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Friesodielsia sahyadrica (Annonaceae), a peculiar new species from the Western Ghats, India

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Introduction

Annonaceae are one of the largest families of the magnoliid angiosperms, comprising trees, shrubs and lianas. Although they exhibit a pantropical distribution, relatively high levels of generic endemism are observed at the continental scale (Doyle and Le Thomas 1997). In India, they are represented by 24 genera and ca. 125 species (Kundu 2006).

During recent field studies in the northern Western Ghats of India, the authors collected some unusual specimens of Annonaceae. These specimens resembled species of the *Desmos* Loureiro (1790: 352) group, with respect to their scandent habit and glaucous abaxial leaf surface, whereas flower morphology resembled that of *Goniothalamus* (Blume 1830: 28) Hooker & Thomson (1855: 105), due to the presence of a coherent inner whorl of petals that conceal reproductive structures by forming a vault over stamens and carpels. However, a thorough investigation of literature on Indian Annonaceae revealed it to be a species of *Friesodielsia* Steenis (1948: 458), which is represented by two other species in India, namely *F. fornicata* (Roxburgh 1814: 94) Das (1963: 93), distributed from the northeastern part of India to Bangladesh and Myanmar, and *F. khoshooi* Vasudeva & Chakrabarty (1985: 435), endemic to the Andaman and Nicobar islands. This report is the first ever record of this genus for the Western Ghats.

Friesodielsia comprises about 51 species (Wang 2009, Chatrou *et al.* 2012), of which ten are recorded from tropical Africa whereas the rest are distributed across tropical Asia. Recent molecular phylogenetic studies (Richardson *et al.* 2004, Couvreur *et al.* 2011, Wang *et al.* 2012) have shown that *Friesodielsia* is polyphyletic with African and Asian species falling into different clades. The African species of *Friesodielsia* are related to the African genus *Monanthotaxis* Baillon (1890: 878), whereas the Asian species are related to the Asian genus *Dasymaschalon* Dalla Torre & Harms (1901: 174) (Couvreur *et al.* 2011, Wang *et al.* 2012). Palynological studies (Walker 1971) have also indicated that African and Asian *Friesodielsia* are distinct in their exine morphology (Table 1). Based on these results, the African species of *Friesodielsia* are likely to be transferred to *Monanthotaxis* or the African *Friesodielsia-Monanthotaxis* clade will be segregated into two or more genera (Wang *et al.* 2012) with the name *Friesodielsia* being retained by the Asian species, which is where the type of the genus occurs. It was therefore essential to assess the position of this new species from the Western Ghats with respect to the existing phylogenetic data.

Certain morphological features observed in the species from Western Ghats such as relative length of outer and inner petals as well as number of seeds per monocarp were consistent with those observed in many African species of *Friesodielsia*. Pollen exine morphology, on the other hand, was similar to that of the Asian group (Table 1, Fig. 2). Biogeographic evidence and molecular dating (Couvreur *et al.* 2011) suggested that clades such as the one consisting of *Friesodielsia* are younger than the time of the Gondwanan breakup and therefore more likely to have dispersed into India from Southeast Asia (Couvreur *et al.* 2011). Under this scenario, the species of *Friesodielsia* from the Western Ghats can be expected to cluster with the Asian group. Due to potentially conflicting evidence from morphology and biogeographic hypotheses, we used a molecular phylogenetic approach to ascertain the position of the new species from the Western Ghats; we wish to assign it to either the African or Asian group. Since *Desmos* shows similar patterns in diversity and distribution to that of Asian *Friesodielsia*, we included *Desmos lawii* Safford (1912: 506), an endemic species confined to the Western Ghats.

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TABLE 1. Comparison of *Friesodielsia sahyadrica* with its African and Asian congeners.

Characters	African *Friesodielsia*	*Friesodielsia sahyadrica*	Asian *Friesodielsia*
Petal length	subequal[1]	subequal	outer much longer[1]
Inner petal	clawed[2]	not clawed	not clawed
Pollen exine	verrucate to microbaculate[3,4]	echinate	echinate[3,4]
Carpels	moniliform[1,5]	non-moniliform	both[5]
Monocarps	stalked	sessile	stalked[5]
No. of seeds	up to 5[1]	up to 5	1 or 2[5]

[1]Verdcourt 1971, [2]Couvreur *et al.* 2012, [3]Walker 1971, [4]Wang 2009, [5]Wang *et al.* 2012

Material and methods

DNA extraction:—Fresh leaves of *Friesodielsia sahyadrica* and *Desmos lawii* were collected in the wild and preserved in silica gel for DNA extraction. DNA was extracted using HiPura plant genomic DNA miniprep kit (Himedia Laboratories, Mumbai, India). Plastid DNA regions, *rbcL*, *matK*, *trnL-trnLF*, *psbA-trnH*, and *ndhF* were amplified with primers and PCR conditions as described in Wang *et al.* (2012). PCR purification and DNA sequencing was done on an ABI 3100 analyser by Amnion Biosciences Pvt. Ltd (Bangalore, India). Accession numbers of the sequences generated in this study are listed in Table 2.

TABLE 2. GenBank accession numbers of taxa analyzed in this study.

Taxon	*rbcL*	*matK*	*trnL-trnLF*	*psbA-trnH*	*ndhF*
Friesodielsia sahyadrica	KC933934	KC933936	KC933938	KC933940	KC933942
Desmos lawii	KC933935	KC933937	KC933939	KC933941	KC933943

Phylogenetic analysis:—The dataset from Wang *et al.* (2012) along with our sequences for the above two taxa were merged and realigned using Muscle (Edgar 2004) and manually corrected using Se-Al v2.0a11 (Rambaut 2002). Missing portions of the sequences were coded as unknown using question marks. Inverted repeats and hypervariable regions omitted in the Wang *et al.* (2012) dataset were similarly omitted. The best-fit models of nucleotide evolution for each partition were selected using jmodeltest v 0.1.1 (Posada 2008) under the Akaike information criterion (AIC). Phylogenetic analyses were performed using PAUP* v. 4b10 (Swofford, 2003), MrBayes v. 3.1.2 (Ronquist and Huelsenbeck 2003) and RAxML 7.2.6 (Stamatakis 2006).

Bayesian inference (BI) involved 10 million runs of a five-gene partitioned dataset with four independent chains of Metropolis coupled Markov chain runs with sampling at every 1000 runs. Convergence of probabilities was checked with Tracer v. 1.5 (Rambaut and Drummond 2007), and the trees were summarized eliminating the first 20% as burnin. For maximum likelihood bootstrapping (ML), 100 replications were run in RAxML under the GTR+G+I model and the thorough bootstrapping method. The bootstrap percentages were included on the best-scoring ML tree. Parsimony bootstrapping (MP) was done with 100 bootstrap replicates. Each replicate consisted of 10 heuristic searches with random addition of sequences and tree bisection reconnection (TBR).

SEM study:—Pollen from crushed anthers was mounted on an aluminum stub with double-sided carbon tape and sputter coated with gold palladium in a Bal-Tec SCD 500, Liechtenstein sputter coating unit. The chosen thickness of the coating was 10 nm. Pollen exine morphology was examined using a scanning electron microscope (SEM) Quanta 200 ESEM, FEI, Netherlands.

Results

The combined dataset used to build the phylogenetic tree consisted of 5398 aligned characters with 557 variable characters, of which 447 were potentially parsimony informative. The heuristic search resulted in 1890 equally parsimonious trees with a tree length of 1611 steps, a consistency index (CI) of 0.72 and a retention index (RI) of 0.75. A strict consensus of the most parsimonious trees resulted in a topology in which all resolved clades were identical to the Bayesian and ML analyses. The Bayesian tree had shorter branch lengths at deeper nodes (Fig. 1). Clade A consisting of members of African *Friesodielsia* and *Monanthotaxis* (clade B3 in Wang *et al.* 2012) as well as clade B3 consisting of *Dasymaschalon* were collapsed (Fig. 1) in the strict consensus tree.

The new species, *Friesodielsia sahyadrica,* falls in the Asian clade (B1 clade of Wang *et al.* 2012) with good support (parsimony bootstrap percentage, MP, = 99, maximum likelihood bootstrap percentage, ML, = 99, Bayesian inference posterior probability, BI, = 1.0; Fig. 1). However, the position of this species within the B1 clade is not fully supported (Fig. 1). *Desmos lawii* falls in clade B2 of Wang *et al.* (2012) with good support (MP = 100, ML = 96, BI = 1.0; Fig.1). However, the exact position of *D. lawii* could not be ascertained due to lack of support. Results from SEM show echinate exine ornamentation in *F. sahyadrica* (Fig. 2), as has been documented in other species of the Asian *Friesodielsia* clade (Walker 1971, Wang 2009).

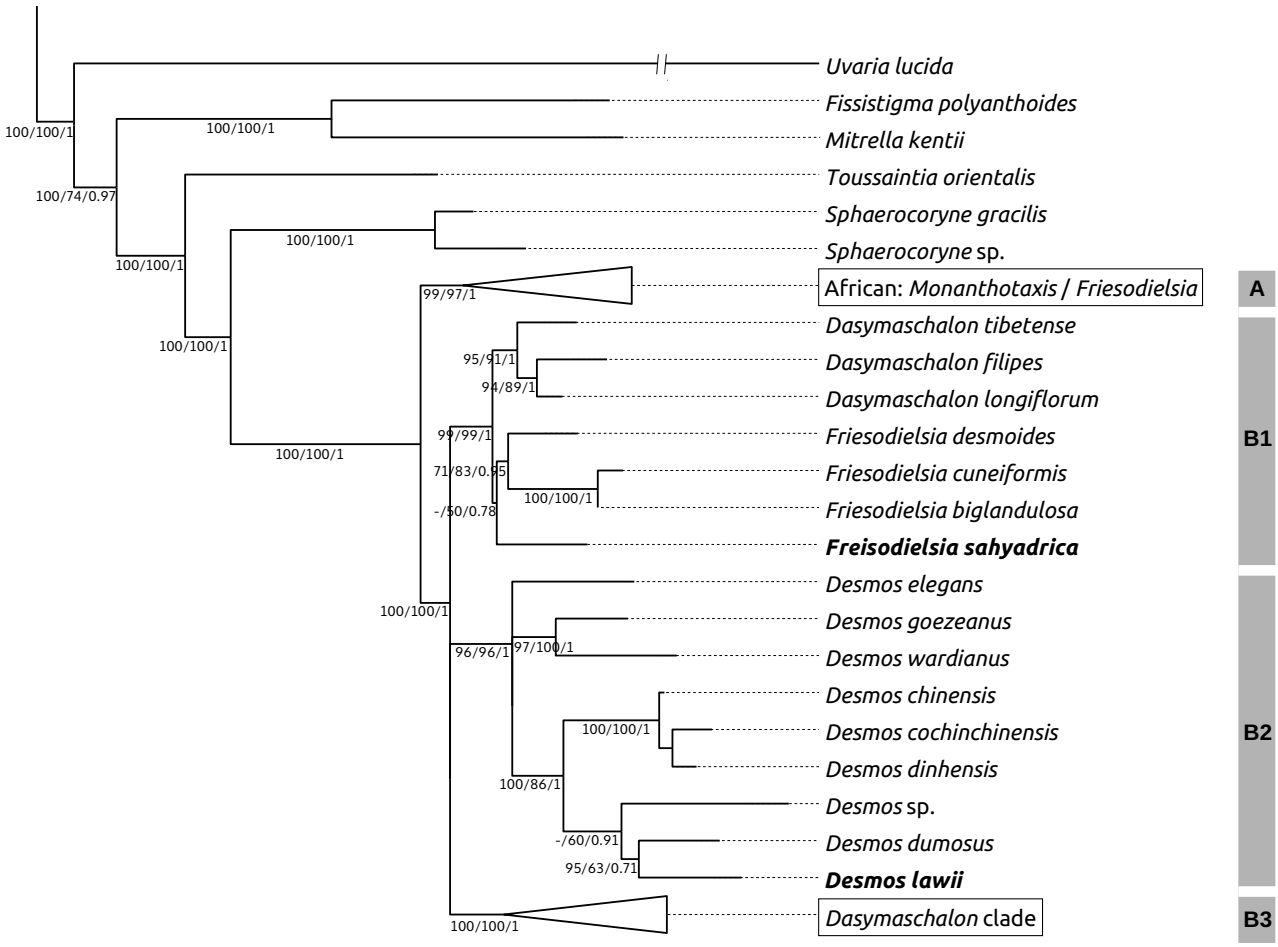

FIGURE 1. Maximum likelihood analysis tree of *Desmos*, *Friesodielsia* and *Dasymaschalon* species using plastid DNA (*matK*, *ndhF*, *psbA-trnH*, *rbcL*, *trnL-F*). The numbers indicated below the branches are maximum parsimony (MP) bootstrap >50%, maximum likelihood (ML) bootstrap >50% and Bayesian inference posterior probabilities (BI) >0.7, respectively (MP/ML/BI). Taxa sequenced for this study are indicated in bold.

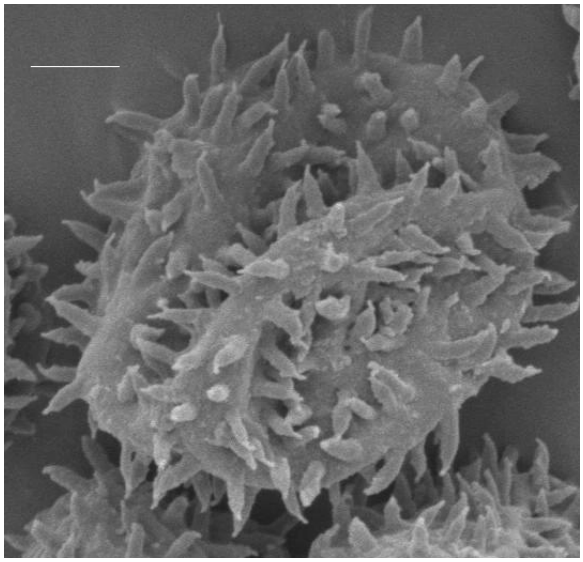

FIGURE 2. SEM image of a pollen grain of *Friesodielsia sahyadrica* showing echinate exine ornamentation. Scale bar represents 5 µm.

Description

Friesodielsia sahyadrica N.V.Page & S.Surveswaran, *sp.nov.* (Figs. 3, 4)

Friesodielsia sahyadrica differs from the rest of the Asian *Friesodielsia* due to its five-seeded monocarps and subequal petal length (Table 1).

Type:—INDIA. Karnataka: Uttar Kannada district, Kathlekan Reserve Forest, 110 m, 13 November 2012, *Page* 110949 (holotype CAL!; isotypes BSI!, FRLH!, JCB!)

Scandent shrubs, often erect and spreading in absence of the host tree. Branches slender, terete, occasionally twining around the host plant, young parts brown-strigose, older glabrous and lenticellate. Leaves simple, alternate, distichous, oblong-lanceolate, (abruptly) acuminate, broadest above the middle, base narrowed, slightly rounded, 12–20 × 3–6 cm; midrib impressed above; petiole 0.8 cm, channeled; secondary nerves 10–13 pairs, looping; tertiary nerves loosely percurrent; margin entire; upper surface glabrous, shining, lower glaucous white. Flowers axillary or extra-axillary, solitary; pedicel stout, 0.4–0.5 cm long, rufous-puberulous, with a minute ovate bract near the middle, bract ca. 0.2 × 0.1 cm; sepals three, green, valvate, 0.4–0.5 × 0.3–0.4 cm, puberulous outside, broadly ovate, lanceolate, connate at the base; petals six, in two whorls, yellow, valvate; outer whorl 0.8–1.0 × 0.5–0.7 cm, ovate-lanceolate, spreading, concave in the lower half, glabrous within, sparsely hairy on the outside; inner similar in shape, 0.6–0.8 × 0.4–0.5 cm, connivent along margins and vaulted over stamens and ovaries, opening only basally by narrow slits. Stamens numerous (ca. 35), 0.10–0.15 × 0.07–0.09 cm; connectives apically dilated, truncate. Ovaries oblong, densely strigose 0.10–0.11 × 0.08–0.09 cm; stigma glabrous, clavate. Monocarps oblong, cylindric, sessile, up to five seeded, tapering at both ends, slightly constricted between seeds, glabrous, 2.0–3.0 × 0.4–0.5 cm, scarlet when mature; seeds cylindrical, 0.6–0.7 × 0.4–0.5 cm. Figs. 3, 4.

Distribution and Habitat:—The species is so far known only from the type locality. It seems to be restricted to evergreen forest between 80–125 m. It occurs in the understory, often climbing up along trunks of trees. It is sympatric with *Desmos lawii*, a closely related genus from the desmoid clade that exhibits similar habit and ecology but shows a much wider distribution.

Etymology:—The specific epithet refers to 'Sahyadri', the vernacular name for the Western Ghats, where this species is distributed.

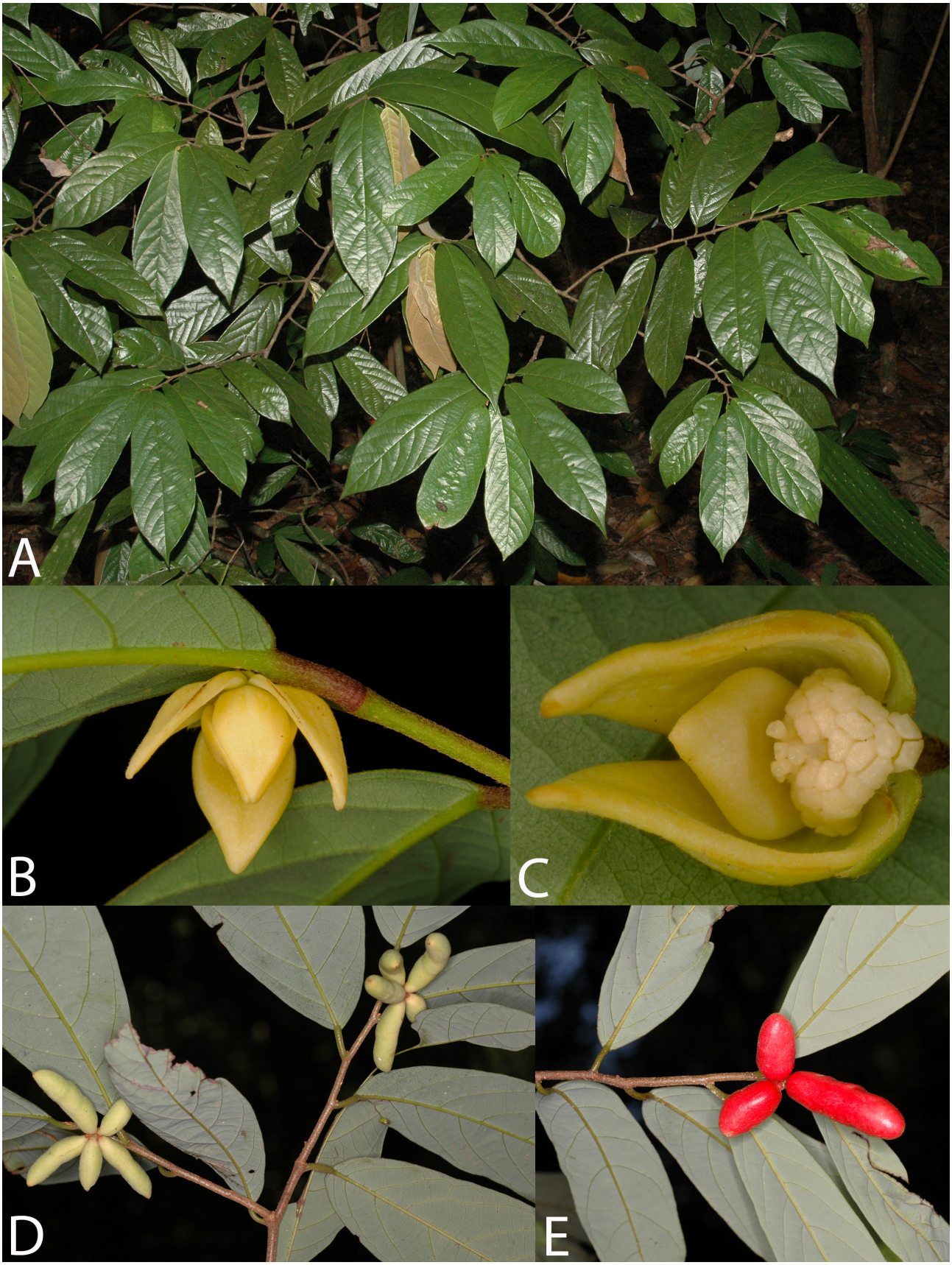

FIGURE 3. *Friesodielsia sahyadrica*. A. Branch with young and mature leaves. B. Open flower. C. Flower with two inner and one outer petal removed to show arrangement of stamens and carpels. D. Fruiting branch showing immature fruits and glaucous abaxial nature of the leaves. E. Mature fruit. Photos by Navendu Page

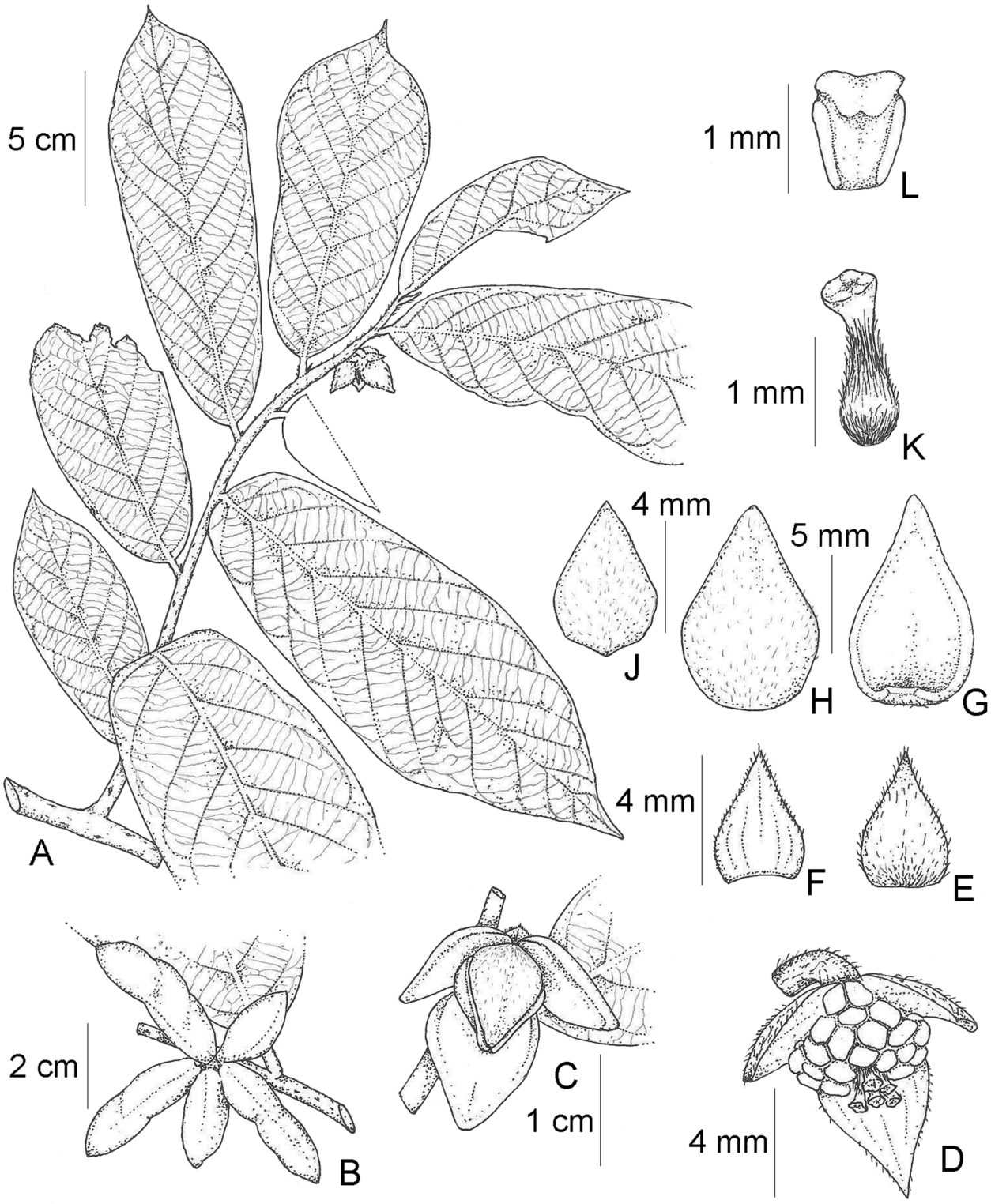

FIGURE 4. *Friesodielsia sahyadrica* (*Page 110949*) A. Flowering branch. B. Fruit. C. Flower. D. Flower with all petals removed showing parts. E. Sepal (abaxial). F. Sepal (adaxial). G. Outer petal (adaxial). H. Outer petal (abaxial). J. Inner petal (adaxial). K. Carpel. L. Stamen (abaxial). Illustrated by Shreekant Deodhar.

Conservation Status:—Number of individuals observed in the locality was less than 150, occupying an area less than 10 km^2. We, therefore, categorize the species as Endangered (EN) (D; B1+2a; IUCN 2010). The type locality falls within the boundaries of the Kathlekan Reserve Forest. Apart from a minor degree of logging, there are no apparent threats to this species. However, proximity to the Gairsoppa Reservoir could pose a potential threat to the species in the future as increasing the water-holding capacity of the dam may lead to submergence of its habitat.

Notes:—Based on the molecular results, it is clear that *Friesodielsia sahyadrica* belongs to the Asian desmoid clade, which consists of *Dasymaschalon*, Asian *Friesodielsia* and *Desmos*. The Asian and the African *Friesodielsia* group are distinguished primarily based on three morphological characters: echinate pollen exine (Fig. 2), relative length of outer to inner petals and number of seeds per monocarp (Verdcourt 1971, Walker 1971). Features such as differences in petal lengths and number of seeds per monocarp (Table 1) are contrary to what has been documented so far in the Asian species, but instead are characteristic of the African group that is related to *Monanthotaxis*. In Asian *Friesodielsia*, however, the outer petals are much longer than the inner petals (Verdcourt 1971), which are not observed in *F. sahyadrica*. Wang *et al.* (2012) inferred multiple transitions from multi-seeded monocarps to maximally one to two-seeded monocarps within the desmoid clade. All species of Asian *Friesodielsia* known previously exhibit one or maximally two-seeded monocarps (Verdcourt 1971, Wang *et al.* 2012). *Friesodielsia sahyadrica* is unique among Asian species of *Friesodielsia* in having five-seeded monocarps. African *Friesodielsia,* however, often have monocarps with up to five seeds. Another peculiar feature of *F. sahyadrica* is its cylindrical sausage-shaped monocarps with no distinct constrictions between seeds (Fig.3, 4). All species belonging to the Asian desmoid clade as well as African *Friesodielsia* exhibit distinctly moniliform monocarps when the number of seeds per monocarp is equal to or greater than two. Further and broader studies are required to determine the phylogenetic relationships of these and other taxa endemic to India.

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