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COMPARATIVE MORPHOLOGY AND MOLECULAR SYSTEMATICS OF AFRICAN PODOSTEMACEAE-PODOSTEMOIDEAE, WITH EMPHASIS ON *DICRAEANTHUS* AND *LEDERMANNIELLA* FROM CAMEROON

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The Podostemaceae (eudicots, Malpighiales) are adapted to rivers that exhibit distinct high-low water seasonality, mainly in the tropics. They attach to submerged rocks with ribbonlike or crustose green roots that cover the substrate like a carpet. Pronounced root dorsiventrality resulted in disklike crusts lacking root caps. African Podostemoideae show a bewildering array of forms not known from other flowering plants, such as (i) foliage leaves having a basis with two sheaths (e.g., *Ledermanniella linearifolia*), (ii) modular shoot construction with repeated stem cups (*Ledermanniella ledermannii*), (iii) endogenous origin of flowers along stems (*Dicraeanthus africanus*), and (iv) epiphyllous flowers (*Ledermanniella letouzeyi*). Important morphological transformations specific to African podostemoids include a shift from erect to inverted flowers in the spathella and unilocular ovaries arising via septum loss. New *mat*K sequence data and new morphological data for eight African Podostemaceae species of the genera *Dicraeanthus*, *Djinga*, and *Ledermanniella* are combined with previously published sequences representing all major groups to test the placement of the African taxa in the family. All podostemoids studied from continental Africa form a clade that is sister to the Madagascan genera *Endocaulos* and *Thelethylax*. The sister of this African-Madagascan lineage is the clade comprising all Asian podostemoids and the American genus *Podostemum*, whereas all other New World podostemoids and the subfamily Tristichoideae are more basal.

Keywords: African Podostemaceae, comparative morphology, *Dicraeanthus*, *Djinga*, *Ledermanniella*, Malpighiales, molecular systematics, structural diversity, water plants.

Introduction

The knowledge of the enigmatic family Podostemaceae has significantly increased over the past 10 years. However, the African Podostemoideae are still not well studied. Our understanding of the comparative morphology and the molecular systematics of the African taxa is especially limited. The last taxonomic treatment of African Podostemoideae dates to nearly 20 years ago (Cusset 1987), and the placement of these taxa within the subfamily is unknown. The phylogenetic relationships of the African clade have yet to be addressed. The availability of newly collected plants from western Central Africa prompted our analysis and contributes to a better—but still preliminary—understanding of the evolution of this group.

Biodiversity and biogeography of Podostemaceae in Africa. The Podostemaceae (river weeds), including Tristichaceae of some authors, are the largest family of strictly aquatic angiosperms, comprising 48 genera and ca. 280 species (Cook 1996; Cook and Rutishauser 2007). They occur worldwide in tropical climates. Many of these species are endemic to small geographical areas. Most species are restricted to tropical and subtropical rivers and waterfalls; few species reach into temperate regions (e.g., *Podostemum ceratophyllum*). Africa (including Madagascar) has 16 genera and ca. 77 species. Engler ([1928] 1930), Colette Cusset (1973, 1980, 1984*a*, 1984*b*, 1987), and other botanists have described a number of genera and species of the Podostemaceae-Podostemoideae from Africa and Madagascar. Most of the African podostemoids are in the genus *Ledermanniella* (ca. 46 species). Many of the genera and species in Africa occur in Cameroon and Gabon (Baker and Wright 1909; Engler [1928] 1930; Cusset 1987; Cook 1996). The genera present in this region are, among others, *Dicraeanthus*, *Djinga*, and *Ledermanniella*. Only two of the species included in this study expand their range outside of Cameroon and Gabon: *Ledermanniella ledermannii*, which occurs in five tropical West African countries (including Cameroon), and *Ledermanniella bifurcata*, occurring in three countries (also including Cameroon). Identification keys, drawings, and updated descriptions of all taxa mentioned in this article are available on a Web site on African Podostemaceae at http://www.systbot.unizh.ch/podostemaceae (Rutishauser et al. 2004). Of all Podostemaceae genera worldwide, 41% are monotypic, and this proportion is even higher (53%) among the African taxa. The high degree of endemism on the generic level might be an artifact of the limited knowledge of species, especially the African ones. A more complete sampling is necessary to evaluate this situation but cannot be provided in this article.

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Comparative morphology of Podostemaceae, mainly Podostemoideae, in Africa and Madagascar. The interpretation of the vegetative body is controversial in Podostemaceae. Many Podostemaceae have a flattened photosynthetic body that adheres to a hard substrate. It has been called a "thallus" because the conventional demarcation into stem, leaf, and root is usually not obvious, and various botanists have denied or doubted the homology of this vegetative body with roots, stems (caulomes), and leaves (phyllomes) of other angiosperms (Cusset 1974; Mohan Ram and Sehgal 1992; Schnell 1998). These botanists think that the vegetative body of all (or most) Podostemaceae represents a unique architectural type. However, for convenience, we adopt the classical root-shoot (CRS) model with its structural categories roots and shoots (including stems and leaves), as used by Warming (1881, 1891, 1899), Jäger-Zürn (2000), Rutishauser (1995, 1997), Rutishauser and Moline (2005), Moline et al. (2006), and Cook and Rutishauser (2007). Thus, we avoid terms such as "thallus" and "thalloid." We use the term "root" for a cylindrical to completely flattened photosynthetic structure when adhesive hairs and endogenous shoot buds are developed but no exogenous leaves are present. The term "stem" is applied to a cylindrical or slightly flattened photosynthetic body that develops exogenous leaves.

Early comparative work on Podostemaceae was carried out by Warming (1881, 1891, 1899). These and several later studies were mainly on Podostemaceae found in the New World and Asia (Rutishauser 1997; Cook and Rutishauser 2007). Compared with these Podostemaceae, those of Africa have not been studied extensively. A few comparative morphological studies on African and Madagascan Podostemaceae have been done by Gérard Cusset (1974), Jäger-Zürn (2000), and Ameka et al. (2002, 2003). The enormous degree of phenotypic plasticity makes comparative morphological studies of the African Podostemaceae, especially the members of the species-rich subfamily Podostemoideae, a challenging task. Several morphological peculiarities of African podostemoids presented in this article are new to science. Thus, the preliminary character of this publication with respect to molecular data will be counteracted by careful morphological analyses, including a nonmolecular data matrix of unique characters.

Phylogenetic placement of Podostemaceae. Recent studies indicate that Podostemaceae are in the Malpighiales clade in the eurosids I (Soltis et al. 1999; Chase et al. 2000; Savolainen et al. 2000; APG II 2003). Gustafsson et al. (2002) found Podostemaceae nested in the Clusiaceae, as sister of the subfamily Hypericoideae. Morphological evidence that the Podostemaceae are related to the Clusiaceae is observable in the androecium. In both groups, polyandrous flowers, with centrifugal stamen inception (e.g., *Mourera fluviatilis*; Rutishauser and Grubert 2000), seem to represent the ancestral condition. Some members of both Podostemaceae (e.g., *Mourera*, *Weddellina*) and Clusiaceae have latex tubes in their stems (Rutishauser and Grubert 1994).

Molecular data corroborate the distinction of three subfamilies in Podostemaceae: the two smaller podostemaceous subfamilies Tristichoideae (five genera, 10 species) and Weddellinoideae (monotypic, with *Weddellina squamulosa*) and the large and morphologically distinct subfamily Podostemoideae, with its 42 genera and ca. 270 species (Cook 1996; Rutishauser 1997; Kato et al. 2003; Cook and Rutishauser 2007). The Tristichoideae have been found to be sister to the Weddellinoideae and Podostemoideae. The New World genera *Apinagia*, *Marathrum*, *Mourera*, and *Oserya* form a clade that is sister to all other Podostemoideae (Les et al. 1997; Kita and Kato 2001). Until now, the placement within the family and the relationship among the African Podostemoideae have been unknown because there were no molecular data published, except for the two Madagascan genera *Endocaulos* and *Thelethylax* (Kita and Kato 2001).

Objectives. The objectives of this study are (i) to describe morphological novelties and developmental peculiarities of African Podostemoideae; (ii) to elucidate their phylogenetic relationships within Podostemaceae using molecular data (*matK*) of three different genera, *Dicraeanthus*, *Djinga*, and *Ledermanniella*; (iii) to assess the monophyly of the African Podostemoideae; (iv) to perform a combined analysis of molecular and morphological data across the entire family; and (v) to describe the evolutionary history of six morphological characters in African podostemoids as compared to the whole of the Podostemaceae.

Material and Methods

Taxon sampling. The sampling aimed to include representatives of important groups of African Podostemaceae to infer their infrageneric relationships and biogeography. *Dicraeanthus africanus*, *Djinga felicis*, *Ledermanniella bifurcata*, *Ledermanniella bowlingii*, *Ledermanniella* cf. *bosii*, *Ledermanniella ledermannii*, *Ledermanniella letouzeyi*, and *Ledermanniella linearifolia* were collected in Cameroon and Ghana. Herbarium specimens, silica gel–dried samples for molecular studies, and ethanol (70%)–fixed samples for morphological studies were collected for each specimen. Vouchers (table A1) are housed in YA, GC, and Z/ZT. All species of our morphological studies were also included in the phylogenetic data set, except for *Saxicolella amicorum* and *Saxicolella submersa* from Ghana (Ameka et al. 2002) because silica gel–dried material of these two species is still lacking.

Two accessions of *D. felicis* from two different locations were included because they differ considerably in shoot size, a character that has been used to delimit species in other podostemoid genera (e.g., *Oserya*; Novelo and Philbrick 1997). Two accessions of *L. ledermannii* (both from Lobé Falls, Cameroon) were included because this species shows significant phenotypic plasticity that could comprise different entities.

The selection of species outside the African Podostemaceae was intended to encompass the global diversity of Podostemaceae and comprised a range of species from all major groups. Sequences of the non-African and Madagascan Podostemaceae were extracted from GenBank (http://www.ncbi.nlm.nih.gov/genbank/), following Kita and Kato (2001). The data matrix included 35 terminal taxa comprising 31 species and two outgroup species. The tree was rooted using *Clusia major* and *Hypericum perforatum* (both Clusiaceae) because Clusiaceae have been found to be closely related to Podostemaceae (Gustafsson et al. 2002; APG II 2003).

Molecular data: DNA extraction, amplification, sequencing, and alignment. The molecular work followed standard

Table 1

List of Primers Used in This Study

Name	Sequence (5′–3′)	Direction	Reference
Pod2Rm	GGTTTTGTGTTTCCGAGCCAAGG	Reverse	Kita and Kato 2001
Pod5Fm	GATTCAGATATTATTGACCG	Forward	Kita and Kato 2001
Pod6Rm	AAATGATACGTAGTGCGATA	Reverse	Kita and Kato 2001
Pod8Fm	TTTTTACTCTATCGCACTA	Forward	Kita and Kato 2001
Pod41Fm	CTTCGTTACTGGTTAAARGATCC	Forward	Modified from Kita and Kato 2001
Pod7Rm	TTCCTTGATAGCGAATATAATG	Reverse	Modified from Kita and Kato 2001
PodAFm	ATCCACTTATCTTTCAGGAG	Forward	Modified from Kita and Kato 2001
Podnew1Rm	ATACATTCGAGTAATTAAACG	Reverse	Modified from Kita and Kato 2001

protocols. Total genomic DNA was extracted from silica-dried samples collected in the field using DNeasy plant extraction kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. Amplifications were performed using 1.5 mM buffer, 0.625 mM $MgCl_2$, 0.2 mM dNTPs, 0.05 U/μL Taq DNA polymerase (Amersham Biosciences), 0.325 μM primer, and 5 ng/μL DNA template. PCR profiles included 33 cycles of 94°C for 1 min, 53°C for 1 min, and 72°C for 3 min. For PCR and subsequent sequencing, we used the primers listed in table 1. Purification of PCR products was done with the Qiagen QIAquick PCR purification kit and GFX PCR DNA purification kit (Amersham Biosciences). Cycle sequencing was carried out using ABI PRISM BigDye Terminator Cycle Sequencing, version 2.0, Ready Reactions (PE Biosystems) according to the manufacturer's specification. Sequencing was done using an ABI PRISM (PE Biosystems) 3100 genetic analyzer. Sequences were aligned using ClustalX (Higgins and Sharp 1988) with the default settings and then manually adjusted in MacClade, version 4.05 (Maddison and Maddison 2002). GenBank accessions are in table A2, and the aligned data set is available as accession SN2579-10242 in Treebase.

Morphological data. For the scanning electron microscopy, the dissected specimens were critical-point dried and sputter-coated (Au). The micrographs were taken with a JEOL scanning electron microscope at 20 kV. Overall, 39 morphological characters were coded in a matrix (app. B, table B1). Many of the characters (see explanations in app. C) are based on the descriptions by Cusset (1987), Ameka et al. (2003), and Rutishauser et al. (2004). Several new data are provided in this article. Character states were verified using ethanol-fixed and herbarium specimens (table A1). The morphological data set of 17 species of Podostemoideae compiled by Moline et al. (2006) was chosen as the starting point for our data matrix. Invariable characters resulting from the different taxon set were excluded, the scoring of other characters was modified in many cases, and new characters relevant for the African species were added. All characters were equally weighted, and all were treated as unordered, except for two characters: first, the flower orientation in the bud, because the developmental inversion of the preanthetical flower passes through an oblique state as intermediate condition; and second, the root shape, because the flattened roots in the different species are part of a transition series from cylindrical roots to ribbonlike and further to crustose roots (described in more detail in Rutishauser and Moline 2005; Koi et al. 2006).

Phylogenetic analyses. Parsimony analyses were performed using PAUP*, version 4.0b10 (Swofford 2000). Parsimonious uninformative characters were excluded before the analysis. In heuristic searches, 200 random addition replicates were calculated, keeping only 10 trees per replicate in memory (nchuck = 10, chuckscore = 1). This step aimed to shallowly explore the entire tree space to find all islands of optimal trees. Subsequently, in a second step, all trees from the first run were used for an additional heuristic search, swapping these trees to completion. Heuristic searches used tree bisection-reconnection (TBR) branch swapping and MULPARS in effect, and with "maxtrees" set to 50,000. Additionally, Bayesian inference of phylogeny using the *matK* data set was explored using MrBayes (Huelsenbeck and Ronquist 2001). The GTR + G model was selected by the Akaike Information Criterion in MrModeltest (Nylander 2004). Two million generations were performed. Trees from the first 5000 generations were discarded.

Morphological and molecular data were analyzed separately to test congruence. The trees from the individual data sets were found to be congruent when they contained no hard incongruent groupings, thus suggesting that they could be combined (Johnson and Soltis 1998; Wiens 1998; Eldenäs and Linder 2000). "Hard" incongruence was arbitrarily defined as conflicting nodes that have at least 70% bootstrap support (Mason-Gamer and Kellogg 1996). In absence of hard incongruence between the trees from the different data sets, the data sets were combined in a total-evidence approach.

The degree of support for each node was assessed using bootstrap analysis (Felsenstein 1985). Bootstrap values were obtained with PAUP* (Swofford 2000), using all characters (including parsimonious uninformative characters) and running 1000 bootstrap replicates, each replicate comprising 10 random addition sequence replicates (nchuck = 10, chuckscore = 1), using TBR branch swapping and MULPARS in effect.

Character optimization. The character optimization for six morphological characters has been carried out using Winclada, version 1.00.08 (Nixon 1999–2002). The following six morphological characters were mapped on one of the two most parsimonious trees based on the combined data: (*a*) tepal number per flower, (*b*) presence of septa in the ovary/capsule, (*c*) flower orientation in the bud, (*d*) root shape, (*e*) vegetative stem length, and (*f*) presence of double-sheathed leaves. For each character, the effects of both the fast optimization and the slow optimization have been explored. Fast optimization is equivalent to Acctran (Swofford and Maddison 1987)

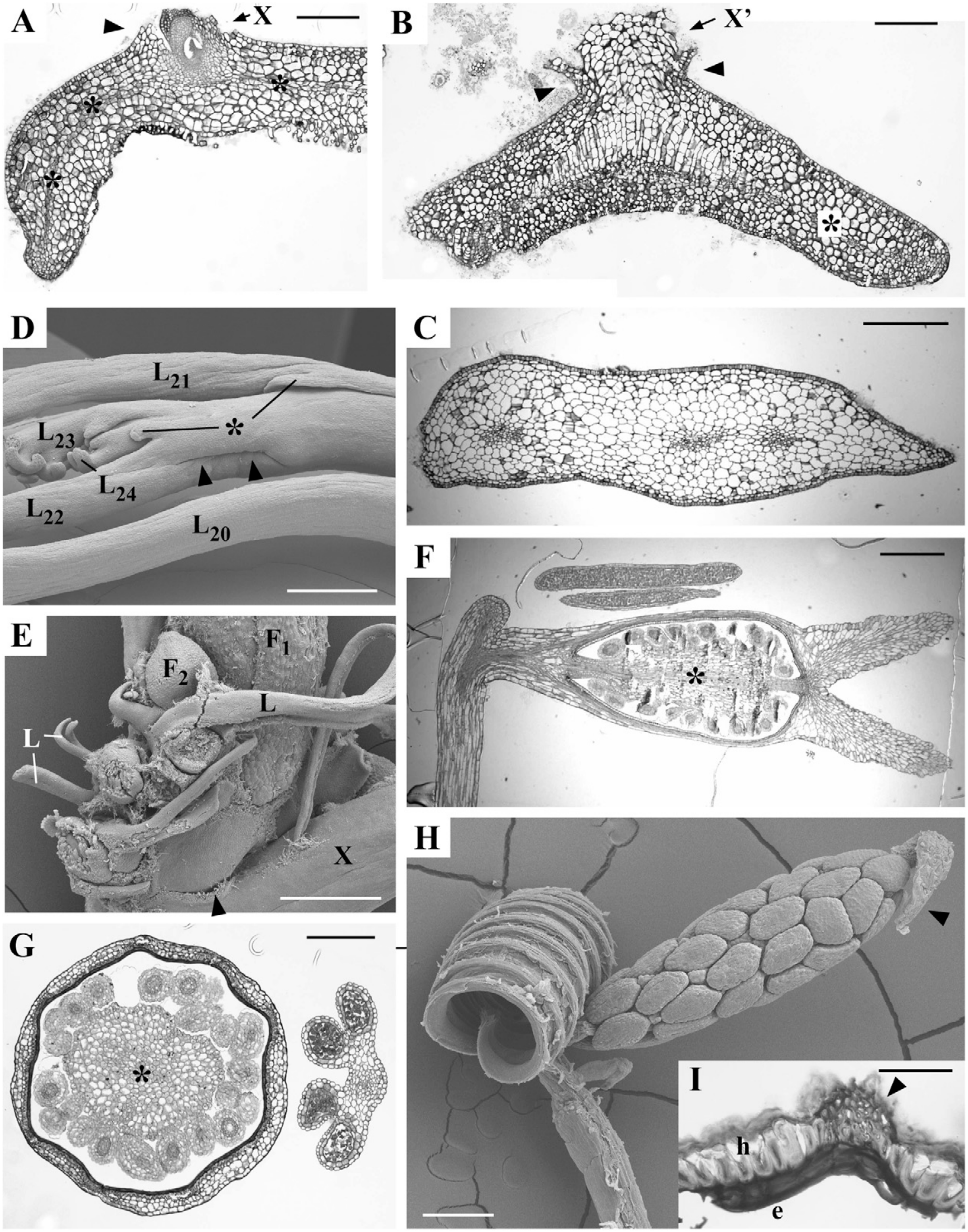


Fig. 1 *Dicraeanthus africanus* (GHO-1413). *A*, *B*, Cross sections of crustose roots with young endogenous shoot bud (X) and basal portion of fully grown stem (X'). Arrowheads point to ruptured root cortex. Note inconspicuous layer of vascular tissue (asterisks) and conspicuous vertical cell rows in the root below stem base X'. Scale bar = 250 μm. *C*, Cross section of stalklike basal portion of compound foliage leaf. Scale bar = 500 μm. *D*, Tip of 30-cm-long shoot. Insertion zone of uppermost leaves L_{20}–L_{24} in distichous order. Note presence of attached stipules (asterisk). Arrowheads point to stem furrow that will give rise to endogenous flower cluster. Scale bar = 500 μm. *E*, Flower cluster along stem (X) with endogenous origin and not subtended by leaf. Flowers (F_1, F_2) covered by spathellas. L = additional small leaves arising from flower

and accelerates transitions between character states, whereas slow optimization is equivalent to Deltran (Swofford and Maddison 1987) and delays transitions between character states.

Results

Morphological Characters of Eight African Members of Podostemoideae Belonging to Dicraeanthus, Djinga, Ledermanniella *subg.* Ledermanniella, *and* Ledermanniella *subg.* Phyllosoma

Roots as ribbons and disklike crusts. Roots in African Podostemoideae are photosynthetic broad ribbons or crustose (disklike) structures. They branch exogenously, giving rise to daughter roots, i.e., daughter ribbons or lateral lobes. Root-borne shoots arise from endogenous buds. In *Dicraeanthus africanus*, the roots are dorsiventral and crustose, resembling foliose lichens, with a layer of rudimentary vascular tissue (fig. 1*A*, 1*B*). Stems arise from endogenous buds along the upper surface of these roots (fig. 1*A*). During shoot establishment, the root below is stabilizing the shoot by the formation of a cushionlike zone with many parallel cell rows running perpendicular to the surface of the disklike crust (fig. 1*B*). In *Ledermanniella ledermannii* and *Ledermanniella* cf. *bosii* (both members of subgenus *Phyllosoma*), the elongated ribbonlike roots have a cross section about three times as wide as it is thick (fig. 2*A*, 2*B*). The lower root side facing the substrate is partially covered with adhesive hairs, and some specimens show a furrow along the axis facing the substrate. There is a single flattened vascular bundle in this ribbonlike root. A root cap is lacking (fig. 2*A*). More proximal root zones show endogenously formed shoots that arise along the root flanks. Each shoot bud is positionally associated with a disklike holdfast that arises as an exogenous lateral lobe from the root flank (figs. 2*C*, 3*C*). In *Ledermanniella letouzeyi* and *Ledermanniella linearifolia* (both members of *Ledermanniella* subg. *Ledermanniella*), the roots are again completely crustose, resembling those of *Dicraeanthus* (figs. 5*A*, 6*A*), whereas in *Ledermanniella bifurcata* (i.e., a third species studied from the same subgenus), there are ribbonlike roots like those described for *L. ledermannii*. The root-borne shoots arise from endogenous buds along the root margin and the upper root surface (*L. linearifolia*; fig. 6*A*, 6*B*), or they are formed in the distal angles of exogenous daughter roots (*Ledermanniella bowlingii*; fig. 4*D*; Ameka et al. 2003). Crustose roots are also observable in *Djinga felicis* (fig. 4*E*).

Endogenous flower formation in the stem cortex. More than 100 flower buds can occur along a single stem in *Ledermanniella letouzeyi* (figs. 4*B*, 5*C*), with the stems at most 10 cm long. The flowers arise from endogenous shoot buds, which are formed inside the stem cortex (fig. 5*E*). Parenchyma cells in the stem cortex dedifferentiate and divide into meristematic cells, giving rise to endogenous buds. They finally protrude the stem periphery, rupturing the epidermis and the outer cortex layers (fig. 5*D*). The morphogenetic capacity to form endogenous shoot buds inside the undamaged stem cortex is also found in *D. africanus*. Here, the leaves are inserted in a distichous manner along two rows, whereas the floriferous short shoots are arranged in a third row along the stem (fig. 4*A*; Rutishauser et al. 2004). They start as endogenous buds from keyholelike stem furrows located below the base of each young leaf (fig. 1*D*, 1*E*). The linear arrangement of these “keyholes” explains the later restriction of flower fascicles along a single stem sector. Besides in *D. africanus* and *L. letouzeyi*, endogenous flower formation along stems—in addition to the regular exogenous flower buds on the shoots—also occurs in *D. felicis* and *L. bowlingii* but without restriction to a single stem sector.

Double-sheathed and terminal leaves. Double-sheathed leaves are leaves that have a basis with two sheaths. This is a morphological peculiarity of many members of the subfamily Podostemoideae (Rutishauser et al. 2003). For example, root-borne short shoots of *L. linearifolia* (subg. *Ledermanniella*) have first foliage leaves with one sheath each, all arranged in the same plane (L_1–L_4 in fig. 6*A*, 6*C*). Then, the next leaf is a double-sheathed leaf (L_5 in Fig. 6*C*), containing a flower stalk in the right sheath and a vegetative daughter bud (with first leaf L′) in the left sheath. Similar double-sheathed leaves are also observable in short shoots of *L. bifurcata* and *D. felicis*. Double-sheathed leaves occupying terminal positions in repeated shoot modules occur in *L. bowlingii*. Several foliage leaves (with a single sheath each) are arranged in a distichous order (e.g., L_{17}–L_{19} in fig. 4*C*). Below the double-sheathed leaf (T_1) terminating the mother shoot, there are two extra-axillary daughter modules (D_I) that end in another terminal leaf (T_2) after two regular leaves are formed. Then, two second-order daughter modules (D_{II}) arise on the left and right side of leaf T_2. Double-sheathed leaves giving rise to daughter shoots or flower buds on both sides are seemingly lacking (or have not been observed) in *D. africanus*, *L. letouzeyi*, *L.* cf. *bosii*, and *L. ledermannii*. The proper identification of double-sheathed leaves was not possible in all African taxa studied because some of them lacked shoot tips with young leaves in the fixed material.

Compound ribbonlike leaves and epiphyllous flowers in L. letouzeyi. The foliage leaves of this species are entire or forked ribbons up to 30 cm long. Toward their base the leaves are tapering into narrow petioles that are inserted in distichous order along the stem (fig. 4*B*). The flattened leaf segments are equifacial, i.e., with identical cell patterns on both sides; a single segment (up to 1 cm broad) may contain

cluster. Arrowhead points to collar of ruptured stem cortex. Scale bar = 1 mm. *F*, Longitudinal section of gynoecium before anthesis. Unilocular ovary with several ovules along central placenta (asterisk). Note presence of two conspicuous conical (beaklike) stigma lobes. Scale bar = 500 μm. *G*, Cross section of unilocular ovary with central placenta (asterisk) surrounded by several ovules. Anther with four pollen sacs. Note presence of six fibrous ribs, i.e., three ribs per valve. Scale bar = 250 μm. *H*, Mature capsule after dehiscence with the two coiled and ribbed valves sticking together. Seeds attached to placenta. Arrowhead points to persisting deflexed stigma lobe. Scale bar = 300 μm. *I*, Cross section of mature capsule wall with rib of fibers (arrowhead). Outer soft layers dropped. Inner layers (with thick cell walls) consist of inner epidermis (*e*) with transversally elongated cells and hypodermis (*h*) with longitudinally elongated cells. Scale bar = 70 μm.

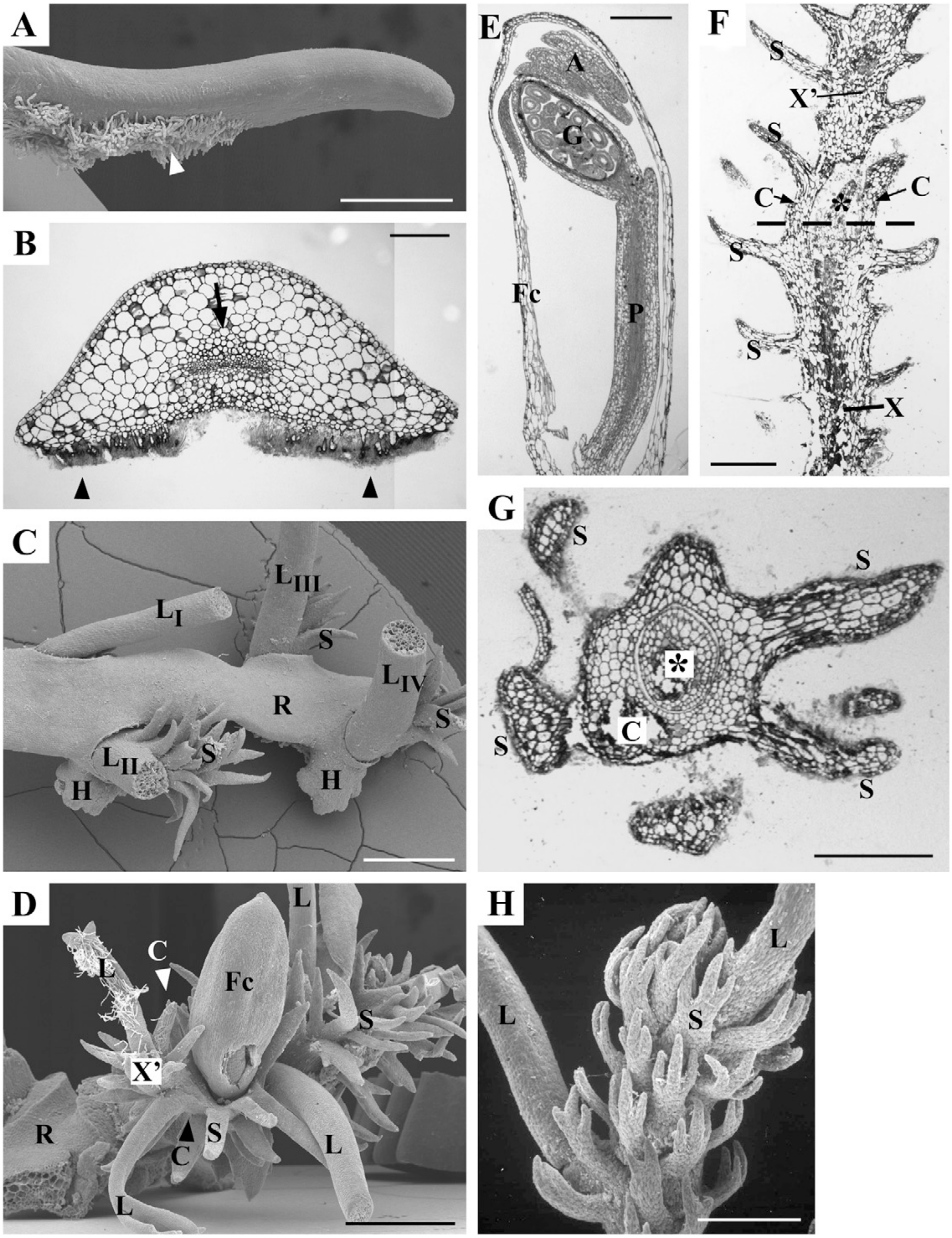


Fig. 2 *Ledermanniella* subg. *Phyllosoma*: *Ledermanniella ledermannii* (GAR-021018-02). *A*, Tip of ribbonlike root lacking cap. Arrowhead points to adhesive hairs along the lower root surface. Scale bar = 500 μm. *B*, Cross section of mature portion of ribbonlike dorsiventral root. Arrow points to vascular tissue; arrowheads indicate adhesive hairs. Scale bar = 250 μm. *C*, Mature portion of ribbonlike root seen from above. Root flanks with four endogenously formed shoots, each one with basal foliage leaf (L_I–L_{IV}), shoot bud with many scales (*S*), and root-borne exogenous holdfast (*H*). Scale bar = 1 mm. *D*, Reproductive short shoot along root (*R*). Flower bud in spathella (*Fc*) arising from a cup (*C*) consisting of fused basal portions of foliage leaves (*L*) and scales (*S*). Bud of next higher shoot module (X') arises from the upper rim of cup *C*. Scale bar = 1 mm. *E*, Longitudinal section of inflated spathella (*Fc*) shortly before rupturing. Note that ovary (*G*) and androecium (*A*) already started to turn upright on the elongating pedicel (*P*). Scale bar = 500 μm. *F*, Longitudinal section of reproductive shoot with two consecutive modules *X* and X'. Dashed line indicates position of cup (*C*) of stem unit *X*. *S* = stem scales. Scale bar = 500 μm. *G*, Cross section of stem cup (*C*) along dashed line in *F*. Inside the cup is the base of a flower unit (asterisk). Scale bar = 500 μm. *H*, Upper portion of short shoot with two foliage leaves (*L*) and several tridentate scales (*S*). Scale bar = 500 μm.

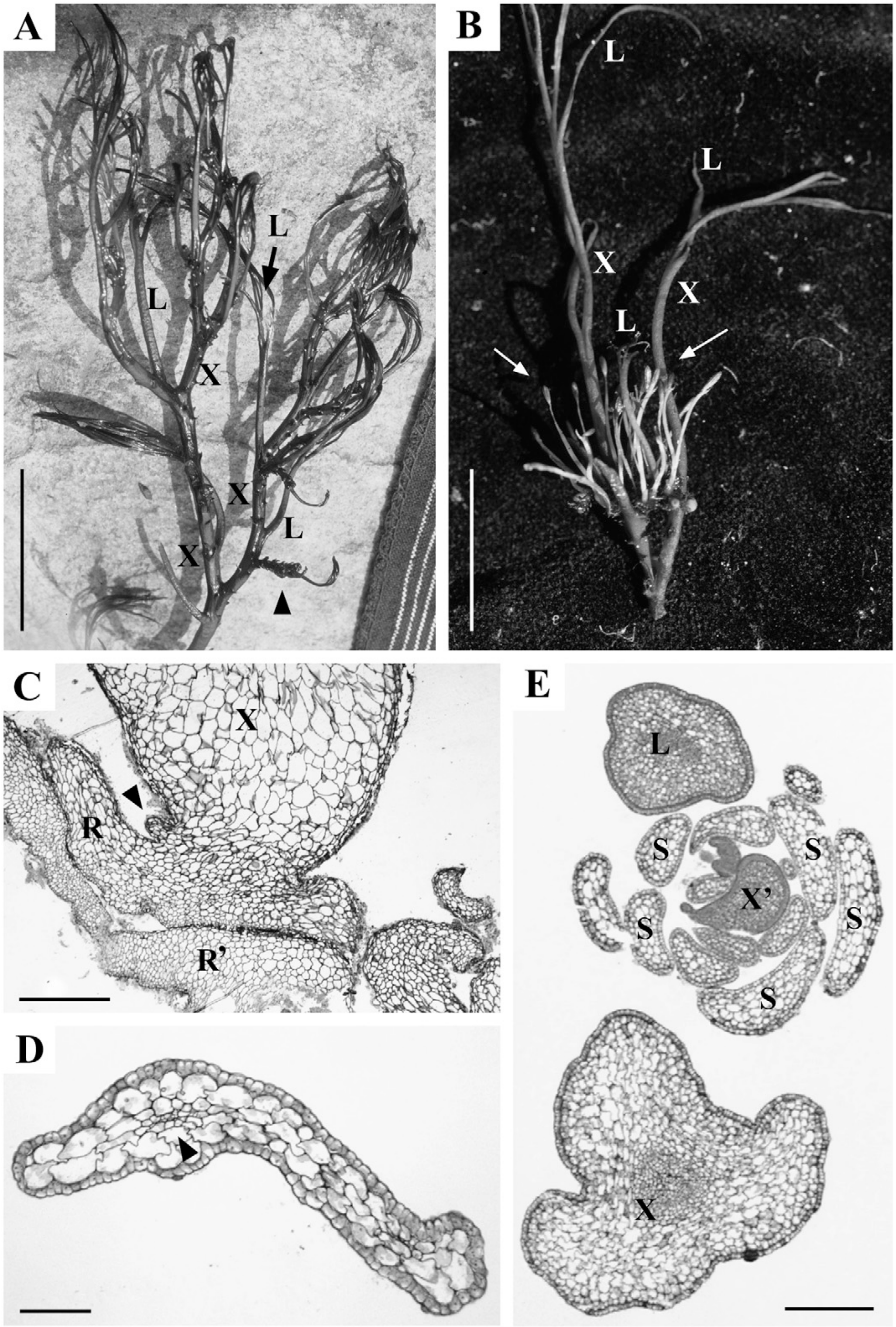


Fig. 3 *Ledermanniella* subg. *Phyllosoma*: *Ledermanniella* cf. *bosii* (GAR-021018-01). *A*, Branched vegetative shoot (whole length 15 cm) with stems (*X*) having rough surface because of minute scales. *L* = forked foliage leaves. Arrowhead points to lateral short shoot densely covered with stem scales. Scale bar = 2.5 cm. *B*, Older stage of branched shoot with distal stem portions (*X*) and foliage leaves (*L*) damaged. Arrows point to stalked capsules arising from lateral short shoots. Scale bar = 2.5 cm. *C*, Longitudinal section of stem foot (*X*) attached to root (*R*), which creeps over another root (*R*′). Arrowhead points to remnants of ruptured root cortex. Scale bar = 500 μm. *D*, Cross section of distal leaf segment. Arrowhead indicates rudimentary vascular bundle. Scale bar = 100 μm. *E*, Cross section of elongate stem (*X*) and leaf stalk (*L*). A lateral short shoot (*X*′) is covered by several scales (*S*). Scale bar = 300 μm.

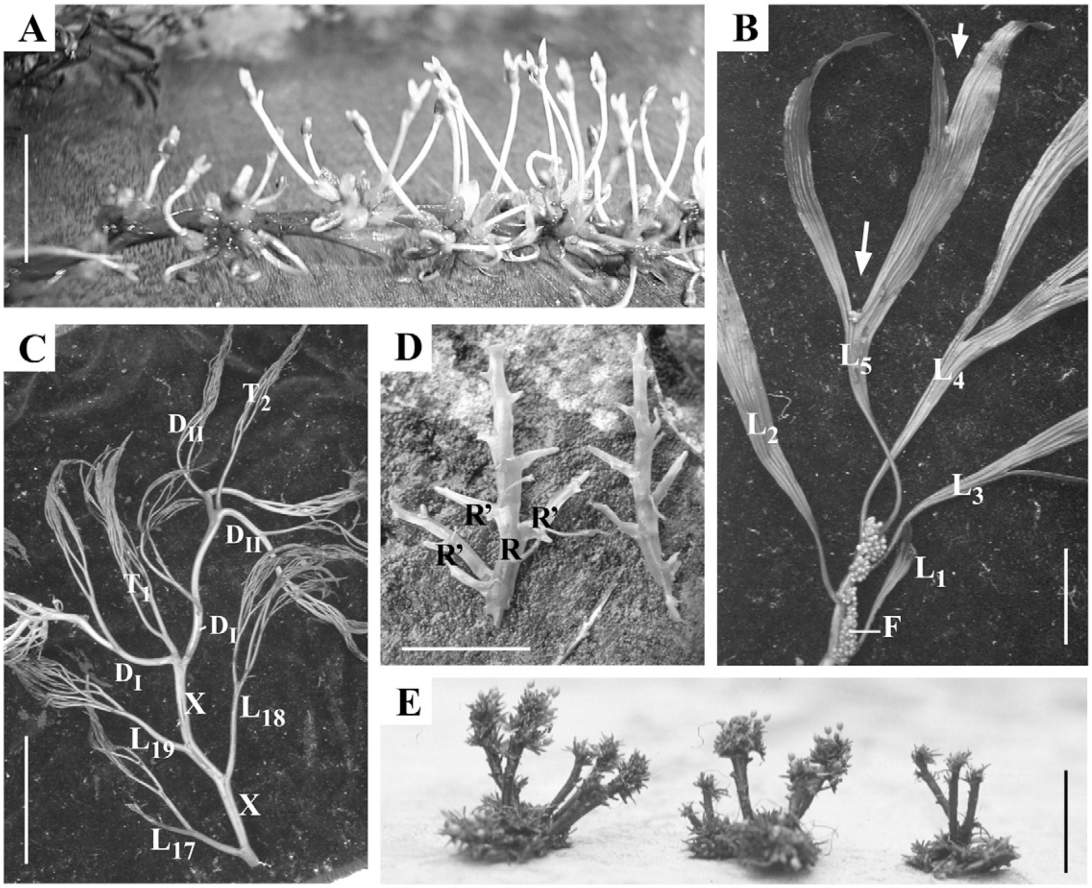


Fig. 4 African Podostemoideae. *A*, *Dicraeanthus africanus* (GHO-1413). Portion of 20-cm-long flowering shoot with stalked flower fascicles restricted to upper stem sector, main foliage leaves pointing downward. Scale bar = 1.5 cm. *B*, *Ledermanniella letouzeyi* (GAR-021023-12). Flowering shoot with densely arranged flower buds (*F*) along one stem sector. Leaves (L_1–L_5) in distichous order; each leaf entire or forked once or twice with lanceolate segments provided with several parallel ribs. Arrows point to additional epiphyllous flowers in angle between leaf segments. Scale bar = 6 cm. *C*, *D*, *Ledermanniella bowlingii* (AR-021010). *C*, Distal portion of 70-cm-long floating shoot with elongate stem (*X*) and compound leaves in distichous order (L_{17}–L_{19}) below stem bifurcation with two daughter shoot modules (D_I) and a terminal leaf (T_1) in between. The right daughter module gives rise to another terminal leaf (T_2) in the fork of two second-order daughter modules (D_{II}). Scale bar = 4 cm. *D*, Broad ribbonlike roots (*R*) fixed to the rock with exogenously produced lateral root ribbons (*R′*). Scale bar = 2 cm. *E*, *Djinga felicis* (GAR-021020-08). Three groups of root-borne stems (up to 1 cm long) with distal and subdistal tufts of subulate leaves and stalked capsules. Scale bar = 1 cm. (Photographs: *A*, J.-P. Ghogue; *B–E*, R. Rutishauser.)

up to 10 parallel ribs with a little bit of vascular tissue each, preventing a clear identification of phloem and xylem elements (fig. 5*B*). *Ledermanniella letouzeyi* is also able to produce epiphyllous flowers, arising from the clefts of the blade forks (fig. 4*B*).

Inversion of flowers in the spathella. Young flowers of *Dicraeanthus* and all *Ledermanniella* species studied are completely inverted (i.e., hanging upside down) within a saclike cover known as the spathella (fig. 5*F*). The ovary can start turning erect already within the still-unruptured but strongly elongating spathella before anthesis, as found in *L. ledermannii* (fig. 2*E*). In *D. felicis*, each flower bud inside the young spathella is oblique, showing an inclined ovary before anthesis but never being completely inverted (R. Rutishauser, unpublished data).

Pollen in monads and dyads. All species studied show tricolpate pollen. The pollen of *D. africanus* and *D. felicis* are in dyads. In *Djinga*, these dyads easily decay into monads. Pollen of five out of the six *Ledermanniella* species studied here is arranged in dyads (e.g., *L. linearifolia*; fig. 6*F*). There is only *L. bifurcata* showing pollen as monads, as is also observable in various other *Ledermanniella* species (R. Rutishauser, unpublished data).

Gynoecium features. The ovaries and immature capsules of *D. africanus* and all *Ledermanniella* species studied are ovoid to ellipsoid or fusiform, with three ribs per valve, running the entire length of the capsule. In *Djinga*, the ovary is globose or subglobose (fig. 4*E*), with the two carpels slightly unequal. While the ovary is completely smooth, the two valves of the young capsule are provided with three broad and inconspicuous ribs each. The valves of all African podostemoids studied are equal or slightly unequal, and one or both valves can be persistent, depending on the species. The ovary of *D. africanus* is topped by two conspicuous conical

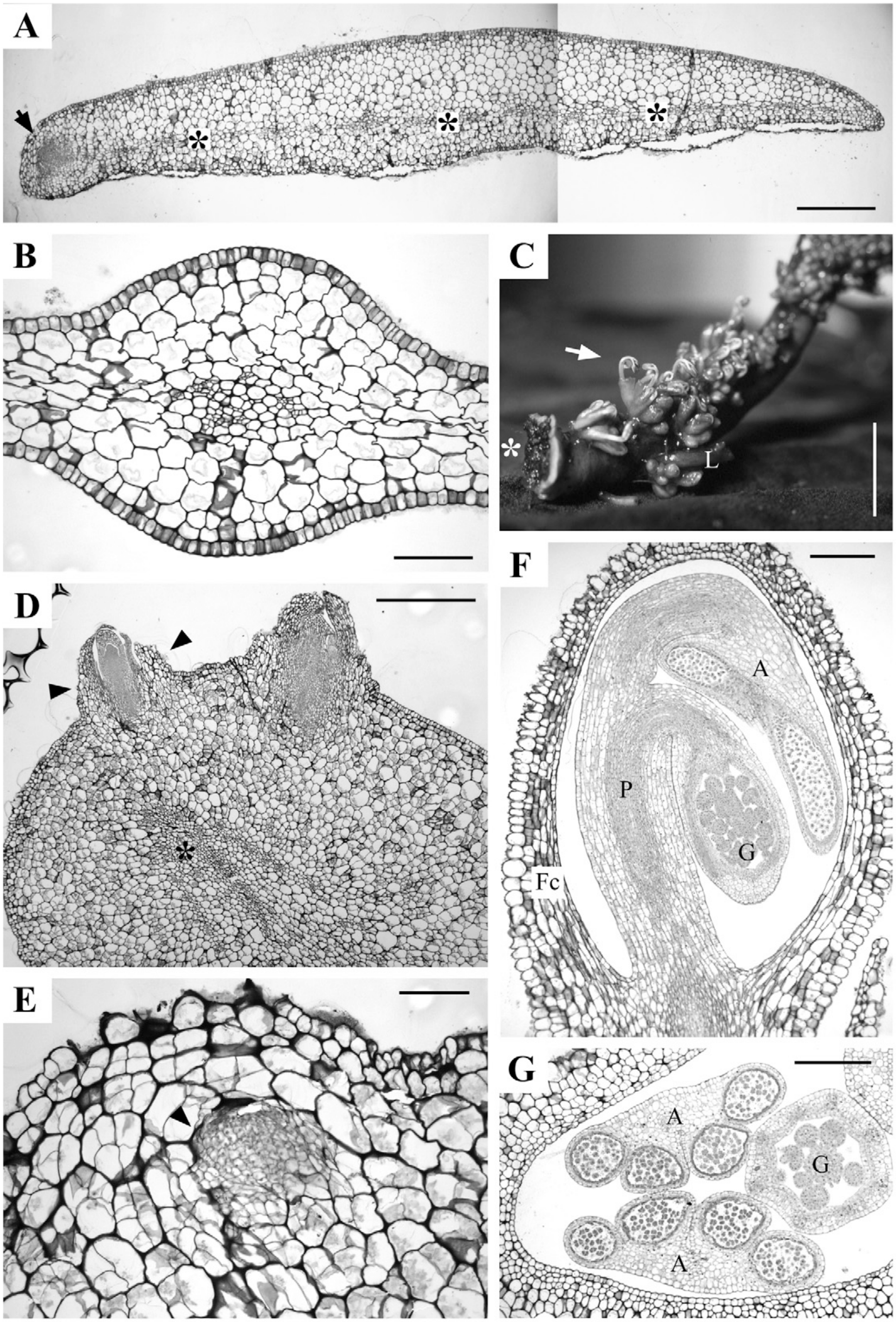


Fig. 5 *Ledermanniella* subg. *Ledermanniella*: *Ledermanniella letouzeyi* (GAR-021023-12). *A*, Cross section of crustose root. Arrowhead points to endogenous shoot bud. Note inconspicuous layer of vascular root tissue (asterisks). Lower epidermis (facing the substrate) slightly damaged. Scale bar = 500 μm. *B*, Close-up of one of the parallel ribs in the isolateral leaf blade. Vascular tissue lacking differentiation into phloem and xylem. Scale bar = 100 μm. *C*, Stem basis with disklike holdfast (asterisk) and many flowers and flower buds along one stem sector

(beaklike) stigma lobes during anthesis, which are spreading and often persistent in fruit (fig. 1*F*). The stigmas of most *Ledermanniella* species are linear, spreading, or reflexed (figs. 2*E*, 6*E*). They may show a slightly stalked ovary; i.e., the ovary and the resulting capsule have a short gynophore above the insertion level of androecium and tepals (e.g., *L. linearifolia*; fig. 6*E*, 6*G*). All species checked in *Dicraeanthus*, *Djinga*, and *Ledermanniella* as yet described lack a septum in the bicarpellate gynoecium, resulting in a unilocular ovary with several ovules inserted around a central placenta (figs. 1*G*, 5*G*, 6*G*).

Stem scales of subgenus Phyllosoma. The members of *Ledermanniella* subgenus *Phyllosoma* (represented here by *L.* cf. *bosii* and *L. ledermannii*) are characterized by additional stem scales with entire or toothed margins. *Ledermanniella* cf. *bosii* usually shows entire scales, whereas scales of *L. ledermannii* are usually tridentate (more rarely entire or quadridentate). These stem scales (i.e., scaly leaves) are present in addition to compound leaves, which usually are much larger (fig. 2*C*, 2*D*; fig. 3*A*). The foliage leaves are arranged in a distichous order, whereas the stem scales are inserted irregularly around the stem (figs. 2*H*, 3*E*). Only foliage leaves and stems are provided with vascular bundles. There is no vascular tissue in the stem scales (fig. 2*F*, 2*G*). In young (i.e., still short) shoots, the scales are imbricate, covering the stem surface completely, whereas mature and strongly elongated stem portions (internodes) may finally have only loosely scattered scales (e.g., *L.* cf. *bosii*; fig. 3*A*, 3*B*).

Modular shoot construction with stem cups in L. ledermanni. Each shoot in this species is composed of several modules, with the maximum stem length reaching 2–5 cm (fig. 2*F*). Each flower (with its spathella) arises at the end of a shoot module, surrounded by a stem cup, which consists of the fused bases of various scales and foliage leaves (fig. 2*D*). Except in one bundle (next to C in fig. 2*G*), this cup lacks vascular tissue completely. The next higher shoot module arises on the rim of this stem cup (see *X′* in fig. 2*D*, 2*F*). A longitudinal section of two consecutive shoot modules clearly shows the cavity where the flower basis is inserted (asterisk in fig. 2*F*). The next higher shoot module (*X′* in fig. 2*F*) again adds a few millimeters to the total stem length before it ends with a new stem cup, surrounding the next higher flower bud. Further studies will show whether these stem cups are found in *L. ledermannii* only; we did not yet observe similar stem cups in *L.* cf. *bosii* and other species of *Ledermanniella* subg. *Phyllosoma*.

Molecular and Morphological Phylogeny of Selected African Podostemoideae as Compared to Non-African Taxa

Separate analysis and congruence. The molecular data yielded two most parsimonious trees, resulting in a highly resolved strict consensus tree (fig. 7*A*; Consistency Index [CI] = 0.59, Retention Index [RI] = 0.75). The African clade contained the single unresolved node. The analysis of the morphological data yielded 1512 most parsimonious trees, resulting in a not completely resolved strict consensus tree, with the backbone resolved but not the African clade (fig. 7*B*; CI = 0.41, RI = 0.73). The Bayesian analysis supported the results of the parsimony study, yielding a topology almost identical to that of the strict consensus tree (not shown). Only the position of *L. linearifolia* differed, by being placed as sister group to *D. felicis*. Because of high bootstrap support of the corresponding node in the parsimony study, we use this topology for our discussion. The topologies of the molecular and morphological analyses conflicted with respect to the placement of the two species pairs, (1) *Podostemum ceratophyllum* and *Podostemum rutifolium* and (2) *Endocaulos mangorense* and *Thelethylax minutiflora*. The molecular data resolved the two species of *Podostemum* as sister to the African clade, and these clades in turn are sister to the Asian clade, whereas the morphological data yielded the Asian clade as sister to the African clade, with these in turn sister to *Podostemum*. *Endocaulos* and *Thelethylax* form a clade that is placed as sister to the African clade by the molecular data, but the morphological data placed the two species nested in the Asian clade. However, the respective nodes in the morphological tree received very low bootstrap support (fig. 7*B*). Thus, the degree of conflict does not preclude the combination of the two data sets.

Combined analysis. The analysis of the combined data yielded two well-resolved and well-supported cladograms (CI = 0.57, RI = 0.74; one shown in fig. 8). Combining morphology and molecular data increased resolution within the African clade. The support increased on most nodes for the combined data, with three exceptions: (1) the African and Madagascan Podostemoideae clade (molecular = 83% vs. combined = 81%), (2) the node below *Podostemum* and the African and Madagascan Podostemoideae clade (molecular = 69% vs. combined = 65%), and (3) within the Asian clade, where support values drop 4%–14% on several nodes in the combined data compared to the molecular data, but the support for the whole clade is slightly improved (molecular = 97% vs. combined = 98%).

The African podostemoid species included in the current combined analysis were monophyletic, with the two Madagascan genera *Endocaulos* and *Thelethylax* as sisters (fig. 8). The Ghanaian endemic *L. bowlingii* (subg. *Ledermanniella*) was sister to a Cameroonian clade consisting of *L. letouzeyi* (subg. *Ledermanniella*) and *D. africanus*. These three species were in turn sister to a clade comprising *L. linearifolia* (subg. *Ledermanniella*) and the monotypic genus *Djinga* (both Cameroon). *Ledermanniella bifurcata* (subg. *Ledermanniella*)

Fig. 5 (*Continued*) without being subtended by leaves (*L* = leaf base). Arrow points to flower just starting anthesis coming out of ruptured spathella but still with inclined ovary. Scale bar = 1 cm. *D*, Cross section of stem sector (stem diameter 7 mm). Two shoot buds are formed endogenously, rupturing the stem cortex (arrowheads). Note presence of vascular tissue (asterisk). Scale bar = 1 mm. *E*, Close-up of stem cortex toward the periphery. Arrowhead points to endogenous shoot bud surrounded by stem cortex. Several parenchyma cells started to divide their content into meristematic cells (as part of dedifferentiation). Scale bar = 100 μm. *F*, Longitudinal section of flower bud inside spathella with inverted gynoecium (*G*) and androecium (*A*) on elongated pedicel (*P*). Note papillose outer epidermis of spathella (*Fc*). Scale bar = 200 μm. *G*, Cross section of flower bud before anthesis inside spathella. Note presence of unilocular ovary (*G*) and two tetrasporangiate anthers filled with pollen dyads. Scale bar = 200 μm.

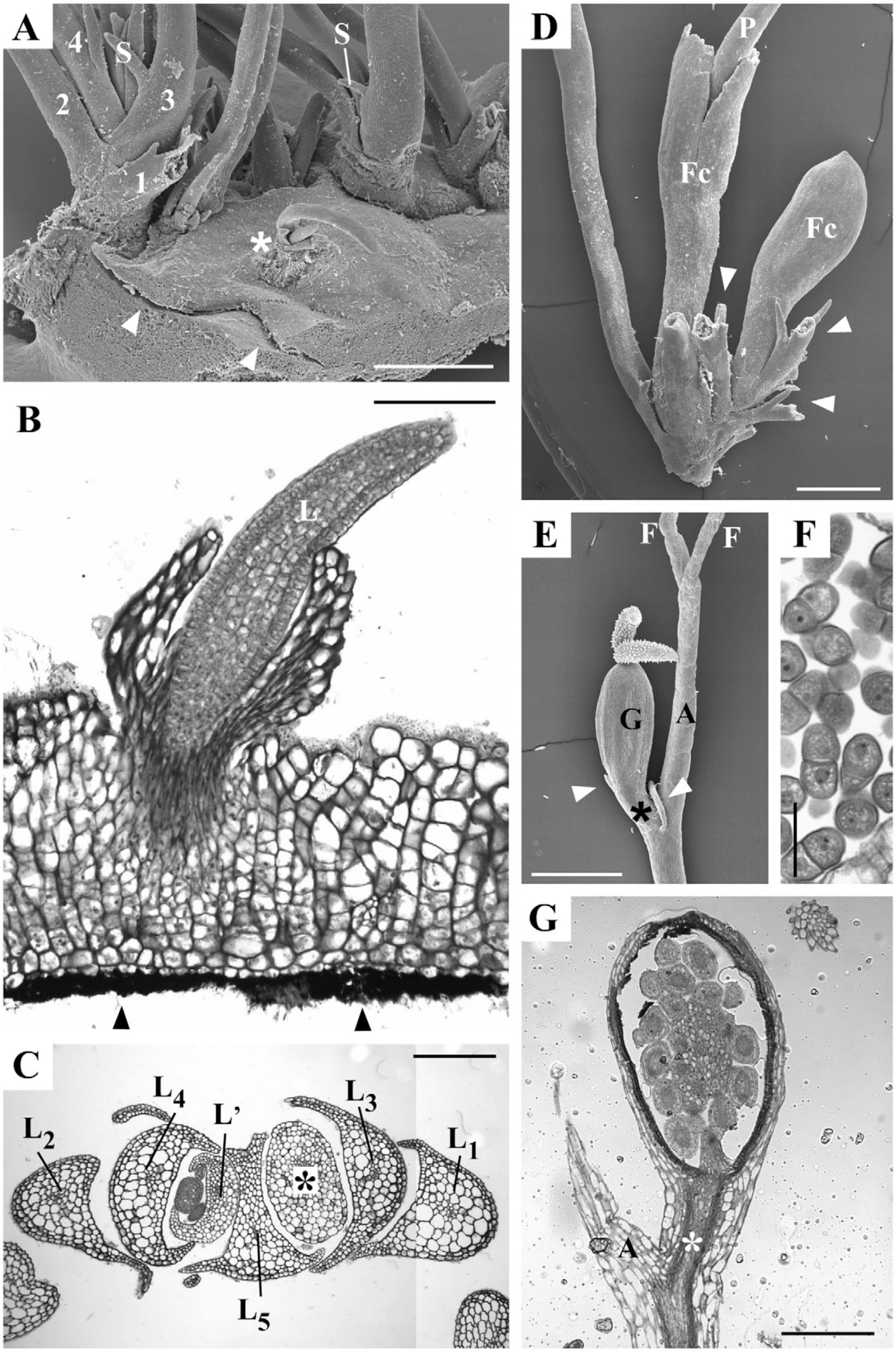


Fig. 6 *Ledermanniella* subg. *Ledermanniella*: *Ledermanniella linearifolia* (GHO-1415). *A*, Crustose roots creeping over each other with narrow margin (arrowheads). Shoots arise from endogenous buds along upper root surface (asterisk). Shoots stay short with leaves (*1–4*) in

was then sister to the clade comprising all aforementioned African species. *Ledermanniella* subg. *Phyllosoma* (*L. ledermannii*, *L.* cf. *bosii*) was paraphyletic to all other Podostemoideae studied from the African continent (including *Dicraeanthus, Djinga*, and *Ledermanniella* subg. *Ledermanniella*). The second cladogram of the combined analysis differed from the above described topology only in the placement of *L. bowlingii* and was otherwise identical. In the second cladogram, *L. bowlingii* was sister to a clade comprising *Djinga*–*L. linearifolia* and *Dicraeanthus*–*L. letouzeyi* instead of being sister to *Dicraeanthus*–*L. letouzeyi* only.

The African, Madagascan, Asian, and Australian Podostemoideae formed a clade together with the American genus *Podostemum*. *Podostemum ceratophyllum* (but not *P. rutifolium*) had a very divergent sequence resulting in a very long branch (fig. 8). The Asian-Australian Podostemoideae formed a monophyletic group. The Weddellinoideae (northern South America) were sister to the Podostemoideae, with the New World genera *Apinagia*, *Marathrum*, *Mourera*, *Oserya*, and *Vanroyenella* as basal members. Podostemoideae and Weddellinoideae were sister to the pantropical Tristichoideae.

Character Optimization of Morphological Characters on the Combined Tree

Each of the three subfamilies possesses a typical number of tepals (fig. 9*A*; CI = 0.75, RI = 0.87). The derived conditions originated only once in Tristichoideae (three tepals in a complete whorl) and Weddellinoideae (five tepals in a complete whorl) but twice in Podostemoideae (two to three tepals on one side of the flower) from the ancestral condition with six to 12 tepals in a complete whorl. The lack of a septum in the ovary (capsule) is a synapomorphy of the African species with one additional origin in the Asian *Hydrobryum micrantherum* (fig. 9*B*; CI = 0.50, RI = 0.90). The inverted orientation of the flower in the bud is a synapomorphy of the African Podostemoideae (fig. 9*C*; CI = 0.66, RI = 0.94). The oblique-inclined flower in the Madagascan species (*E. mangorense*, *T. minutiflora*) represents an intermediate condition between the erect flower (plesiomorphic condition) and the inverted flower in the bud. This character shows a tendency toward reversal in *D. felicis*. The shape of the dorsiventral root has been flattened toward the crustose condition in at least three lineages in parallel (fig. 9*D*; CI = 0.33, RI = 0.50). The fast and slow optimizations yielded different results for the African clade. The fast optimization (shown in fig. 9*D*) suggested that the crustose roots originated once in a subclade of the African Podostemoideae (*D. africanus*, *D. felicis*, *L. letouzeyi*, *L. linearifolia*), with one reversal in *L. bowlingii*. The slow optimization suggested that the crustose root shape originated twice independently, once in the clade comprising *Dicraeanthus* and *L. letouzeyi* and once in the clade comprising *Djinga* and *L. linearifolia*. Crustose roots also evolved twice outside Africa (*Hydrobryum*, *Zeylanidium olivaceum*). The vegetative stem length in Podostemaceae ranges from less than 1 cm to more than 30 cm (fig. 9*E*; CI = 0.14, RI = 0.33). The slow and fast optimizations suggested that the plesiomorphic vegetative stem length in Podostemaceae is 10–30 cm or more and that the vegetative stems have four times been reduced in length to a few millimeters ("loss"). The results of the fast optimization (shown in fig. 9*E*) suggested a loss at the base of the Tristichoideae and at the base of the Asian and African Podostemoideae, with a reversal in the latter, and a secondary loss in *L. linearifolia* and *E. mangorense*. In the slow optimization, the vegetative stem gets lost (i.e., reduced in length to a few millimeters) in *L. linearifolia*, in *E. mangorense*, in the Tristichoideae pro parte (*Malaccotristicha malayana*, *Terniopsis sessilis*), and at the base of the Asian Podostemoideae. Differences between the fast and slow optimizations are restricted to the backbone of the tree and the character states in the Tristichoideae. (i) The fast optimization yields a stem length of more than 10–30 cm from the basal node of the Podostemaceae until the basal node of the Podostemoideae. (ii) In the slow optimization, this character state reaches further up into the tree until the basal node of the African Podostemoideae. In the Tristichoideae, the stem length of more than 10–30 cm is the plesiomorphic condition, with two independent reductions to 1–10 cm in *Tristicha trifaria* and *Dalzellia zeylanica* and a loss of the stem for the node uniting *M. malayana* and *T. sessilis*. The presence of double-sheathed leaves appears to have originated four times within the Podostemoideae (fig. 9*F*; CI = 0.20, RI = 0.66): in *Zeylanidium subulatum*, in the American genus *Podostemum*, in the remaining American Podostemoideae, and in the African Podostemoideae, excluding *Dicraeanthus*, *L.* cf. *bosii*, *L. ledermannii*, and *L. letouzeyi*.

Discussion

Phylogeny and Taxonomy

Both morphology and *matK* sequence data provide phylogenetic signals (figs. 7, 8). The two data sets yielded no conflicting nodes with a bootstrap higher than 70%. For those nodes that do conflict in the morphological data compared to the molecular data, the conflict is probably due to a lack of a strong phylogenetical signal from the morphological

Fig. 6 (*Continued*) distichous order. Note presence of attached lateral stipules (*S*). Scale bar = 1 mm. *B*, Cross section of crustose root with endogenous shoot bud arising from upper surface. *L* = young linear leaf. Arrowheads point to remnants of substrate fixed to the lower root surface. Scale bar = 200 μm. *C*, Cross section of root-borne short shoot with all organs arranged in one plane. After four leaves (L_1–L_4), there is a fifth one (L_5), which is a double-sheathed leaf containing a spathella stalk (asterisk) in the right sheath and a vegetative daughter bud (with L' as first leaf). Scale bar = 300 μm. *D*, Another root-borne short shoot with two stalked and clavate spathellas (*Fc*), the left one ruptured and releasing the elongating flowering stalk (*P* = pedicel). Leaves removed except for stipulate bases (arrowheads). Scale bar = 1 mm. *E*, Flower in anthesis. Stalked ovary (*G*) topped by two papillose stigma lobes. Long andropodium (*A*) carrying two filaments (*F*), anthers out of frame. Note two threadlike tepals (arrowheads) on either side of gynophore (asterisk). Scale bar = 900 μm. *F*, Section of pollen sac with pollen dyads. Scale bar = 40 μm. *G*, Nearly median longitudinal section of postanthetic ovary with gynophore (asterisk) above insertion area of andropodium (*A*). Scale bar = 250 μm.

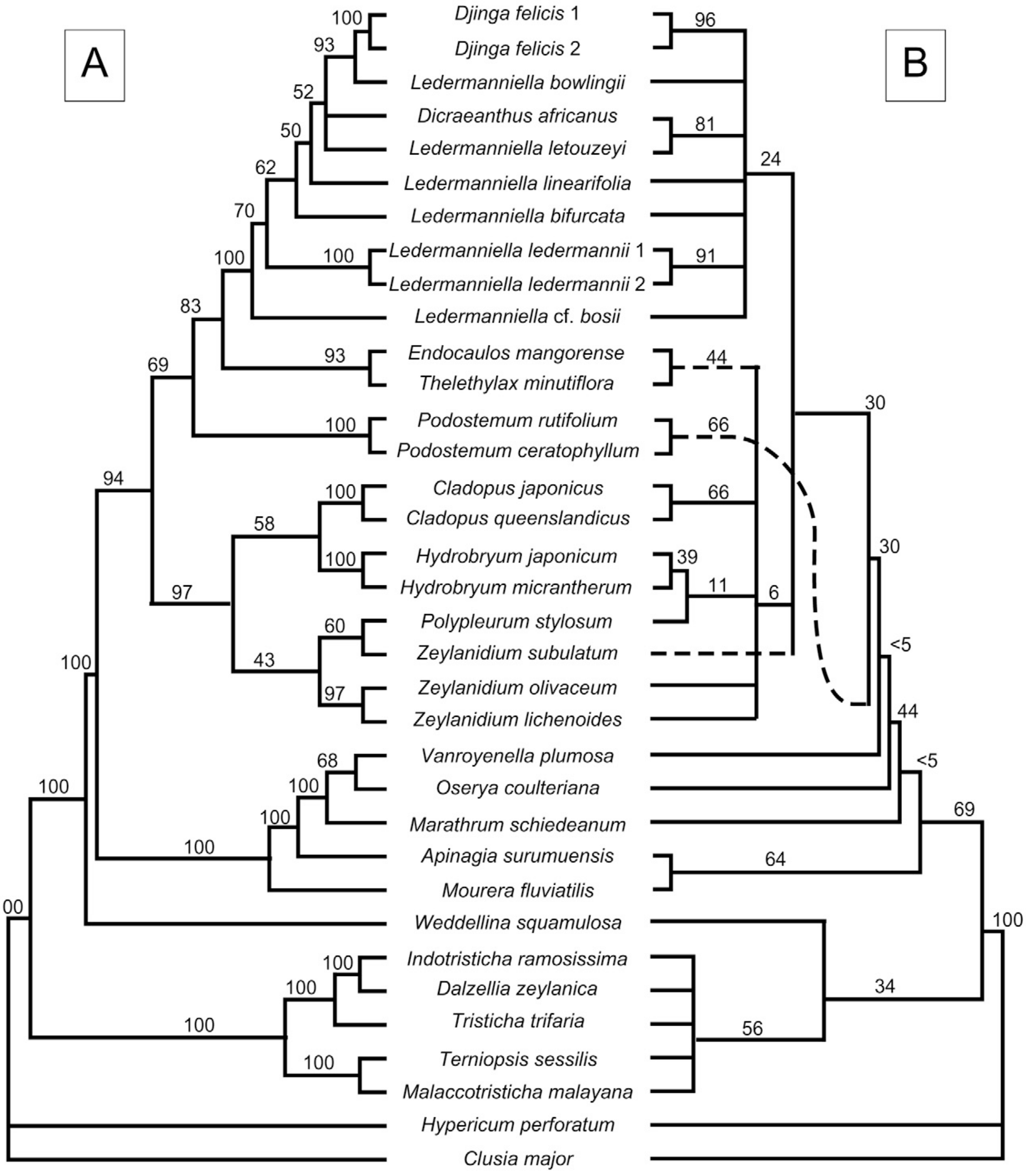
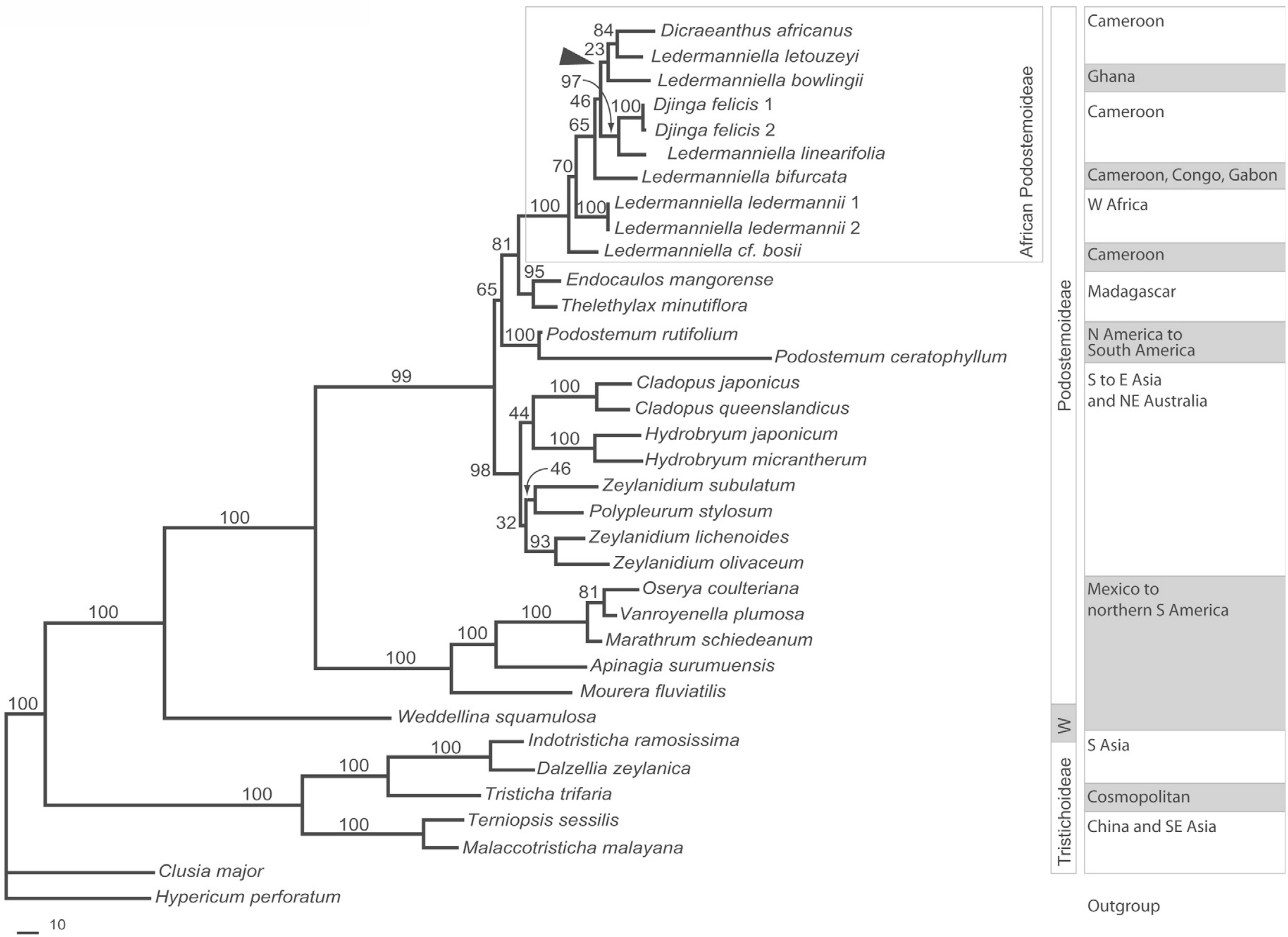


Fig. 7 Results of the separate analysis of the morphological and molecular data. *A*, Strict consensus tree of the two most parsimonious trees based on the molecular data. *B*, Strict consensus tree of 1512 most parsimonious trees based on the morphological data. The conflicting placement in the morphological tree relative to the molecular tree is indicated by broken lines. Bootstrap support is indicated above branches.

characters leading to stochastic resolution of the topology and low support values. The conflicting placement of *Podostemum* is probably a reflection of its morphological characters (Philbrick and Novelo 2004; Moline et al. 2006). The combined tree has strongly supported clades, except for some nodes within the African taxa. Our strict consensus showed a topology similar to that of the phylogenetic hypotheses presented by Kita and Kato (2001, 2004) and Gustafsson et al. (2002), but these studies did not include species from the African continent. Although our taxon sample covered only parts of the African Podostemoideae, we infer from the phylogeny that the generic delimitations require revision.


Dicraeanthus africanus
Ledermanniella letouzeyi
Ledermanniella bowlingii
Djinga felicis 1
Djinga felicis 2
Ledermanniella linearifolia
Ledermanniella bifurcata
Ledermanniella ledermannii 1
Ledermanniella ledermannii 2
Ledermanniella cf. bosii
Endocaulos mangorense
Thelethylax minutiflora
Podostemum rutifolium
Podostemum ceratophyllum
Cladopus japonicus
Cladopus queenslandicus
Hydrobryum japonicum
Hydrobryum micrantherum
Zeylanidium subulatum
Polypleurum stylosum
Zeylanidium lichenoides
Zeylanidium olivaceum
Oserya coulteriana
Vanroyenella plumosa
Marathrum schiedeanum
Apinagia surumuensis
Mourera fluviatilis
Weddellina squamulosa
Indotristicha ramosissima
Dalzellia zeylanica
Tristicha trifaria
Terniopsis sessilis
Malaccotristicha malayana
Clusia major
Hypericum perforatum
84
23
97
46
100
65
70
100
100
81
95
65
100
99
100
44
100
98
46
32
93
81
100
100
100
100
100
100
100
100
100
100
10
African Podostemoideae
Podostemoideae
W
Tristichoideae
Cameroon
Ghana
Cameroon
Cameroon, Congo, Gabon
W Africa
Cameroon
Madagascar
N America to South America
S to E Asia and NE Australia
Mexico to northern S America
S Asia
Cosmopolitan
China and SE Asia
Outgroup

Ledermanniella (with ca. 46 species) is the largest podostemoid genus in Africa. This genus with its two subgenera *Ledermanniella* and *Phyllosoma*, as described by Cusset (1984*a*, 1984*b*), seems to be an artificial one. Our results indicate that the "smooth" subgenus *Ledermanniella* is paraphyletic, with the small genera *Dicraeanthus* (two species) and *Djinga* (monotypic) nested in it. In addition, the "scaly" subgenus *Phyllosoma* (*Ledermanniella* cf. *bosii*, *Ledermanniella ledermannii*) appears to be paraphyletic without the inclusion of the "smooth" subgenus *Ledermanniella* (*Ledermanniella bifurcata*, *Ledermanniella bowlingii*, *Ledermanniella letouzeyi*, *Ledermanniella linearifolia*).

In addition to the imperfect generic delimitation, the problem of adequate species definitions is still very pronounced in African taxa. For example, Cusset's (1987) original diagnosis of *Djinga felicis* was based on a very fragmentary herbarium specimen and is thus very vague, lacking clear diagnostic characters. The three specimens of *Ledermanniella* subg. *Phyllosoma* from Lobé Falls have been determined using the available species circumscriptions. Although the assignment to the subgenus was straightforward, the species definitions proved to be inadequate for the variation found in the specimens. The collection GAR-021018-01 contained specimens of subgenus *Phyllosoma* with scaly stems of up to 15 cm. These relatively large plants were determined as *Ledermanniella* cf. *bosii* in this study, although this species is still badly defined and known only from two South Cameroonian waterfalls (Lobé, Ntem).

Morphological Evolution in African Podostemoideae

The optimization of morphological data onto the combined tree of Podostemaceae allows us to tentatively trace the evolution of certain morphological traits (fig. 9).

Tepal number (fig. 9A*).* The distribution of the different tepal numbers per flower (fig. 9*A*) is congruent with the taxonomic delimitation of the subfamilies (Cook 1996; Rutishauser 1997; Kita and Kato 2001). This character has long been used in the taxonomic treatment of the family (Engler [1928] 1930), and it is consistent with the combined phylogeny presented here.

Unilocular ovaries (fig. 9B*).* There are three carpels (capsule valves) in the Tristichoideae and two carpels (capsule valves) in Podostemoideae and Weddellinoideae. The prominent central placenta is often completely covered with ovules. The ovary of many Podostemoideae is bilocular, with a thin septum. Bilocular ovaries represent the plesiomorphic condition in podostemoids, occurring in all New World members (except *Castelnavia*) as well as all non-African Old World members (except *Hydrobryum micrantherum*). Cusset (e.g., 1984*a*, 1984*b*, 1987) emphasized the number of locules to delimit African genera. The majority of African Podostemoideae (including all members considered in this study) show unilocular ovaries. Bilocular ovaries seem to be restricted to the endemic Madagascan genera *Endocaulos* and *Thelethylax*, as well as to *Saxicolella pro parte* (Ameka et al. 2002) and *Sphaerothylax* (Jäger-Zürn 2000). Ameka et al. (2003) found a rudimentary septum in the basal part of the otherwise unilocular ovary of *L. bowlingii*. Thus, the single locule (as observable in many African podostemoids) is probably due to septum loss, i.e., a precocious growth stop of the septum, during preanthesis development.

Inverted flower buds (fig. 9C*).* The Podostemoideae are distinguishable from the two other subfamilies by the presence of a thin nonvascularized cover, the spathella, which encloses the young flower (fig. 5*F*). The inverted flowers in the spathella are a synapomorphy of various African (Madagascan) members (indicated by arrow in fig. 9*C*), whereas erect flower buds are plesiomorphic. In many Podostemoideae (all non-African groups), the flower buds are upright (erect) and sessile (or nearly so) inside the unruptured spathella. The flower is completely inverted in most African Podostemoideae, including *Dicraeanthus* and all members of the large genus *Ledermanniella*. Only 11 podostemoid species in Africa and Madagascar have erect (or slightly inclined) flower buds inside the spathella (Ameka et al. 2002). Floral inversion is correlated with the presence of an elongating flower stalk (pedicel) inside the young spathella (Engler [1928] 1930; Cusset 1987). Growth of the flower bud and pedicel elongation usually lead to the rupture of the spathella. Few African Podostemoideae are able to continue with spathella dilatation and elongation for a while, a fact not yet described in literature. This allows the young flower of *L. ledermannii* to give up the completely inverted position by turning upward and becoming nearly erect already within the intact spathella before anthesis (fig. 2*E*). An intermediate condition of flower bud position inside the spathella is found in the Madagascan genera *Endocaulos* and *Thelethylax*, again in the monotypic genus *Letestuella*, from Western to Southern Africa, where the flower buds are weakly to strongly inclined but never totally inverted (Cusset 1973). Similarly, the flower buds in *Djinga* are oblique (i.e., only slightly inclined) in the spathella (fig. 9*C*).

Creeping and flattened photosynthetic roots (fig. 9D*).* There are a few podostemaceous species that lack obvious roots, for example, *Dalzellia zeylanica* (Tristichoideae) and *Mourera fluviatilis* (basal Podostemoideae), although other species in the same genera are clearly provided with roots (Warming 1899; Rutishauser and Grubert 1994; Mathew et al. 2001). In contrast to many American members (e.g., *Podostemum* spp.) and a few non-American ones (e.g. *Cladopus* spp., *Thelethylax minutiflora*), the roots of most Old World Podostemoideae are devoid of an obvious cap (fig. 2*A*; Cusset 1974; Rutishauser and Pfeifer 2002; Rutishauser and Moline 2005; Koi et al. 2006; Moline et al. 2006). These roots are dorsiventrally flattened to a considerable degree. The roots of most non-American podostemoids are ribbons with endogenous shoot buds and exogenous lobes (such as

Fig. 8 One of the two most parsimonious trees based on the combined molecular and morphological data. Bootstrap support is indicated above branches, and branch length is proportional to the amount of changes. The three subfamilies of Podostemaceae are indicated on the right of the tree. *W* indicates the subfamily Weddellinoideae. The generalized distribution of the included species is given at the far right. The arrowhead indicates the collapsed node in the strict consensus tree.

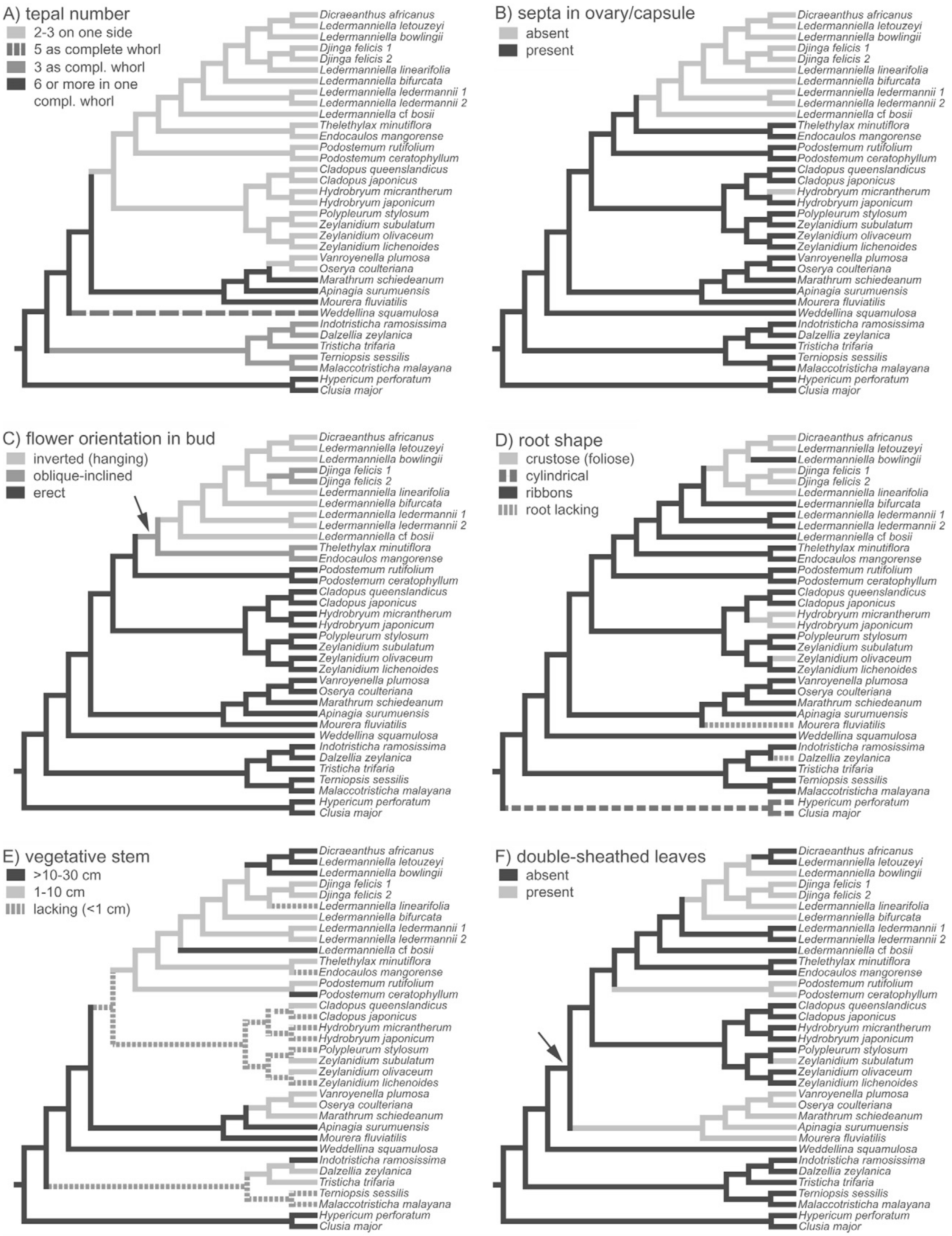


Fig. 9 Results of the character optimization for six selected morphological characters. Legends indicate the different characters and their character states. For explanation, see "Results" and "Discussion"; for the coding of the morphological characters, see appendixes B and C.

fingerlike holdfasts and/or lateral root lobes) along the flanks (fig. 2*B*, 2*C*; fig. 4*D*). Less frequently, the roots of non-American podostemoids are completely flattened resembling crustose (foliose) lichens, covering the rock like a carpet. They are provided with endogenous shoot buds that also arise from the upper root surface (fig. 1*A*, 1*B*; fig. 5*A*; fig. 6*A*, 6*B*). It is possible to arrange the roots of most podostemoids along a transformation series. A progressive elaboration of the root is most obvious in Asian Podostemoideae, where it is accompanied by a size reduction of the root-borne shoots (fig. 9*E*; Kita and Kato 2004; Rutishauser and Moline 2005; Koi et al. 2006). In the African Podostemoideae studied so far, crustose roots evolved once (or twice) and occur in *Dicraeanthus*, *Djinga*, and many species of *Ledermanniella* subg. *Ledermanniella* (fig. 9*D*), with a possible reversal in *L. bowlingii* (Ghana), which instead has broad root ribbons (width up to 7 mm; fig. 4*D*; Ameka et al. 2003). Crustose roots are also found in other African taxa not included in this study, e.g., species of *Ledermanniella* subg. *Ledermanniella* (such as *Ledermanniella thalloidea*), *Ledermanniella* subg. *Phyllosoma* (such as *Ledermanniella gabonensis*, *Ledermanniella torrei*), and also in some but not all species of *Macropodiella*, *Sphaerothylax*, and *Stonesia* (Warming 1891; Cusset 1987; Schnell 1998; Jäger-Zürn 2000; Rutishauser et al. 2004).

Prominent shoots with nonaxillary branching and double-sheathed leaves (fig. 9E, 9F*).* In most angiosperms, axillary stem branching entails the production of a lateral shoot bud in the distal axil of a subtending, single-sheathed leaf. Such "typical" axillary branching occurs only rarely in Podostemoideae, e.g., in *Saxicolella submersa* (Ameka et al. 2002). In many podostemoids (but never in the other two subfamilies), there are leaves with two sheaths that are inserted laterally and opposite each other. Such leaves have been called double sheathed or "dithecous" by Warming (1881) and others (Rutishauser 1997 and literature cited therein). The occurrence of double-sheathed leaves (among single-sheathed ones) allows the stem to branch by a peculiar process that, for lack of a more appropriate term, was called "bifurcation" (Rutishauser et al. 2003). As long as a stem unit (shoot order) is developing single-sheathed leaves, it grows in a monopodial manner. Then, a double-sheathed leaf appears in a terminal (or nearly terminal) position, giving rise to new shoot modules (daughter shoots) in each sheath (fig. 4*C*), or one of the two sheaths is occupied by a flower instead of a daughter shoot (fig. 6*C*). The presence of double-sheathed leaves in Podostemoideae seems to be restricted to taxa with stems at least 1–10 cm long, whereas most taxa having very short root-borne shoots only (including most Asian podostemoids) are devoid of double-sheathed leaves because short shoots less than 1 cm long usually do not branch and produce a single terminal flower each (fig. 9*E*, 9*F*; Jäger-Zürn 2000). This indicates that these two characters are correlated, but then the character optimization of the double-sheathed leaves on the combined tree might not be reliable. It seems more parsimonious to assume that double-sheathed leaves have originated only once at the base of the Podostemoideae (indicated by an arrow in fig. 9*F*) instead of the four independent origins suggested by the optimization. Indeed, when *L.* cf. *bosii* is coded as having double-sheathed leaves—a probable coding for this still little-known species—the optimization yields a single origin of double-sheathed leaves at the base of the Podostemoideae (data not shown). There are few African exceptions regarding the correlation of stem branching and presence of double-sheathed leaves. *Ledermanniella linearifolia* has double-sheathed leaves, although there are only short shoots (less than 1 cm high) in this species (fig. 6*C*, 6*D*). *Dicraeanthus africanus* and *L. letouzeyi* have shoots of 30 cm or more in length but lack double-sheathed leaves, although *L. bowlingii* as their common sister has them (fig. 4*C*). It seems as if *D. africanus* and *L. letouzeyi* could have lost double-sheathed leaves, instead using another and perhaps more efficient mean to produce lateral shoots (floral short shoots) by endogenous formation inside the stem cortex (fig. 1*D*, 1*E*; fig. 5*D*, 5*E*).

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Appendix A

Table A1

List of Voucher Specimens

Taxon	Collection number	Origin	Description	Collection date
Ingroup: African Podostemaceae-Podostemoideae:				
Dicraeanthus africanus Engler	GHO-1413	Cameroon (near coast), Lobé Falls, ca. 10 km from Kribi Campo Road; altitude 15 m	Herbaceous plant with shoots up to 75 cm long fixed on rock	February 18, 2003, by Ghogue
Djinga felicis C. Cusset (=*D. felicis* 1)	GAR-021020-08	Cameroon (northwest), Juafef Waterfall in Fundong; altitude 1400 m	Very small aquatic herb fixed on the rock in water-saturated atmosphere	October 20, 2002, by Ghogue, Ameka, and Rutishauser
D. felicis C. Cusset (=*D. felicis* 2)	GAR-021021-09	Cameroon (northwest), Anyanjua River upper waterfall; altitude 1500 m; 5 km east of Belo, 23 km south of Fundong	Aquatic small herb with erect small branches (up to 10 cm tall) in water-saturated atmosphere	October 21, 2002, by Ghogue, Ameka, and Rutishauser
Ledermanniella subg. *Ledermanniella*:				
Ledermanniella bifurcata (Engler) C. Cusset	GHO-1597	Cameroon (Nyong), Makak; altitude 600 m; waterfall of very fast stream passing at the middle of the forest reserve; invasive on a wet rock in a rapid stream	Erect small herb ca. 1 cm tall; abundant flowers	January 24, 2004, by Ghogue
Ledermanniella bowlingii (J. B. Hall) C. Cusset	AR-021010	Ghana (Kwahu District), Asuboni River, 4 km on road from Kwahu Nteso to Kofi Opare village; altitude 20 m	Aquatic herb fixed on rock in flowing water, with shoots up to 1 m long	October 10, 2002, by Ameka and Rutishauser
Ledermanniella letouzeyi C. Cusset	GAR-021023-12	Cameroon (southwest), Ngomboku waterfall, ca. 15 km north of Nyasoso; altitude 1200 m	Aquatic herb in river rapids fixed on the rock; grows with leaves up to 30 cm long	October 23, 2002, by Ghogue, Ameka, and Rutishauser
Ledermanniella linearifolia Engler	GHO-1415	Cameroon (near coast), Lobé Falls, ca. 10 km from Kribi Campo Road; altitude 15 m	Bright green plant forming a dense lawn fixed on rock	February 16, 2003, by Ghogue
Ledermanniella subg. *Phyllosoma*:				
Ledermanniella cf. *bosii* C. Cusset	GAR-021018-01	Cameroon (near coast), Lobé Falls, 10 km south of Kribi, north side of waterfall; altitude 15 m	Aquatic herb fixed on the rock in flowing water, ca. 15 cm long	October 18, 2002, by Ghogue, Ameka, and Rutishauser
Ledermanniella ledermannii C. Cusset (=*L. ledermannii* 1)	GAR-021018-02	Cameroon (near coast), Lobé Falls, 10 km south of Kribi, south side of waterfall; altitude 15 m	Small aquatic herb fixed on rock in flowing water, with stems ca. 1.5 cm tall	October 18, 2002, by Ghogue, Ameka, and Rutishauser
Ledermanniella ledermannii C. Cusset (=*L. ledermannii* 2)	GHO-1414	Cameroon (near coast), Lobé Falls, ca. 10 km from Kribi, north side of waterfall; altitude 15 m	Branched erect plant fixed on rock in flowing water with stems up to 5 cm tall	February 16, 2003, by Ghogue
Outgroup: Clusiaceae:				
Clusia major (Jacq.) L.	Thiv 4094 (STU)	Germany, Stuttgart; cultivated in Wilhelma		July 1, 2004, by Thiv
Hypericum perforatum L.	Thiv 4015 (STU)	Germany, Stuttgart, north meadow between Pragsattel and Rosensteinpark; altitude 300 m		June 28, 2004, by Thiv

Table A2

Species Included in the Cladistic Analysis

Species	Author	Synonym	GenBank accession	Reference
Apinagia surumuensis	(Engler) Royen		AB048367	Kita and Kato 2001
Cladopus japonicus	Imamura		AB038189	Kita and Kato 2001
Cladopus queenslandicus	(Domin) C. D. K. Cook and Rutish	*Torrenticola queenslandica*	AB038199	Kita and Kato 2001
Clusia major	(Jacq.) L.		DQ168430	This study
Dalzellia zeylanica	(Gardner) Wight		AB038190	Kita and Kato 2001
Dicraeanthus africanus	Engler		DQ168442	This study
Djinga felicis 1	C. Cusset		DQ168433	This study
D. felicis 2	C. Cusset		DQ168434	This study
Endocaulos mangorense	(Perrier) C. Cusset		AB038191	Kita and Kato 2001
Hydrobryum japonicum	Imamura		AB038192	Kita and Kato 2001
Hydrobryum micrantherum	(Royen) C. D. K. Cook and Rutish	*Synstylis micranthera*	AB038205	Kita and Kato 2001
Hypericum perforatum	L.		DQ168438	This study
Indotristicha ramosissima	(Wight) Royen		AB038193	Kita and Kato 2001
Ledermanniella bifurcata	(Engler) C. Cusset		DQ168439	This study
Ledermanniella bowlingii	(J. B. Hall) C. Cusset		DQ168429	This study
Ledermanniella cf. *bosii*	C. Cusset		DQ168431	This study
Ledermanniella ledermannii 1	(Engler) C. Cusset		DQ168432	This study
L. ledermannii 2	(Engler) C. Cusset		DQ168442	This study
Ledermanniella letouzeyi	C. Cusset		DQ168435	This study
Ledermanniella linearifolia	(Engler) C. Cusset		DQ168437	This study
Malaccotristicha malayana	(Dransfield and Whitmore) C. Cusset and G. Cusset		AB048827	Kita and Kato 2001
Marathrum schiedeanum	(Cham.) Tul.		AB038195	Kita and Kato 2001
Mourera fluviatilis	Aublet		AB038200	Kita and Kato 2001
Oserya coulteriana	Tul.		AB048375	Kita and Kato 2001
Podostemum ceratophyllum	Michx.		DQ168440	Moline et al. 2006
Podostemum rutifolium	Warming			
Subsp. *ricciforme*	(Liebm.) A. Novelo and C. T. Philbrick	*Podostemum ricciforme*	DQ168441	Moline et al. 2006
Polypleurum stylosum	(Wight) J. B. Hall		AB066174	Kita and Kato 2004
Terniopsis sessilis	H. C. Chao		AB048377	Kita and Kato 2001
Thelethylax minutiflora	(Tul.) C. Cusset		AB038196	Kita and Kato 2001
Tristicha trifaria	(Bory ex Willd.) Spreng.		AB038198	Kita and Kato 2001
Vanroyenella plumosa	A. Novelo and C. T. Philbrick		AB048378	Kita and Kato 2001
Weddellina squamulosa	Tul.		AB038206	Kita and Kato 2001
Zeylanidium lichenoides	(Kurz.) Engler		AB048828	Kita and Kato 2001
Zeylanidium olivaceum	Engler		AB038207	Kita and Kato 2001
Zeylanidium subulatum	Gardner	*Podostemum subulatum*	AB038202	Kita and Kato 2001

Appendix B

Table B1

Morphological Data Matrix Used in This Study

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39
Dicraeanthus africanus	1	1	0	0	2	1	0	2	1	0	1	2	0	1	1	0	0	0	1	2	0	0	1	0	1	0	2	0	1	1	0	1	0	1	0	1	(01)	1	2
Ledermanniella letouzeyi	1	1	0	0	2	1	0	2	1	0	1	2	0	1	1	0	0	0	1	2	0	0	1	0	1	0	2	0	(01)	1	0	3	0	1	0	0	0	0	0
Ledermanniella bowlingii	1	1	0	0	2	1	0	3	0	0	1	2	0	1	1	0	0	0	1	2	0	0	0	0	1	0	2	1	(01)	(01)	0	2	(01)	0	0	1	(01)	(01)	1
Djinga felicis 1	1	1	0	0	2	1	0	3	0	0	(01)	2	0	1	1	1	0	0	0	1	1	0	1	0	1	0	(01)	1	(012)	(01)	0	1	1	0	0	1	0	1	2
D. felicis 2	1	1	0	0	2	1	0	3	0	0	(01)	2	0	1	1	1	0	0	0	1	1	0	1	0	1	0	1	1	(012)	(01)	0	1	1	0	0	1	0	1	2
Ledermanniella linearifolia	1	1	0	0	2	1	0	2	1	0	1	2	0	1	1	(01)	0	0	1	2	0	0	1	0	1	0	0	1	2	0	0	1	1	0	0	1	0	1	2
Ledermanniella bifurcata	1	1	0	0	2	1	0	2	1	0	0	2	0	1	1	0	0	0	1	2	0	0	0	0	1	0	1	1	2	0	0	1	(01)	0	0	1	0	(01)	2
Ledermanniella ledermannii 1	1	1	1	0	2	1	0	2	1	0	1	2	0	1	1	0	0	0	1	2	0	0	0	0	0	0	1	0	(02)	2	0	1	(01)	0	0	1	0	0	0
L. ledermannii 2	1	1	1	0	2	1	0	2	1	0	1	2	0	1	1	0	0	0	1	2	0	0	0	0	0	0	1	0	(02)	2	0	1	(01)	0	0	1	0	0	0
Ledermanniella cf. *bosii*	1	1	0	0	2	1	0	2	1	0	1	2	0	1	1	0	0	0	1	2	0	0	0	0	0	0	2	0	(02)	0	0	2	(01)	0	0	1	0	0	0
Endocaulos mangorense	1	1	0	0	2	1	0	2	1	0	1	2	0	1	2	1	0	1	0	1	2	0	0	?	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0
Thelethylax minutiflora	1	1	0	0	2	1	0	2	1	(01)	1	2	0	1	1	(01)	0	1	0	2	2	0	0	1	0	0	1	0	0	(01)	0	3	(01)	1	0	(01)	(01)	0	0
Podostemum ceratophyllum	1	1	0	0	2	1	0	2	1	1	1	2	0	1	1	1	0	1	0	0	0	0	0	1	0	0	2	1	2	0	0	2	0	0	0	1	0	1	1
Podostemum rutifolium	1	1	0	0	2	1	0	2	1	1	1	2	0	1	1	1	0	1	0	0	0	0	0	1	0	0	1	1	2	0	0	0	(01)	0	0	1	0	1	1
Cladopus japonicus	1	1	0	0	2	1	0	3	0	0	1	2	0	0	0	1	0	1	0	0	1	0	0	1	1	0	0	0	0	0	0	0	1	0	0	1	0	(01)	2
Cladopus queenslandicus	1	1	0	0	2	1	0	3	0	0	1	2	0	1	(12)	1	0	1	0	0	1	0	0	1	1	0	1	0	0	0	0	0	1	0	0	1	0	(01)	2
Hydrobryum japonicum	1	1	0	0	2	1	0	2	1	0	1	2	0	1	2	0	0	1	0	0	0	0	1	0	1	0	0	0	0	0	0	0	1	0	0	1	0	0	0
Hydrobryum micrantherum	1	1	0	0	2	1	0	3	0	0	1	2	0	1	2	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	1	0	0	1	0	0	0
Zeylanidium subulatum	1	1	0	0	2	1	0	2	0	0	1	2	0	1	1	1	0	1	0	0	0	0	0	0	1	0	1	1	2	0	0	1	1	0	0	1	0	0	0
Polypleurum stylosum	1	1	0	0	2	1	0	2	0	0	1	2	0	1	1	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	1	0	0	0
Zeylanidium lichenoides	1	1	0	0	2	1	0	(23)	0	0	1	2	0	1	1	1	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	1	0	0	0
Zeylanidium olivaceum	1	1	0	0	2	1	0	2	0	0	1	2	(01)	1	1	1	0	1	0	0	0	0	1	0	1	0	1	0	0	0	0	0	1	0	0	1	0	0	0
Oserya coulteriana	1	1	0	0	2	1	0	(23)	(01)	0	0	2	0	1	1	1	0	1	0	0	0	0	0	1	0	0	1	1	2	0	0	(12)	0	0	1	1	1	1	1
Vanroyenella plumosa	(01)	1	0	0	2	1	0	2	0	1	0	2	0	1	1	0	0	1	0	0	0	0	0	1	0	1	1	1	2	1	1	(23)	0	0	0	0	1	1	1
Marathrum schiedeanum	0	1	0	1	0	(01)	1	1	0	0	0	2	0	1	1	0	0	1	0	0	0	0	0	1	0	1	1	1	2	2	(01)	(23)	0	0	0	0	1	1	1
Apinagia surumuensis	0	1	0	1	0	0	1	0	0	0	0	2	0	1	1	0	0	1	0	0	0	0	0	1	0	0	2	1	2	2	0	2	0	1	0	0	1	1	1
Mourera fluviatilis	0	1	0	1	0	0	(12)	0	0	0	0	2	0	1	1	0	0	1	0	0	0	1	. . .	. . .	. . .	0	2	1	3	0	0	3	0	1	0	0	1	1	1
Weddellina squamulosa	0	0	0	1	1	0	1	(12)	0	0	0	1	2	1	1	0	0	1	0	. . .	0	0	0	?	0	0	2	0	0	0	0	0	1	0	0	2	0	0	0
Dalzellia zeylanica	1	0	1	1	3	0	(01)	(23)	0	0	0	0	0	1	1	. . .	1	1	0	. . .	0	1	. . .	. . .	. . .	1	1	0	0	1	0	0	1	0	0	2	0	0	0
Indotristicha ramosissima	1	0	1	1	3	0	1	3	0	0	0	0	0	1	1	. . .	1	1	0	. . .	0	0	0	1	0	0	2	0	(01)	(01)	0	0	1	0	1	2	0	0	0
Tristicha trifaria	1	0	0	1	3	0	0	(23)	0	0	0	0	0	1	1	. . .	1	1	0	. . .	0	0	0	0	0	0	1	0	(01)	0	0	0	1	0	1	2	0	0	0
Malaccotristicha malayana	1	0	0	1	3	0	1	2	0	0	0	0	0	1	1	. . .	1	1	0	. . .	0	0	0	1	0	0	0	0	0	0	0	0	1	0	1	2	0	0	0
Terniopsis sessilis	1	0	0	1	3	0	1	2	0	0	0	0	0	1	1	. . .	1	1	0	. . .	0	0	0	1	0	0	0	0	0	0	0	0	1	0	1	2	0	0	0
Clusia major	0	0	0	1	0	0	2	0	0	0	0	1	0	0	0	. . .	1	1	0	. . .	1	0	2	1	0	0	2	0	1	0	0	2	1	. . .	0	0	0	0	0
Hypericum perforatum	0	0	0	1	0	0	2	0	0	0	0	1	0	0	0	. . .	1	1	0	. . .	0	0	2	1	0	0	2	0	1	0	0	1	1	1	0	0	0	0	0

Note. Taxa are in the same order as in the trees in figures 7 and 8; the two outgroup species are included at the end of the table. Two or three numerals in parentheses indicate that certain characters have two or three states that are found in a single taxon.

Appendix C

The 39 Morphological Characters Used in This Study (See Data Matrix in Table B1)

1. Showiness of flower: 0 = white to pink, 1 = greenish, inconspicuous.
2. Spathella: 0 = absent, 1 = present.
3. Cupule: 0 = absent, 1 = present.
4. Shape of tepals: 0 = linear, often elongate, 1 = triangular, often short.
5. Tepal number: 0 = six or more in one complete whorl, 1 = five as complete whorl, 2 = two or three on one side, 3 = three as complete whorl.
6. Perianth symmetry: 0 = radial as whorl around ovary, 1 = confined to one side.
7. Stamen whorl(s): 0 = incomplete, restricted to one side, 1 = at least one complete whorl, 2 = additional stamens with centrifugal initiation.
8. Maximal stamen number: 0 = >13, 1 = 3–12, 2 = two (or three but never more), 3 = one.
9. Andropodium: 0 = absent, 1 = present.
10. Third tepal on andropodium: 0 = absent, 1 = present.
11. Pollen union: 0 = monads, 1 = dyads.
12. Pollen apertures: 0 = pantoporate (polyporate with up to 12 pores), 1 = tricolporate, 2 = tricolpate (or tetracolpate, pentacolpate).
13. Shape of stigma lobes: 0 = each lobe entire, linear or conical, 1 = lobes branched (multilobed), 2 = one style with capitate stigma.
14. Ribs of mature capsule: 0 = absent, 1 = present.
15. Number of ribs per capsule valve: 0 = none observable, 1 = three ribs per valve, 2 = five or more ribs per valve.
16. Valve symmetry (if two carpels present): 0 = two equal valves (isolobous), 1 = two unequal valves, one persistent.
17. Carpel number per ovary: 0 = two carpels, 1 = three carpels.
18. Presence of septa in ovary and capsule: 0 = absent (unilocular ovary), 1 = present (bilocular or trilocular ovary).
19. Gynophore: 0 = absent, 1 = present (at least one-sixth of ovary length).
20. Ovary position of flower buds in spathella: 0 = erect, 1 = oblique inclined, 2 = inverted (hanging).
21. Ovary shape: 0 = fusiform (spindle-shaped) to ellipsoidal (at least twice as long as thick), 1 = globose to subglobose (less than twice as long as thick), 2 = obovoid, tapering toward base.
22. Roots: 0 = present, 1 = absent.
23. Root shape: 0 = narrow to broad ribbons with outgrowth of endogenous shoots (flowers) only along flanks, 1 = crustose (disklike) with outgrowth of endogenous shoots (flowers) on upper surface, 2 = cylindrical and subterranean.
24. Root cap: 0 = absent, 1 = present (at least rudimentary).
25. Root branching: 0 = endogenous, 1 = exogenous outgrowth of daughter roots (lobes).
26. Stem shape or rosette symmetry (cross section): 0 = cylindrical or nearly so, 1 = flattened, dorsiventral, disklike.
27. Vegetative stem length (including ramuli in Tristichoideae): 0 = lacking or <1 cm, 1 = 1–10 cm, 2 = at least 10–30 cm.
28. Double-sheathed leaves: 0 = absent (or not yet observed), 1 = present.
29. Inflorescence branching: 0 = root-borne short shoots with few leaves (scales) and one terminal flower, 1 = lateral buds along elongated leafy stem, 2 = forked stem due to double-sheathed leaves, 3 = spikelike with basipetal initiation of double-sheathed bracts.
30. Flower bud protection and origin along stem: 0 = free leaf sheaths in addition to spathella, 1 = endogenous origin in stem cortex, 2 = endogenous pockets between fused leaf sheaths.
31. Flower number per sheath site: 0 = only one flower, 1 = two to nine flowers in fascicle.
32. Maximum leaf length (except ramuli in Tristichoideae): 0 = <1 cm, 1 = 1–5 cm, 2 = 6–20 cm, 3 = >20 cm.
33. Blade division of foliage leaves (except ramuli in Tristichoideae): 0 = pinnate (or plumose) or forked once (or more), 1 = simple, broad, or threadlike (filamentous).
34. Ultimate leaf divisions: 0 = threadlike (except hair brush), 1 = slightly flattened with (hairy) groove.
35. Scales on leaf rachides: 0 = no additional scales present, 1 = scales present, irregular to pseudowhorls.
36. Number of parallel vascular bundles in (broadened) leaf portions: 0 = two or more bundles, 1 = only one bundle, 2 = no vascular tissue in leaves.
37. Blade vernation (circinate): 0 = tip and subunits straight, 1 = tip and lateral lobes coiled.
38. Stipule presence: 0 = absent (sheath only), 1 = present (any type, for at least some leaves).
39. Stipule type: 0 = absent (sheath only), 1 = intrapetiolar sheathing stipule (i.e., median or axillary stipule), 2 = two lateral teeth attached to leaf sheath.

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