

PHYLOGENETIC RELATIONSHIP AND MORPHOLOGY OF *DALZELLIA GRACILIS* (PODOSTEMACEAE, SUBFAMILY TRISTICHOIDEAE) WITH PROPOSAL OF A NEW GENUS

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Dalzellia gracilis, an enigmatic species of Podostemaceae, is characterized by the subcylindrical creeping roots and dorsiventral ribbonlike shoots borne on the roots. To reveal the phylogenetic relationships of *D. gracilis*, molecular phylogenetic and morphological analyses were performed. A *matK* tree indicates that it is most likely sister to a clade of its congeners and *Indotristicha ramosissima*. *Dalzellia gracilis*, like *Tristicha*, has subcylindrical, capless roots, and it exhibits an endogenous developmental pattern in both shoots and associated holdfasts shared by *Terniopsis*, *Indotristicha*, and *Tristicha*. The dorsiventral ribbonlike adventitious shoots, with the dorsiventral compressed shoot apical meristem producing dimorphic leaves on the dorsal and ventral-lateral sides, are extremely similar to the crustose shoots of the other species of *Dalzellia*. *Dalzellia gracilis* is devoid of rosettes, although the other congeners have them. The many free leaves subtending the base of the pedicel are also unique to the species, whereas the trimerous flower is common to subfamily Tristichoideae. On the basis of these molecular and morphological results, we describe a new genus *Indodalzellia* for *D. gracilis* to draw attention to these morphological distinctions. *Dalzellia*, *Indodalzellia*, and *Indotristicha* have remarkable morphological differences compared to the inconspicuous morphological diversity in *Tristicha* and *Terniopsis* and between the two genera.

Keywords: *Dalzellia*, *Indodalzellia*, molecular phylogeny, morphology, Podostemaceae, taxonomy.

Online enhancement: appendix.

Introduction

Dalzellia gracilis Mathew, Jäger-Zürn, & Nileena was recently described from Kerala, southern India, as an enigmatic species of subfamily Tristichoideae of the unusual aquatic angiosperm family Podostemaceae (Mathew et al. 2001). These aquatic plants are submerged, adhering to rock surfaces, in the rapids and waterfalls of the tropics and subtropics during the rainy season and are then exposed and flower during the dry season, when the water level is lower. *Dalzellia gracilis* has a special set of characters. It differs from *Dalzellia zeylanica* (Gardner) Wight and other congeners in having roots and lacking cupules, i.e., special envelopes containing flower buds (Rutishauser 1997; Jäger-Zürn and Mathew 2002). On the other hand, *D. gracilis* and *D. zeylanica* share flattened, dorsiventral shoots bearing small dorsal leaves and large marginal leaves, and they are both leafless on the ventral surface (Jäger-Zürn 1995, 1997; Mathew and Satheesh 1997). On the basis of these shared characters, the two species were treated as congeneric. Mathew et al. (2001) suggested that *D. gracilis* links the rootless *D. zeylanica* to the rooting members of the subfamily.

A molecular phylogenetic analysis showed that *D. zeylanica* is sister to *Indotristicha ramosissima* (Wight) P. Royen, among the species examined (Kita and Kato 2001). In marked contrast to that of *Dalzellia*, however, the vegetative plant of *I. ramosissima* consists of a branched, subcylindrical root with markedly large, radially organized shoots on the flank (Rutishauser and Huber 1991). Therefore, *D. gracilis* may be a key species that is involved in the remarkable diversification in the subfamily Tristichoideae, but it has not been examined by phylogenetic studies. This study examines the molecular phylogenetic relationships of *D. gracilis* and provides additional morphological and anatomical information. The results lead us to propose a new genus for *D. gracilis*.

Material and Methods

Material

Plants of *Dalzellia gracilis* for morphological observations were collected in February 2005 and December 2006 in the Punnathurthi River, a tributary of the Periyar River at Urulathannayy (near Pooyamkutty), Idukki District, Kerala, India. Vouchers are deposited in the Herbarium (CALI) of the Department of Botany, University of Calicut (Kerala, India), and the Herbarium (TNS), Department of Botany, National Museum of Nature and Science (Tsukuba, Japan). We also used material of reproductive stages (MRPII/430, MRPII/470) collected by

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C. R. Mathew from the same river and fixed in 15% formaldehyde solution (Mathew et al. 2001).

In addition to *D. gracilis*, 14 samples of nine species of subfamily Tristichoideae and eight samples of undescribed species were collected in the field for molecular phylogenetic analysis (see the appendix in the online edition of the *International Journal of Plant Science*). Eighteen samples of six species assigned to four genera of Tristichoideae, five species of five genera of Podostemoideae, and one species of Weddellinoideae were used from GenBank (appendix). *Clusia cruiva* Cambess. of Clusiaceae and *Cratoxylum ligustrinum* Blume of Hypericaceae were collected in the Tsukuba Botanical Garden, National Museum of Nature and Science (Tsukuba, Japan). These two families, along with Podostemaceae, are members of Malpighiales, and Hypericaceae is suggested to be a sister group to Podostemaceae (Savolainen et al. 2000; Soltis et al. 2000; Gustafsson et al. 2002; APG 2003; Davis et al. 2005; Tokuoka and Tobe 2006). Sequence data of species of other members of Malpighiales—Ixonanthaceae, Linaceae, Salicaceae, and Violaceae—were obtained from GenBank (appendix).

Morphology

For morphological observations, materials were fixed with FAA (formalin : acetic acid : 50% ethyl alcohol = 5 : 5 : 90). For anatomical observations, the fixed materials were dehydrated in an ethyl alcohol series, embedded in Historesin Plus (glycol methacrylate; Leica, Heidelberg), cut into sections 2 μ m thick, and stained with safranin, toluidine blue, and orange G (Jernstedt et al. 1992). For SEM observations, the fixed materials were dehydrated in an ethyl alcohol series, soaked in isoamyl acetate, critical-point dried with an HCP-2 (Hitachi, Tokyo), sputter-coated with platinum-palladium using a JFC-1200 (Jeol, Tokyo), and observed in a JSM-5410LV (Jeol, Tokyo) at 5 kV.

Phylogeny

For phylogenetic analysis, materials were dried with silica gel. The *matK* gene was sequenced by methods slightly modified from those described by Kita and Kato (2004). Total DNA was extracted from the dried materials with DNeasy Plant Mini Kit (Qiagen, Valencia, CA). The *matK* region was amplified by PCR (35 cycles with a profile of 45 s at 94°C, 45 s at 52°C, and 180 s at 72°C) with Ampdirect (Shimadzu, Kyoto) and TaKaRa Ex Taq (Takara, Tokyo), using 3914F and MK-2rR for forward and reverse primers, respectively (Koi et al. 2008), or, alternatively, trnK-2R for reverse primer (Johnson and Soltis 1994). The PCR products were purified with GFX PCR DNA and Gel Band Purification Kit (GE Healthcare, Amersham). For cycle sequencing, we used an ABI Prism DNA sequencing kit with BigDye Terminator, version 3.1 (Applied Biosystems, Foster City, CA); the amplification primers; internal sequencing primers used previously (Kita and Kato 2001; Kita and Kato 2004; Koi et al. 2008); and newly designated primers MK-F11 (5'-TTACAATCAATTCATTCAAT-ATT-3'), MK-R12 (5'-ATCATTAAAGAAAGAATATC-3'), MK-F13 (5'-TGGATTCCGATATTATTGACCG-3'), MK-F14 (5'-CAGATTGCTTCTTTGATCTTCC-3'), MK-F15 (5'-ACTATCGCACTATGTATC-3'), MK-F16 (5'-CAAAATTTACAATCAATTCA-3'), MK-F18 (5'-GTCTGGTTTCAACCMGAYAA-3'), MK-R19 (5'-TAYTCATGAAGAAASAATCG-3'), MK-R20 (5'-GAYAATGATTCAATYATTGG-3'), and MK-R22 (5'-AAATGGAATATTCAATTAGT-3'). The cycle-sequencing samples were purified by ethanol precipitation. Sequencing was conducted with an ABI PRISM 3130xl Genetic Analyzer (Applied Biosystems).

Phylogenetic analysis was performed with sequences of three subfamilies of Podostemaceae obtained in this study and previous studies (appendix; Kita and Kato 2001, 2004). The sequences were assembled using SeqMan II (DNASTar, Madison,

Table 1

Comparison of Characters among Species and Genera of Subfamily Tristichoideae

Character	<i>Indodalzellia gracilis</i>	<i>Dalzellia</i>	<i>Indotristicha ramosissima</i>	<i>Indotristicha tirunelveliana</i>	<i>Tristicha</i>	<i>Terniopsis</i>	<i>Cussetia</i>
Root	+	–	+	–	+	+	+
Root cap	–	NA	+	NA	–	+	?
Holdfast	+	–	+	+	+	+	?
Shoot size (max. length, mm)	10	30	600	30	20	30, rarely 90	15
Shoot dorsiventrality	+	+	–	+	–	–	–
Short shoot (=ramulus)	–	–	+	+	+	+	+
Phyllotaxis of ramulus	NA	NA	Spiral	4-stichous	3-stichous	3-stichous	3-stichous
Leaf dimorphism	+	+	–	–	–	–	–
Rosette	–	+	–	–	–	–	–
Short shoot associated with flower	NA	NA	+	+	+	+	+
Cupule	–	+	+	+	–	–	–
Membranous leaf (bract)	+	–	–	–	+	+	+
Source	This study; Mathew et al. 2001	Jäger-Zürn and Mathew 2002; Imaichi et al. 2004	Rutishauser and Huber 1991	Sharma et al. 1974; Uniyal 1999	Jäger-Zürn 1970; Koi et al. 2006	Imaichi et al. 1999	Cusset 1973; Kato 2006b

Note. NA = character state not available because of absence of organ.

WI). The sequences were aligned with ClustalX (Thompson et al. 1997) and refined manually with MacClade 4.0 (Maddison and Maddison 2000). Gaps were treated as missing data. Maximum likelihood (ML) and maximum parsimony (MP) analyses were performed with PAUP*, version 4.0b10 (Swofford 2002). In ML analyses, Modeltest, version 3.7 (Posada and Crandall 1998), was used to determine the nucleotide substitution model. The transversion model (Posada and Crandall 1998) + proportion of invariable sites (I) + shape parameter of the gamma distribution (G) was selected for our data set by hierarchical likelihood ratio tests. Nucleotide frequencies were A = 0.3310, C = 0.1493, G = 0.1385, T = 0.3812; the substitution rate matrix was A to C = 1.1396, A to G = 1.4461, A to T = 0.1824, C to G = 1.0073, C to T = 1.4461, and G to T = 1.0000; the proportion of invariable sites was 0.1547; and the gamma distribution shape parameter was 2.826. Heuristic searches were conducted with 100 random addition replicates involving nearest-neighbor-interchange branch swapping. Bootstrap values were calculated with 100 replicates with 10 random addition replicates. In MP analyses, all characters were equally weighted, and heuristic searches were conducted with 1000 random addition replicates involving tree-bisection-reconnection branch swapping. Bootstrap values were calculated with 1000 replicates and 100 random addition replicates. In Bayesian analysis, the general time-reversible + I + G model was selected with MrModeltest 2.2 (Nylander 2004). Markov chain Monte Carlo iterations with four chains were conducted for 2,000,000 generations, sampling a tree every 100 generations, with MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003). The first 4000 trees were discarded as burn-in, and the remaining 16,000 trees, whose standard deviation of split frequency was <0.01, were used to calculate a 50% majority-rule tree and to determine the posterior probabilities for branches. The species of Clusiaceae, Hypericaceae, Ixonanthaceae, Linaceae, Salicaceae, and Violaceae (appendix) were treated as outgroups (Savolainen et al. 2000; Soltis et al. 2000; Gustafsson et al. 2002; APG 2003; Davis et al. 2005; Tokuku and Tobe 2006).

Results

Taxonomy

Indodalzellia Koi & M. Kato, *gen. nov.*

Radices subcylindricae, elongatae, ramosae, firmatissime saxo adpressae, utrinque caulibus; caules repentes, oppositi vel alterni, simplices, isotome vel anisotome ramosi, taeniformi, dorsoventrales, foliis dorsalibus et laterali-marginalibus; folia oblonga, dimorpha, laterali-marginales dorsalibus longiora, uninervia, avascularia; flores singulares, pedicellis basi foliis multis, linearibus raro ovatis, radiati, 3-meri, tepalis 3-lobis, staminibus 3, ovario singulari, 3-syncarpellato, stigmatibus 3; capsulae obovoideae, 9-costatae.

Dalzellia caule dorsoventrali, foliis dimorphis, flore trimero similis, sed radice praesenti, caule taeniformi, rosula absenti, cupula basi pediceli absenti differt, a *Cussetia*, *Indotristicha ramosissima*, *Tristicha*, et *Terniopsis* caule dorsoventrali, foliis dimorphis, ramulo absenti, ab *Indotristicha tirunelveliana* radice praesenti, ramulo absenti differt.

Typus. *Indodalzellia gracilis* (Mathew, Jäger-Zürn, & Nileena) Koi & M. Kato, *comb. nov.*—*Dalzellia gracilis* Mathew, Jäger-Zürn, & Nileena, *Int J Plant Sci* 162:900, figs. 1–13, 2001.

Roots subcylindrical, elongate, branched, adhering to rock surface by rhizoids, with shoots on flank of roots; shoots solitary or in groups of two, creeping, opposite or alternate, simple or isotomously or anisotomously branched, ribbonlike, with holdfasts on ventral side of shoots, dorsiventral with dorsal and marginal (lateral) leaves; leaves oblong, dimorphic, marginal leaves longer than dorsal leaves, both univeined but avascular; no leaf rosette on shoot; flowers solitary, pedicel not surrounded at base by cupules but by linear and ovate leaves (bracts), 3-merous with three calyx lobes, three stamens, a single 3-syncarpous ovary, and three stigmas; fruit-capsules obovoid, rarely obtetrahedral, triquetrous, nine-ribbed.

The diagnostic characters of *I. gracilis* and other members of Tristichioideae are shown in table 1. See also descriptions below.

Ecology. Annual. Submerged, adhering to rock surface, in stream in the rainy season, and exposed and then flowering and dead in the dry season when the water level is lower.

Distribution. Endemic to southern India (Kerala).

Molecular Phylogenetic Relationships

The phylogenetic relationships of *I. gracilis* were deduced from *matK* sequence data (1527 base pairs [bp]). The ML tree

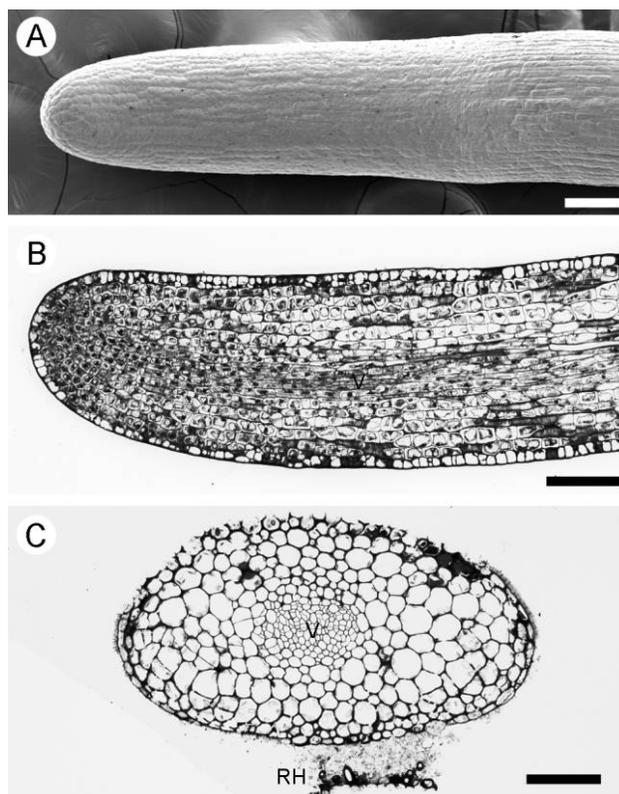


Fig. 2 Roots of *Indodalzellia gracilis*. A, SEM image of dorsal view of root tip, showing naked root apex. B, Longitudinal section of root apical meristem composed of dermal layer and inner meristematic cells. C, Cross section of root. Root is slightly flattened and subcylindrical, and rhizoids arise on the ventral surface. RH = rhizoid; V = nonvascular strand. Scale bars = 100 μ m.

($\ln = -12858.55$; fig. 1) shows that *Dalzellia zeylanica* and its southern and southeastern Asian congeners are monophyletic, with 100% (ML) bootstrap support, and are sister to *I. ramosissima*, with relatively high (89%) support. The topology is the same as those of the strict consensus tree of 14 MP trees and the Bayesian tree (data not shown). The monophyly of the *Dalzellia* species and of *Dalzellia* and *Indotristicha* is supported with bootstrap values of 100% and 81% (MP; data not shown) and posterior probabilities of 1.00 and 1.00 (Bayes; fig. 1), respectively. *Indodalzellia gracilis* is sister to the clade of *Dalzellia* and *Indotristicha*, with robust support (100% in ML and MP, 1.00 in Bayes). There are at least 66 bp of nucleotide substitutions between *Indodalzellia* and *Dalzellia*, 56 bp between *Indodalzellia* and *Indotristicha*, and 50 bp between *Dalzellia* and *Indotristicha*. In comparison, the species of *Dalzellia* are different from each other by 13 bp at most. The three genera are sister to *Tristicha*, with high support, and together they form a clade with *Terniopsis*.

Root

The root is elongate (20 cm long or more), subcylindrical (~ 0.6 mm wide and ~ 0.3 mm high in cross section), and branched, creeps on the rock surface, and has a strand of elongate, nontracheary cells (fig. 2). It is capless, with the root apical meristem naked and composed of a dermal layer and inner meristematic cells (fig. 2A, 2B). The rhizoids are abundant on

the ventral surface of the root, and, together with holdfasts borne on the ventral side of the shoots (not seen in this section), they adhere to the rock surface (fig. 2C).

Shoot

The shoots are borne on the flank of the root and are flattened, ribbonlike (~ 10 mm or more long, ~ 0.7 mm wide, and ~ 0.3 mm thick), and simple or isotomously or anisotomously branched. The shoot bears dimorphic leaves, i.e., small, oblong leaves on the dorsal surface and large, oblong marginal leaves on the flank (fig. 3A, 3B). Both leaves have midribs comprising nonvascular elongate cells. The dorsal and marginal leaf primordia arise facing each other at the shoot apex. The phyllotaxis of the dorsal leaves is apparently irregular. There is a marginal leaf between two daughter shoots (fig. 3C, 3D).

The shoots arise endogenously on the flank of the root and subsequently grow and extrude in association with the breaking of the dermal tissue of the root (fig. 3E; fig. 4A, 4D). Both dorsal and marginal leaves form while the shoot is still embedded in the root. The shoot apical meristem is dorsiventral with respect to oblique orientation and leaf formation and produces dorsal leaves from the upper flank and marginal (lateral) leaves on the lower flank (fig. 3B, 3F; fig. 4B, 4D). The leaves are produced close to each other without internodes and later are separated by cell proliferation and enlargement between the leaves in a subapical zone proximal to the meristem

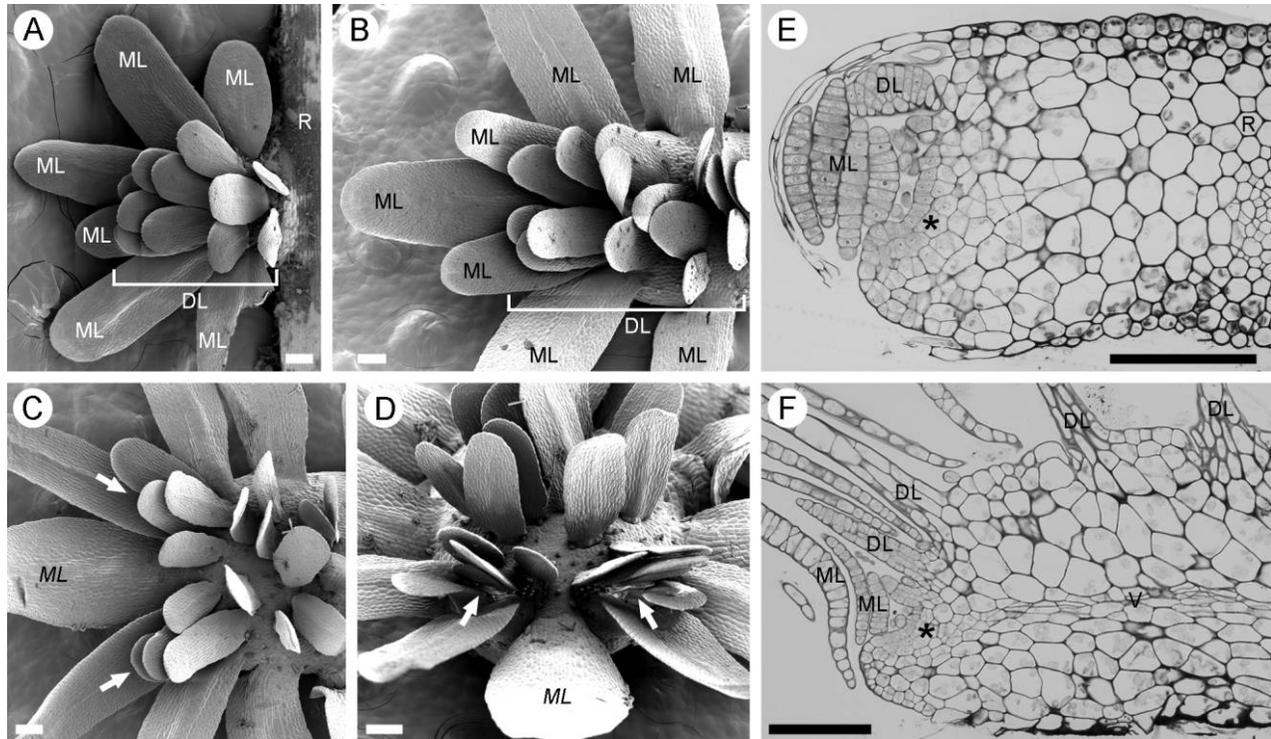


Fig. 3 Shoots of *Indodalzellia gracilis* collected in December 2006. A–D, SEM images of shoots. A, B, Dorsal views of short (A) and long (B) shoots with irregularly arranged dorsal leaves and marginal leaves. C, D, Dorsal and distal views of a just-bifurcated shoot. Note a marginal leaf (ML) between daughter shoots, whose apices are marked by arrows. E, F, Longitudinal sections of shoots. E, Young shoot enclosed by just-broken dermal tissue of a cross-sectioned root. F, Elongating shoot producing dorsal and marginal leaves from the dorsiventral shoot apical meristem. Asterisk indicates shoot apical meristem; DL = dorsal leaf; ML = marginal leaf; R = root; V = nonvascular strand. Scale bars = 100 μ m.

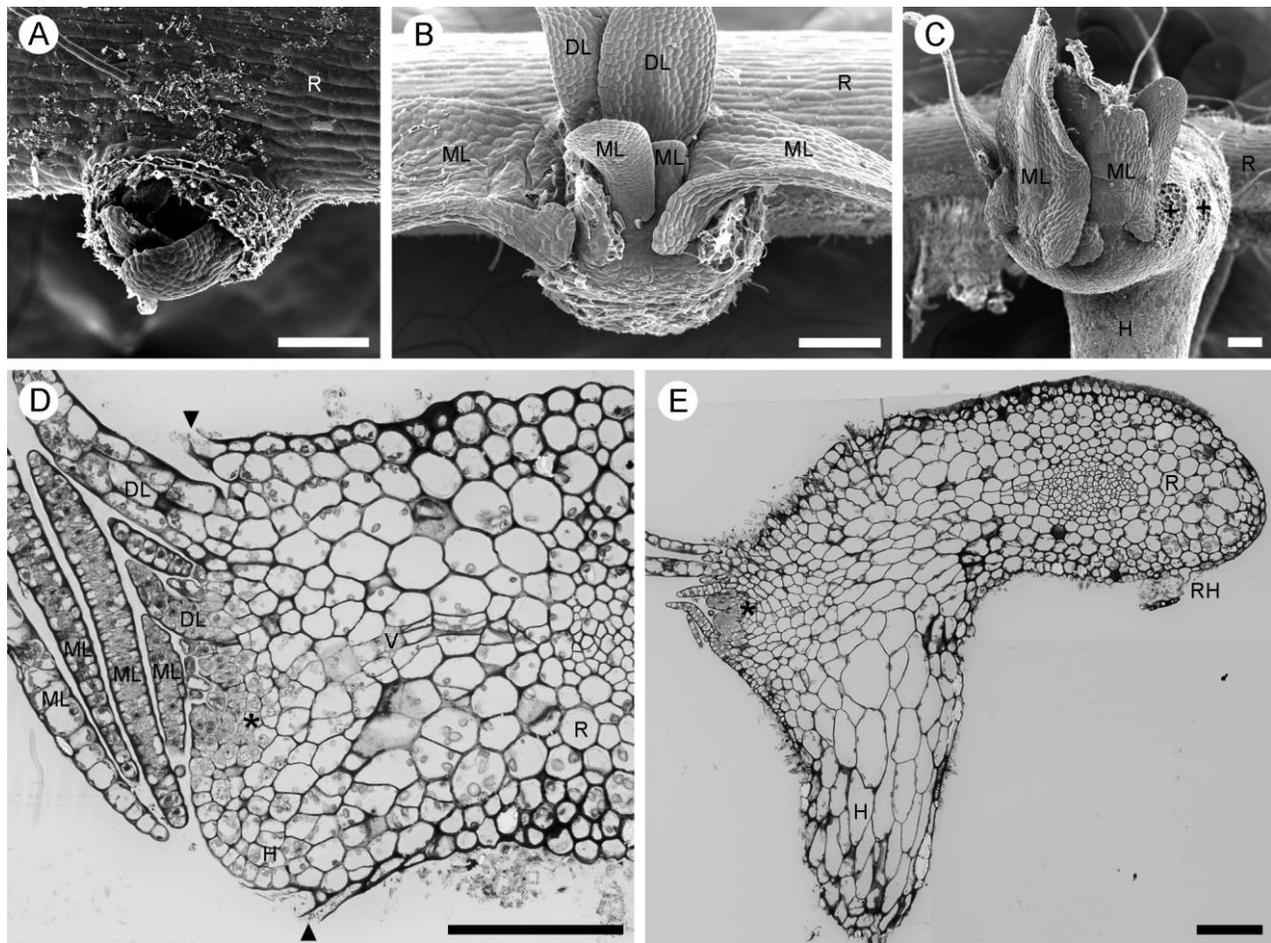


Fig. 4 Shoots of *Indodalzellia gracilis* collected in February 2005. A–C, SEM images of shoots. A, Endogenous shoot emerging from root. B, Older shoot producing dorsal and marginal leaves. C, Developed shoot with elongated holdfast. Marginal-leaf scars (plus signs) are seen. D, E, Longitudinal sections of shoots (i.e., cross sections of roots). D, Protruding shoot at stage between stages shown in A and B. Arrowheads indicate edges of root epidermis. E, Developed shoot and holdfast at stage nearly equivalent to that in C. Proximal dorsal leaves are caducous. Note that holdfast lacks vascular strand. Asterisk indicates shoot apical meristem; DL = dorsal leaf; H = holdfast; ML = marginal leaf; R = root; RH = rhizoid; V = nonvascular strand. Scale bars = 100 μ m.

(fig. 3F). The shoot is supplied by a nonvascular strand consisting of elongate cells that initiate with horizontal divisions of adjacent parenchymatous cells between the shoot apical meristem and the nonvascular strand of the root (figs. 3F, 4D). Unlike in *Dalzellia* (Imaichi et al. 2004), no leaf rosette is borne on the dorsal surface of the shoot.

The holdfast (i.e., hapter) forms exogenously on the ventral side of the young shoot, possibly while the shoot is still embedded in the root (fig. 4D). It may elongate or not, depending on the growing season. In plants collected in December, the holdfast did not develop well even after the shoot extrudes from the root (fig. 3F; S. Koi, stereomicroscopic data, not shown). In plants collected in February, the holdfast elongated soon (fig. 4C, 4E).

Flower

The reproductive shoots are nearly cylindrical, arise from the vegetative shoot apex or, rarely, adventitiously from the vegetative shoot, and yield flowers at the apices (fig. 5C). The

tissue at the base of the reproductive shoot has intercellular space, unlike the vegetative shoot tissue (fig. 5C). The reproductive shoot, like the vegetative shoot, produces imbricate narrow leaves on the dorsal and lateral sides (fig. 5A–5C), which may be elongate and midribbed or short and not midribbed, but there is no such leaf on the ventral side. Two or three membranous leaves (bracts) are borne on the ventral-lateral side below the flower bud. There are some narrower elongated leaves on the dorsal and lateral sides at the base of the pedicel above the bracts. Thus, in the phyllotaxis and morphology, the long and short leaves on the dorsal and lateral sides of the reproductive shoot, respectively, are comparable to the dorsal and marginal leaves on the vegetative shoot. There is no cupule, i.e., no leafy cuplike envelope for a flower bud, at the base of the pedicel.

The flower is terminal at the apex of the pedicel, which is 7–15 mm long at anthesis and ~40 mm long at fruiting time. It is small (2–3 mm long) and trimerous, comprising a three-lobed perianth, three stamens with introrse anthers alternate

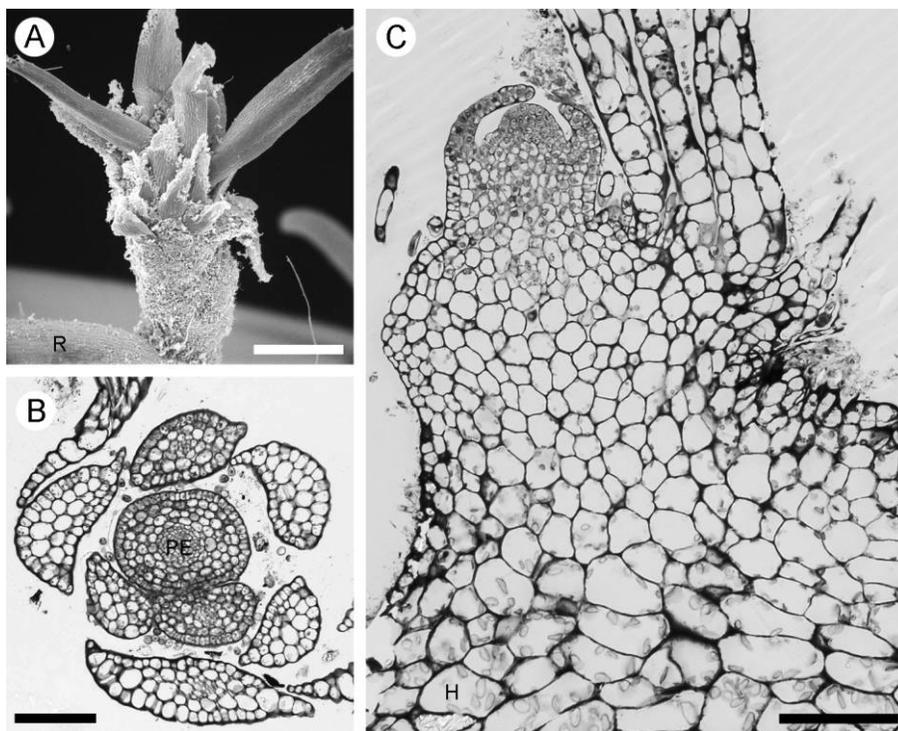


Fig. 5 Reproductive shoots of *Indodalzella gracilis*. *A*, SEM image of reproductive shoot as outgrowth of the narrow, creeping root. Note small, scaly dorsal leaves and large marginal leaves. *B*, Cross section of pedicel base, surrounded by scaly leaves. Note prominent nonvascular strand in pedicel. *C*, Longitudinal section of young, holdfast-bearing reproductive shoot with dorsal leaves and dorsal-leaf scars. Note intercellular spaces at the base of reproductive shoot. Holdfast base is seen at left bottom. *H* = holdfast; *PE* = pedicel; *R* = root. Scale bars = 500 μm in *A*, 100 μm in *B*, *C*.

to the perianth lobes, longer than the pistil, and a single trigonous, 3-locular ovary with three free stigma lobes on the tip (fig. 6*A*, 6*B*). About 20 ovules are borne on the axile placenta in each of locules partitioned by thin septa. Already the young capsule is nine-ribbed (fig. 6*C*). The mature capsule shows septicidal dehiscence, releasing the minute seeds (fig. 6*D*).

Discussion

Phylogenetic Relationships

The results of the molecular phylogenetic analyses indicate that *Indodalzella gracilis* is sister, with a moderately high support, to the clade comprising *Dalzella* and *Indotristicha ramosissima* and that the divergence of *Indodalzella gracilis* was much earlier than the diversification of *Dalzella*. The results oppose the systematic placement of the species in *Dalzella* (Mathew et al. 2001). Instead, along with revealing remarkable morphological differences (table 1), they allow separation of the species at the genus level, as proposed above. This is in accordance with the current classification, in which the subfamily comprises six genera (the five in fig. 1, plus *Cussetia*; Kato 2006*b*). An alternative lumping treatment of assigning them to the single genus *Dalzella* because of their monophyly will veil morphological distinctions among *Dalzella*, *Indodalzella*, and *Indotristicha*, and such an extremely polymorphic “*Dalzella*” is impossible to define (see table 1). This study did not examine *Cussetia*, whose species had been placed in *Dalzella* (Kato

2006*b*), or *Indotristicha tirunelveliana* B. D. Sharma, S. Karthikeyan & B. V. Shetty, which appears to be similar to *Dalzella* and *Indodalzella* (Sharma et al. 1974), because fresh material was not available.

Evolution of Morphology in Tristichoideae

Kita and Kato (2005) hypothesized evolutionary trends in characters of Podostemaceae. *Dalzella* was suggested to have lost both the primary root (radicle) and secondary (adventitious) roots, whereas *I. ramosissima*, along with the basal *Terniopsis* (including *Malaccotristicha*) and most other Tristichoideae, retains at least the secondary roots (table 1). This hypothesis is strongly supported by the robust phylogenetic relationships. It is likely that the loss of the root occurred after the divergence of *Indodalzella* and *Indotristicha*. The root loss may occur in *I. tirunelveliana*, or the long holdfast of the species may be comparable to a transformed root, although this anatomical feature is different from the roots of Tristichoideae (Uniyal 1999).

The histology of the root apical meristem of the capless root of *Indodalzella* is nearly the same as that of *Tristicha* in that the meristem comprises a single dermal layer enclosing inner cells (Koi et al. 2006). Phylogenetically, the capless *Tristicha* is sister to a clade comprising the capless *Indodalzella* and the subclade of the capped *Indotristicha* and the rootless *Dalzella*, and the clade of these four genera is sister to the capped *Terniopsis* (fig. 1; table 1). There are two likely alternatives for root cap loss: the cap may have been either lost recurrently in

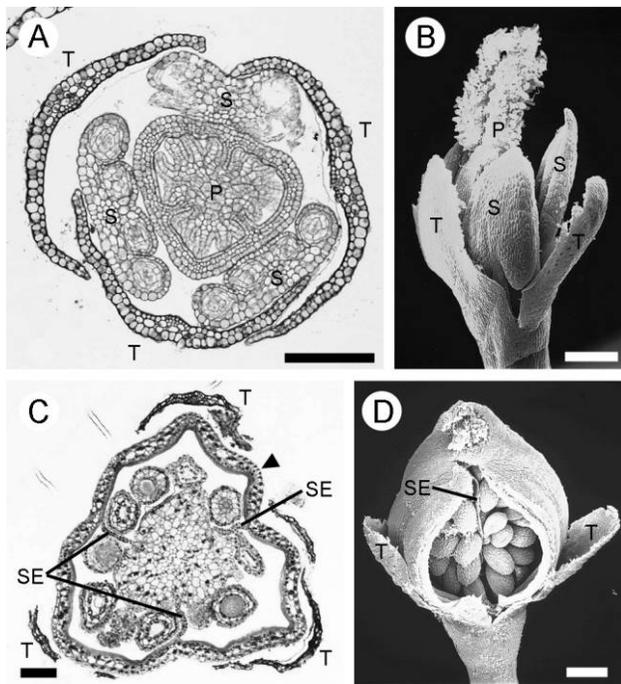


Fig. 6 Flowers and fruits of *Indodalzellia gracilis*. A, Cross section of flower before anthesis. Three tepals ensheath three stamens and a 3-locular ovary. B, SEM image of lateral view of flower at early anthesis. Stamens partially covering ovary topped by three papillate stigma lobes. C, Cross section of immature capsule with nine ribs (arrowhead indicates one of nine). Prominent placentae is separated into three locules by thin septa. D, SEM image of mature septical capsule starting to dehisce. Ellipsoid seeds are borne in locules. P = pistil; S = stamen; SE = septum; T = tepal. Scale bars = 100 μ m in A, C, 300 μ m in B, D.

Indodalzellia and *Tristicha* or lost in the ancestor of the two clades and then regained in *I. ramosissima* (Koi et al. 2006). Similar root cap loss occurred in the subfamily Podostemoideae (Koi et al. 2006; Moline et al. 2007). However, the ancestral root cap developmental patterns differ: in the Tristichoideae *I. ramosissima* and *Terniopsis*, the root cap is produced outwardly from the root apical meristem, as in other angiosperms, while in Podostemoideae, the dorsiventral root cap is produced from the ventral flank of the dorsiventral apical meristem (Koi et al. 2006). This suggests convergence of the corresponding root apical meristem in the capless roots.

Indodalzellia is similar to *Terniopsis* and possibly other root-bearing genera in the endogenous origin of the shoot associated with the holdfast (i.e., hapter). In the exogenous formation of the holdfast on the ventral side of the young shoot while the shoot may still be embedded in the root, *Indodalzellia* is quite similar to *Terniopsis malayana* (J. Dransf. & Whitmore) M. Kato (Imaichi et al. 1999) and *I. ramosissima* (Rutishauser and Huber 1991), although *Terniopsis* is remotely related to the other two and each of the three taxa has a distinct body plan. Jäger-Zürn (1997) stated that *Tristicha trifaria* (Bory ex Willd.) Sprengel lacks a holdfast, but it does have holdfasts, which develop on the ventral side of the endogenous shoots (S. Koi, unpublished data). Although *Indodalzellia* is similar to *Dalzellia* in

the dorsiventral flattened shoot, they differ in the adhesion of plants to rock surfaces. In *Indodalzellia*, adhesion is by the holdfasts and rhizoids on the shoot and by the rhizoids on the root, while in rootless *Dalzellia*, it is by rhizoids on the shoot (Imaichi et al. 2004).

In *Indodalzellia*, as in *Dalzellia* (Jäger-Zürn 1995; Imaichi et al. 2004), the shoot apical meristem and the consequent shoot and phyllotaxis are dorsiventral. *Indodalzellia* is also similar to *I. tirunelveliana* in shoot dorsiventrality (Sharma et al. 1974; Uniyal 1999). It is less similar to the subcylindrical and subdorsiventral base of the shoot of *Terniopsis australis* (C. Cusset & G. Cusset) M. Kato (Kato et al. 2003) and *T. trifaria* (Jäger-Zürn 1970), but all other distal parts of these shoots are radial. The developmental shoot anatomy of *Terniopsis* and *Tristicha*, with special reference to the transition from the subdorsiventral base to the radial distal part, is useful for insight into the evolution of the shoot dorsiventrality in Tristichoideae.

In *Indodalzellia*, the dorsal leaves are formed close to each other at the flank of the shoot apical meristem and are separated by cell proliferation and enlargement in the subapical zone. This development is identical to that of *Dalzellia zeylanica* (Imaichi et al. 2004). The phyllotaxis of the dorsal leaves is apparently irregular, although Mathew et al. (2001) described them as being arranged in two rows. In addition, a marginal leaf is regularly borne between two daughter shoots. In *D. zeylanica*, with a similar marginal leaf between shoot lobes, the horizontally extended shoot apical meristem is divided by formation of marginal leaves, one of which remains between daughter shoot lobes after branching (Imaichi et al. 2004). If the position of such leaves is determined by a similar cause, it will strengthen the correspondence of the meristems of the two species. From the molecular phylogenetic relationships, we infer alternative pathways leading to the similarity: the crustose shoot of *Dalzellia* derived from the ribbonlike shoot of *Indodalzellia* by shoot expansion, or such shoots of the two appeared independently.

Morphological variation is small in *Tristicha*, with the sole species *T. trifaria* (which is perhaps multispecific), and in *Terniopsis*, which has several species. But each genus has considerable molecular variation among populations or species or a long estimated divergence time. Furthermore, morphological differences are small even between the genera, with a very remote relationship to each other in the subfamily Tristichoideae. Unlike that in those genera, the body plan is diverse in the clade of *Dalzellia*, *Indodalzellia*, and *Indotristicha*, as partially implied by Kita and Kato (2001), who proposed saltational evolution for the distinction between *Dalzellia* and *Indotristicha*. The three genera differ in the presence or absence of the root, the capped versus capless root, and the radial versus dorsiventral symmetry of the shoot (table 1). These remarkable differences are probably caused by drastic changes in the juvenile body plan (Imaichi et al. 2004; Kita and Kato 2005). Information on the embryo seedling development of *Indodalzellia* and close relatives will be useful for better understanding the body plan change.

Flower morphology is conserved in Tristichoideae. The 3-merous flower is shared by all genera of Tristichoideae, although there is variation in the number (one to three) of stamens in *Terniopsis* and *Tristicha* (Cusset and Cusset 1988; Kato et al. 2003; Kato 2006b). The flower bud is protected by

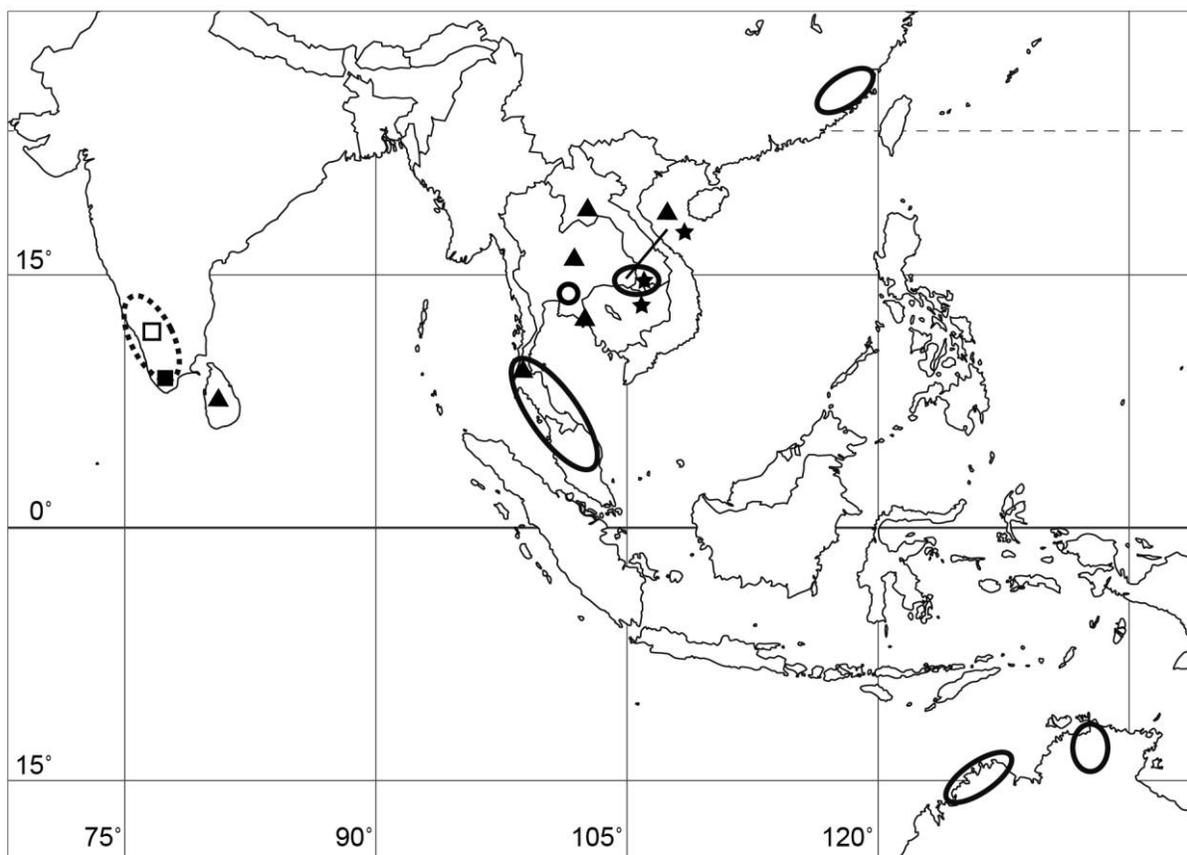


Fig. 7 Map showing distribution ranges of Tristichoideae, excluding Afro-American *Tristicha*. Open square, *Indodalzella gracilis*; dotted line, *Indotristicha ramosissima* and *Dalzella zeylanica*; solid square, *Indotristicha tirunelveliana*; triangles, *Dalzella* spp. (*D. zeylanica* in Sri Lanka); ovals and circle, *Terniopsis* spp.; and stars, *Cussetia* spp.

imbricate leaves and bracts in most species, including *Indodalzella*, while it is protected by a leafy cupule in *Dalzella* and *I. ramosissima* (Rutishauser and Huber 1991; Jäger-Zürn and Mathew 2002; Kato 2006b). Considering the phylogenetic relationship of the subfamily, the cupule is a novel protective organ in the clade of *Dalzella* and *Indotristicha*.

Distribution

Indodalzella gracilis, like *Indotristicha ramosissima* and *Indotristicha tirunelveliana*, is endemic to southern India (fig. 7). *Dalzella zeylanica* is also restricted to southern India and Sri Lanka, but undescribed species may occur in southern India. The other four species of *Dalzella* are distributed in Thailand and Laos (Kato 2006b; S. Koi, unpublished data), and *Terniopsis*, with six species, is distributed in central-eastern China (Fujian), Laos, Thailand, peninsular Malaysia, and northwestern Australia, with a distribution center in Thailand (Kato et al. 2003; Kato 2006a, 2006b). *Cussetia*, with two species and a possible affinity to *Terniopsis* or *Tristicha*, occurs in a narrow region extending over southern Laos, northern Cambodia, and eastern Thailand (Kato 2006a, 2006b). The monotypic (but perhaps multispecific) *Tristicha* is exceptionally widely distrib-

uted in Africa, Madagascar, Central America, and South America (Kita and Kato 2004). The New World *Tristicha* is suggested to have migrated by long-distance dispersal from western Africa (Kita and Kato 2004). It is therefore likely that *Indodalzella* originated from the ancestral tropical Asian Tristichoideae.

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