having evolved several times. It appears that epidermal invaginations could be an apomorphy that unites several taxa within both subfamilies, with a few reversals to flat and undulate leaves. Because deep epidermal invaginations (forming stomatiferous grooves) are generally associated with xerophytic habitats, taxa that possess this feature may have evolved this feature to reduce evapotranspiration in their xeric environment.

Vascular bundle contact with the epidermis seems to vary significantly in the Haemodoraceae. The adaptive significance of the states of this character, if any, is unknown.

Silica body presence in the leaves of the Haemodoraceae was studied by Prychid et al. (2003*a*, 2003*b*). This study confirms that silica bodies are found only in members of the Conostylidoideae, an apparent apomorphy for the subfamily. The fact that not all members of this subfamily were observed to possess them may be explained either as evolutionary losses or because of a scanty occurrence in some taxa. Silica bodies may function in general as a deterrent to herbivory.

Conclusions

In conclusion, several anatomical characters of the root, stem, and leaf in the Haemodoraceae are correlated, show a clear phylogenetic signal, and can be interpreted as nonmolecular apomorphic traits. Many of these anatomical features may constitute adaptations. Most of the adaptations proposed are related to either habitat (xeric vs. at least periodically wet) or possible deterrence of herbivory. However, these adaptive scenarios are based solely on imprecise habitat correlations and not on direct observational or experimental data. These scenarios can be seen only as speculative. Our study points out the need for more precise, quantifiable ecological data in assessing these anatomical features as possible adaptations, the result of selective pressures.

Acknowledgments

We thank Dr. Stephen Hopper for help with fieldwork in Australia collecting specimens of the Conostylidoideae.

Appendix

Taxa and documentation (collector, collection number, and herbarium accession number, where available) for anatomical features. Symbols for organ type: L = leaf; R = root; S = scape.

Haemodoroideae

Barberetta aurea: Ornduff 7661 (R, S, L); *Dilatris corymbosa: Simpson 2223*, SDSU 13867 (L); *Dilatris ixioides: Simpson 2216* (L); *Dilatris pillansii: Simpson 2221*, SDSU 13872 (L); *Dilatris viscosa: Simpson 2224*, SDSU 13868 (R, S, L); *Haemodorum loratum: Aerne 39*, SDSU 17013 (L); *Haemodorum simplex: Simpson 20IX81A*, DUKE 287760 (L); *Haemodorum simulans:*

Aerne 46, SDSU 17014 (L); *Haemodorum spicatum: Aerne 45*, SDSU 17020 (L); *Haemodorum venosum: Simpson 13IX81N*, SDSU 17131 (R, L); *Aerne 34*, SDSU 17019 (L); *Lachnanthes caroliniana: Simpson 14VI80A*, DUKE 287765 (R, L); *Pyrrorhiza neblinae: Boom & Weitzman 5741*, NY 88806 (R, L); *Schiekia orinocensis: B. Maguire 41569*, NY 214485 (R, S, L); *Wachendorfia brachyandra: Simpson 2215*, SDSU 13871 (R, S, L); *Wachendorfia paniculata: Simpson 2218*, SDSU 13870 (L); *Wachendorfia thyrsiflora: Simpson 2217*, SDSU 13874 (L); *Xiphidium caeruleum: Antonio 1201*, MO 1884872 (R, S); *Aerne 2*, SDSU 17269, 17340 (L).

Conostylidoideae

Anigozanthos flavidus: Aerne 23, SDSU 16291 (R); *Simpson 24IX81J*, DUKE 287752 (L); *Anigozanthos humilis: Simpson 9IX81CC*, SDSU 17113 (L); *Anigozanthos preissii: Aerne 61* (L); *Anigozanthos rufus: Simpson 27IX81F*, SDSU 17110, 17111 (R, S, L); *Blancoa canescens: Simpson 18IX81AA*, DUKE 287757, SDSU 17038 (R, S, L); *Conostylis aculeata* subsp. *bromelioides: Aerne 54*, SDSU 16996 (L); *C. aculeata* subsp. *spinuligera: Aerne 31*, SDSU 16997 (R, L); *Conostylis androstemma: Aerne 33*, SDSU 16998 (L); *Conostylis angustifolia: Aerne 32*, SDSU 16999 (L); *Conostylis aurea: Aerne 26*, SDSU 17000 (L); *Conostylis bracteata: Aerne 25*, SDSU 17001 (S, L); *Conostylis candicans: Aerne 62*, KPBG 20020044 (L); *Conostylis canteriata: Aerne 41*, SDSU 17002 (L); *Conostylis caricina* subsp. *caricina: Aerne 60*, SDSU 17003 (L); *Conostylis caricina* subsp. *elachys: Aerne 56*, SDSU 17004 (L); *Conostylis crassinerva* subsp. *absens: Aerne 42*, SDSU 17005 (L); *Conostylis dielsii* subsp. *teres: Aerne 48*, SDSU 17006 (L); *Conostylis festucacea* subsp. *filifolia: Aerne 52*, SDSU 17007 (L); *Conostylis juncea: Aerne 27*, SDSU 17008 (R, L); *Conostylis latens: Aerne 29*, SDSU 17009 (L); *Conostylis micrantha: Aerne 47*, SDSU 17011 (L); *Conostylis misera: Aerne 63*, KPBG 19970155 (L); *Conostylis neocymosa: Aerne 40*, SDSU 17012 (R, L); *Conostylis pauciflora* subsp. *euryhipis: Aerne 24*, SDSU 16915 (R, L); *Conostylis petrophiloides: Aerne 57*, SDSU 16916 (R, L); *Conostylis prolifera: Aerne 51*, SDSU 16917 (L); *Conostylis pusilla: Simpson 5IX81D*, SDSU 17029 (L); *Conostylis resinosa: Aerne 44*, SDSU 16918 (L); *Conostylis robusta: Aerne 49*, SDSU 16985 (L); *Conostylis seminuda: Aerne 35*, SDSU 16986 (R, L); *Conostylis setigera* subsp. *dasys: Aerne 58*, SDSU 16987 (R, L); *Conostylis setosa: Aerne 59*, SDSU 16988 (L); *Conostylis stylidioides: Aerne 64*, KPBG 19971286 (L); *Conostylis teretifolia* subsp. *planescens: Aerne 28*, SDSU 16989 (L); *Conostylis teretifolia* subsp. *teretiflolia: Aerne 36*, SDSU 16990 (L); *Conostylis teretiuscula: Aerne 30*, SDSU 16991 (L); *Conostylis tomentosa: Aerne 43*, SDSU 16992 (L); *Conostylis vaginata: Simpson 27IX81A*, SDSU 17037 (R, L); *Conostylis villosa: Aerne 55*, SDSU 16993 (L); *Conostylis wongonensis: Aerne 53*, SDSU 16994 (R, L); *Macropidia fuliginosa: Simpson 18IX81DD*, DUKE 287754 (R, S, L); *Phlebocarya ciliata: Simpson 13IX81G* (R, S, L); *Simpson 16IX81A*, SDSU 17021 (S); *Phlebocarya pilosissima: Simpson 16IX81K*, SDSU 17023,17024 (R); *Aerne 37*, SDSU 17018 (L); *Tribonanthes australis: Simpson 13IX81C*, SDSU 17017 (R, S, L); *Tribonanthes brachypetala: Simpson 24IX81K*, SDSU 17016 (L); *Tribonanthes longipetala: Simpson 5IX81H*, SDSU 17015 (L).

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