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ELAEOCARPUS PULNEYENSIS, A NEW SPECIES OF *ELAEOCARPUS* (ELAEOCARPACEAE) FROM PENINSULAR INDIA, BASED ON MORPHOLOGICAL AND MOLECULAR EVIDENCE

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A new species of *Elaeocarpus* is described from the Kodaikanal region of Pulney Hills, India; it is morphologically allied to *E. variabilis* in having anther tips without setae or tuft of hairs and 3-locular pyrenes. It differs from the latter in having ovate, light-green sepals, and ellipsoid fruits enclosing ellipsoid pyrenes. Molecular phylogenetic analyses based on ITS and *trn*L–F regions revealed that *Elaeocarpus pulneyensis* is distinct from *E. variabilis* and support the novelty of the taxon.

Keywords. Endemic, Kodaikanal, Oxalidales, Pulney Hills, Rudraksha.
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Introduction

Elaeocarpus L., the most diverse genus in the family Elaeocarpaceae, is represented by about 350 species worldwide (Coode, 2004). Weibel (1968) studied the embryology of the genus, highlighting that most species have straight embryos (sect. *Elaeocarpus*), with few having curved embryos (sect. *Coilopetalum*). The Western Ghats of India is home to ten taxa in three sections: *Elaeocarpus* sect. *Coilopetalum* (*E. blascoi* Weibel, *E. munroii* (Wight) Mast.); *Elaeocarpus* sect. *Elaeocarpus* (*E. gadgilii* A.M.Maya *et al.*, *E. gaussenii* Weibel, *E. serratus* L., *E. variabilis* Zmarzty, *E. weibelii* (Zmarzty) Shareef *et al.*); and *Elaeocarpus* sect. *Monocera* (*E. recurvatus* Corner, *E. tuberculatus* Roxb., *E. venustus* Bedd.). *Elaeocarpus blascoi*, *E. gaussenii* and *E. venustus* are narrow endemics threatened by habitat loss (Gole, 2021, 2022; Raveendran, 2022).

During field excursions in October 2021 to the Kodaikanal region of Pulney Hills, India, a population of *Elaeocarpus* morphologically allied to *E. variabilis* and with flowers and young fruits was discovered at Pambarpuram Shola. The following year, another population of the same species was discovered from Bombay Shola, in the Pulney Hills, Kodaikanal (**Figure 1**). Despite a review of the literature on the genus *Elaeocarpus* from the Indian subcontinent (Hooker, 1874; Gamble, 1915–1936; Fyson, 1920; Murti, 1993; Matthew, 1999; Zmarzty, 2001) and recent reports of endemic species and new taxa (Stewart & Balcar, 2017; Shareef *et al.*, 2020; Manoharan *et al.*, 2021), in addition to critical examination and comparison of vegetative and floral characters of herbarium material,

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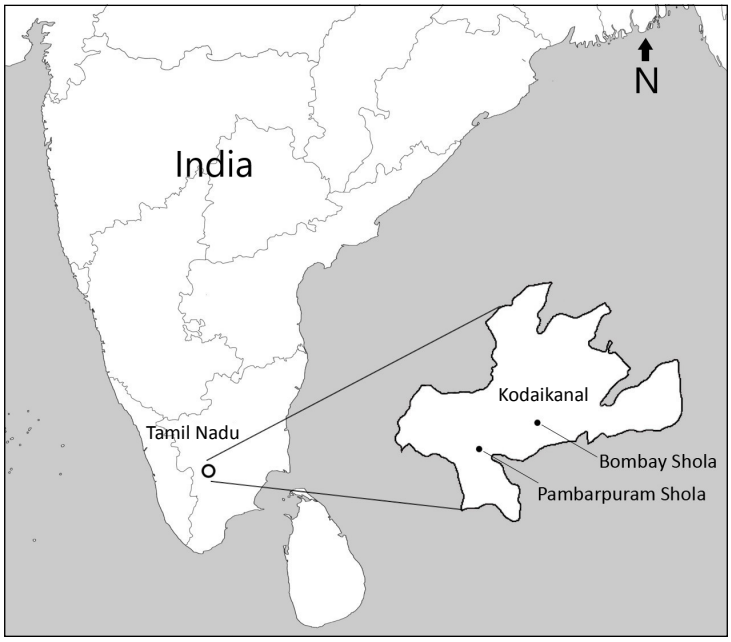

Figure 1. The locations (black circles) of the two populations of the putative new species in India.

it was not possible to assign the plant to any of the described species from the Indian subcontinent, suggesting that the species could belong to a hitherto undescribed species of *Elaeocarpus*.

Materials and methods

Taxonomy

The morphology of populations of all the taxa of *Elaeocarpus* described and reported from peninsular India were studied across different locations of the states of Maharashtra, Karnataka, Kerala, Odisha and Tamil Nadu from 2017 to 2021. Flowering and fruiting material was collected and preserved using a formaldehyde solution for detailed dissections. Herbarium specimens were prepared following the standard protocol of Bridson & Forman (1998). Morphological descriptions and illustrations were prepared for all species. In addition to our own field collections, specimens in the herbaria at BLAT, BSI, CAL, FRLHT, KFRI, MH and SUK, and online herbarium databases at BM, K and US (acronyms according to Thiers, continuously updated), were studied.

Molecular study

Taxon sampling. To confirm the novelty of the putative new species and to investigate its phylogenetic relationship with other species of *Elaeocarpus* sect. *Elaeocarpus* from

peninsular India, a molecular phylogenetic tree was constructed using nuclear and chloroplast markers. We sampled multiple accessions of the five species of *Elaeocarpus* sect. *Elaeocarpus* (*E. gadgilii*, *E. gaussenii*, *E. serratus*, *E. weibelii* and *E. variabilis*) previously reported from peninsular India, along with the putative new species, to reconstruct the molecular phylogeny of this group.

DNA isolation. Leaf tissue samples were collected in the field and their surfaces sterilised using 70% ethanol and preserved in silica gel until further use. Total genomic DNA was extracted from 10 mg of leaf tissue using QIAGEN DNeasy Plant Mini kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. The integrity of DNA samples was checked by electrophoresis on a 1% agarose gel, while DNA quantification was performed on Nanodrop ND-1000 v. 3.7.1 (Thermo Fisher Scientific, Waltham, Massachusetts, USA).

The nuclear ITS (comprising ITS1, 5.8S and ITS2) and plastid *trn*L–F intergenic spacer regions were selected based on their efficacy in differentiating species based on the phylogenetic reconstruction of *Elaeocarpaceae* (Baba, 2013; Phoon, 2015). The ITS region was amplified using primers ITS A–ITS B (Blattner, 1999) or ITS 5–4 (White *et al.*, 1990). The *trn*L–F region was amplified using universal primers c and f (Taberlet *et al.*, 1991). Amplification of both ITS and *trn*L–F was performed in a 20 µL reaction using 10 µL of 2X Kapa PCR Mix containing 1.5 mM dNTP and 10 X Buffer (Kapa Biosystems, Wilmington, Massachusetts, USA), 1 µL of forward and reverse primers (10 pM each), 0.6 µL of dimethyl sulphoxide (0.4 mM), and 0.25 µL of bovine serum albumin (0.4%) and template DNA (30–40 ng), along with PCR-grade water for final adjustment of volume. The amplified PCR products were electrophoresed on 1.5% agarose gel along with GeneRuler 100 bp Plus DNA Ladder (Thermo Fisher Scientific) to confirm the amplification of specific bands. Purification of the amplified products was carried out using either APS PCR clean-up kit or APS Gel elution kit (APS Labs, Pune, India).

PCR cloning. Amplification of the ITS region for the putative new species and *Elaeocarpus serratus* showed preferential amplification of fungal endophytic DNA, even when genus-specific markers were employed as by Baba (2013) and Phoon (2015). Thus, PCR cloning was performed for all accessions of these species, as per manufacturer's instructions, using the StrataClone PCR Cloning Kit (Agilent, Santa Clara, California, USA). The purified DNA was quantified on Nanodrop ND-1000 v. 3.7.1 and subjected to single-pass DNA sequencing using the Sanger di-deoxy method with T3 and M13(–20) universal primers.

Sequence editing and alignment. Sequence quality was checked using the software FinchTV v. 1.4.0 (Geospiza Research Team, 2004–2006). Sequence contigs were prepared using the software BioEdit Sequence Alignment Editor v. 7.2.5 (Hall, 1999). The sequences were manually edited to rectify erroneous base calls or gaps in the conserved region, using MEGA 11.0.11 (Tamura *et al.*, 2021). Multiple sequence alignment was performed in MEGA 11.0.11 or AliView v. 1.17.1 (Larsson, 2014). Concatenation of sequence alignments was performed in MEGA 11.0.11.

Phylogenetic analyses. Phylogenetic analyses were undertaken using maximum-likelihood (ML) and Bayesian inference methods. The best-fit model generalised time reversible (GTR) was identified using jModelTest v. 2.1.3 (Darriba *et al.*, 2012), based on the lowest values of the Akaike information criterion and Bayesian information criterion (BIC). AliView v. 1.17.1 was used for conversion of .fas files into formats appropriate for ML or Bayesian analysis. The Bayesian inference was estimated with MrBayes v. 3.2.6, using the GTR+G+I model (Ronquist *et al.*, 2012), with two independent runs. Each run consisted of four Markov chain Monte Carlo chains. These were sampled every 1000 generations.

The analysis was run for 1,000,000 generations. The initial 25% of the sampling was discarded as burn-in, and the rest was used to calculate posterior probability (PP) values. The ML analyses were performed by using IQ-TREE v. 2.1.2 (Nguyen *et al.*, 2015) in the CIPRES Science Gateway (Miller *et al.*, 2015), with support at nodes calculated with bootstrap analyses (ML–BS) of 1000 repeats. The phylogenetic tree was visualised in FigTree v. 1.4.2 (Rambaut, 2014).

Molecular phylogenetic sampling of *Elaeocarpus* sect. *Elaeocarpus* included *E. gadgilii*, *E. gaussenii*, *E. serratus*, *E. tectorius*, *E. variabilis* and our putative new species. *Elaeocarpus weibelii* could not be included in the analysis due to preferential amplification of a fungal endophyte and failed PCR cloning. Based on an earlier study (Phoon, 2015), six species from the sister clade representing *Elaeocarpus* sect. *Ganitrus* were also included in the study to confirm phylogenetic position of the novel species. Details of the accessions used in the study are given in the **Appendix table**.

Results

Phylogenetic analyses

The final combined ITS and *trn*L–F dataset comprised a total of 1299 characters (including all alignment gaps) for 32 strains (including the outgroup sequence). The aligned datasets of ITS and *trn*L–F were, respectively, 475 and 823 bp long. The matrix has 1850 distinct alignment patterns. According to BIC, GTR+G+I was chosen as the best-fit model. Estimated base frequencies were as follows: A = 0.303, C = 0.227, G = 0.214, T = 0.255; substitution rates, AC = 0.74350, AG = 3.97915, AT = 0.54971, CG = 3.13372, CT = 5.12958, GT = 1.00000; gamma distribution shape parameter α = 0.279. A best-scoring RAxML tree resulted in the value of likelihood −2706.708.

Species of *Elaeocarpus* sect. *Elaeocarpus* from the Western Ghats (*E. gadgilii*, *E. gaussesnii*, *E. serratus*, *E. variabilis* and our putative new species) formed a well-supported clade (91/1.00) and is sister to the other species of *Elaeocarpus* sect. *Elaeocarpus* from the Indo-Malayan region (*E. dongnaiensis*, *E. stipularis*, *E. tectorius*). The new species described in this paper is sister to the clade containing the rest of the species of *Elaeocarpus* sect. *Elaeocarpus* from the Western Ghats. Both ML analysis using IQ-TREE and PP analyses

using MrBayes (Ronquist & Huelsenbeck, 2003) using ITS alone and concatenated datasets concurred regarding the position of the new species, thus validating its novelty. *Elaeocarpus dongnaiensis* and *E. tectorius* formed a basal subclade delineating with high support (89/1.00) from both ML and Bayesian analyses (**Figure 2**).

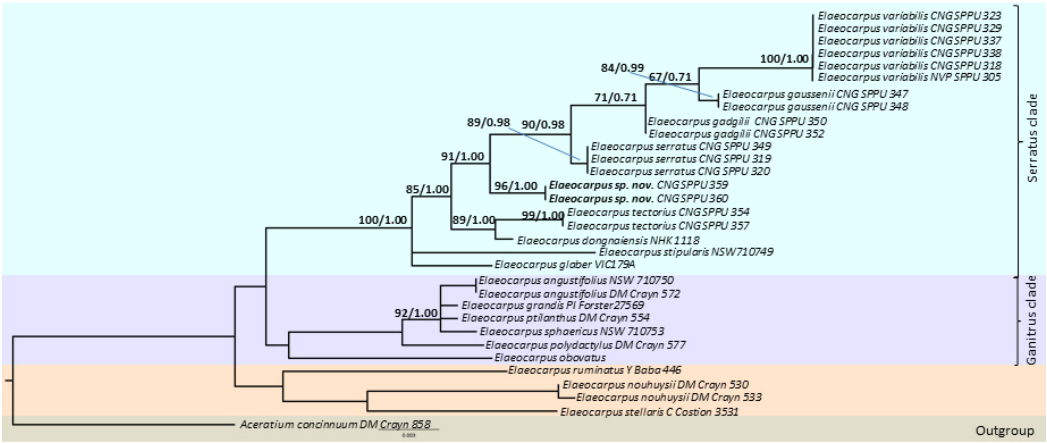

Figure 2. Phylogram inferred from the maximum-likelihood (ML) analysis, using the GTR+G+I model and a combined ITS and *trn*L–F dataset, of species from *Elaeocarpus* sect. *Elaeocarpus* and closely related species in Elaeocarpaceae. Branch support values greater than 65% (ML with bootstrap support) and/or 0.7 posterior probability are indicated at all nodes. The tree is rooted with *Aceratium concinnum* (S.Moore) C.T.White (grey). The new species is highlighted in bold. The Serratus clade is highlighted in light blue, the Ganitrus clade in lilac, and species from other subclades (from Phoon, 2015) in tan.

Taxonomic treatment

Species description

Elaeocarpus pulneyensis Gole, N.V.Page, Sardesai, **sp. nov.**

Elaeocarpus pulneyensis can be distinguished from the majority of species of *Elaeocarpus* from South India and Sri Lanka in having anther tips without setae or tuft of hairs. It is morphologically most similar to *Elaeocarpus variabilis*, the only other species which exhibits anther tips without setae or tuft of hairs and 3-locular pyrenes. *Elaeocarpus pulnyensis* can be distinguished from *E. variabilis* based on light green sepals (vs green or reddish brown), ellipsoid fruits (vs obovoid), pyrenes which are rounded at both base and apex (vs pyrenes which are tapering towards the base and rounded towards the apex), and a pyrene circumference at widest point of 6.3–6.7 cm (vs 4.8–5.4 cm). – Type: India, Tamil Nadu, Dindigul district, Kodaikanal, Pulney Hills, 10°13'29.10"N, 77°28'45.50"E, 2100 m, 10 x 2021, *C.N. Gole* SPPU 359 (holotype BSI!, isotype SPPU!). **Figures 3, 4, 5, 6.**

Trees to 20 m tall, trunk buttressed, branches and branchlets terete, grey-barked, vegetative buds pubescent. *Stipules* reduced, scaly, 0.8–1 mm long. *Leaves* alternate. *Petioles* with pulvinus at base and apex, 2–2.8 cm long, glabrous. *Lamina* green, turning scarlet red during senescence, elliptic-lanceolate, 7–9 × 4–5 cm, apex bluntly and shortly acuminate, margin serrate, serrations 0.4–1 cm apart, secondary nerves 5–7 pairs, domatia present at the axils of primary and secondary nerves. *Inflorescence* racemose, borne in the axils of fallen leaves and below the crown of young leaves, 12–15 cm long, 10- to 20-flowered. *Flower buds* short, conical, tomentose. *Flowers* bisexual, 9–12 mm across, pedicels 3–4 mm long, bracts caducous, bracteoles not seen, sepals 5, c.5 mm long, light green, lanceolate, sparsely hairy outside, glabrous inside, buds short, conical, petals 5, 6–7 mm long, alternating with sepals, white, slightly bulging outwards near the base, laciniate to half their length, margins sparsely hairy on the lower half, disc with fused lobes, yellowish orange when fresh, 2 mm high; stamens 25–30, creamy white when young, brownish black when mature, filaments light green, curved, puberulous, 1.2–1.5 mm long, anthers basifixed, creamy white, puberulous, c.2.5 mm long, anther tips without setae or tuft of hairs, ovary light green, 3-locular bearing 2 ovules each, superior, light green, style short c.2–3 mm long, broader at the base gradually tapering into a stigma. *Fruit* a pulpy drupe, ellipsoid, 2.8–3 cm long, green when young, brownish black at maturity, pyrenes light to dark brown, ellipsoid, with three or very rarely two loculi, rugose, 2.2–2.7 × 1.3–1.5 cm, circumference at widest part 6.3–6.7 cm, rounded at both ends, seed 1, 1.6–1.8 × 0.4–0.6 mm, embryo straight, endosperm and cotyledon not seen.

Distribution. Known only from Pulney Hills, Kodaikanal, Tamil Nadu. Two populations were observed during the study, one from the type locality in Pambarpuram Shola and the other from Bombay Shola (herbarium material not collected from the population in Bombay Shola) (see Figure 1).

Habitat and ecology. High-elevation evergreen shola forest. Flowering July to November, fruiting October to January.

Etymology. The specific epithet *pulneyensis* is derived from the type locality, Pulney Hills, situated in peninsular India.

Proposed IUCN conservation category. The species occurs at only one location, with its extent of occurrence < 100 km^2, and area of occupancy < 10 km^2. Moreover, the habitat quality has declined in the past and is still declining due to clear-felling of trees for monoculture plantations (personal observations); the species therefore meets the requirements of criteria B1ab(iii)+2ab(iii). The number of mature individuals does not exceed 50, fulfilling the Criteria D, of very restricted or small population. Thus, the species is proposed as Critically Endangered (CR) following *IUCN Red List Categories and Criteria*, version 3.1 (IUCN, 2012).

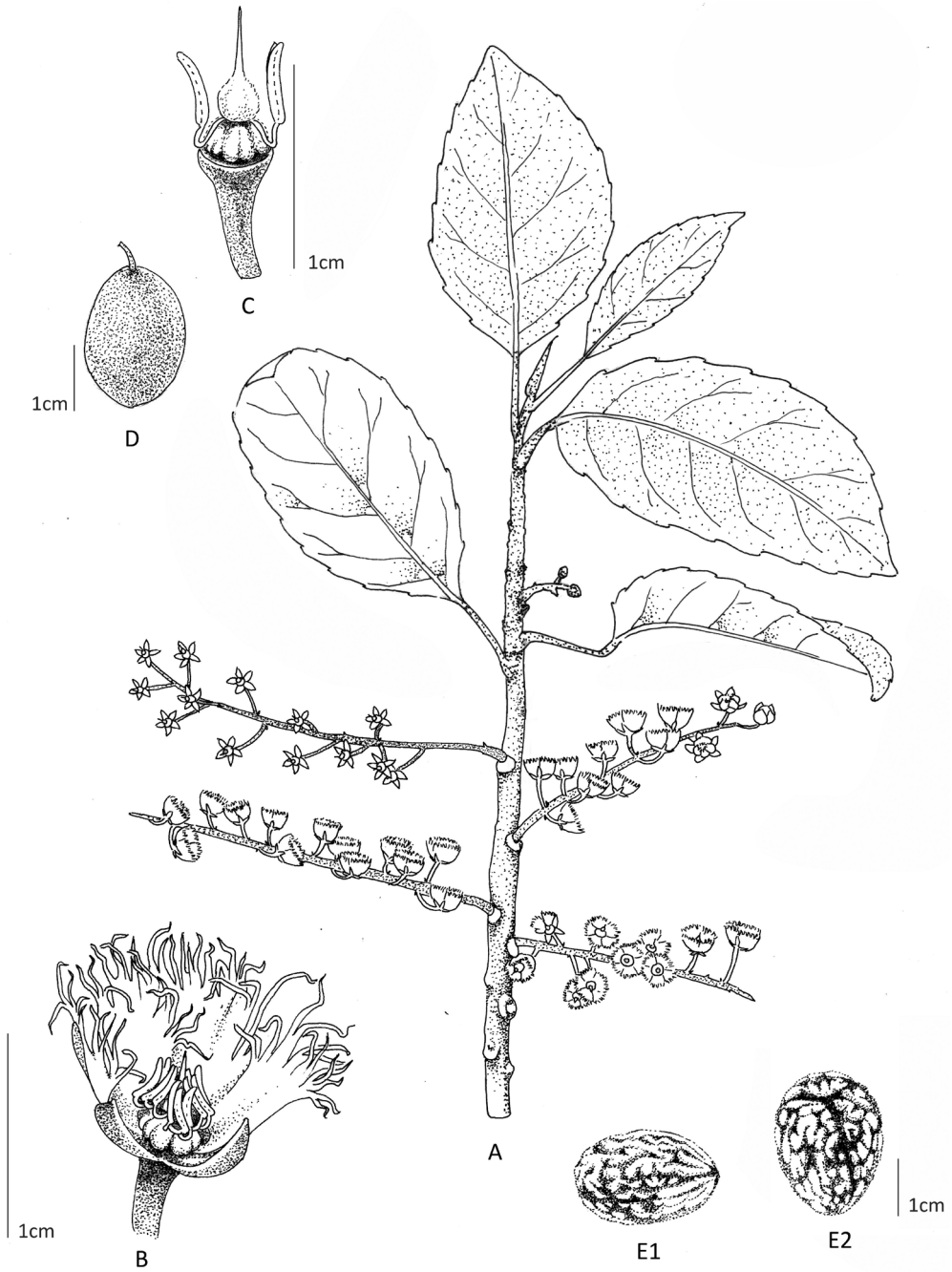

Figure 3. *Elaeocarpus pulneyensis* Gole *et al.*, sp. nov. A, Leafy twig with flowers and fruits; B, dissected flower, showing stamens; C, flower with petals removed to show gynoecium on a disc; D, mature fruit; E1, side view of pyrene; E2, top view of pyrene. Drawn by Renuka Potdar from *C.N. Gole* 359 (SPPU).

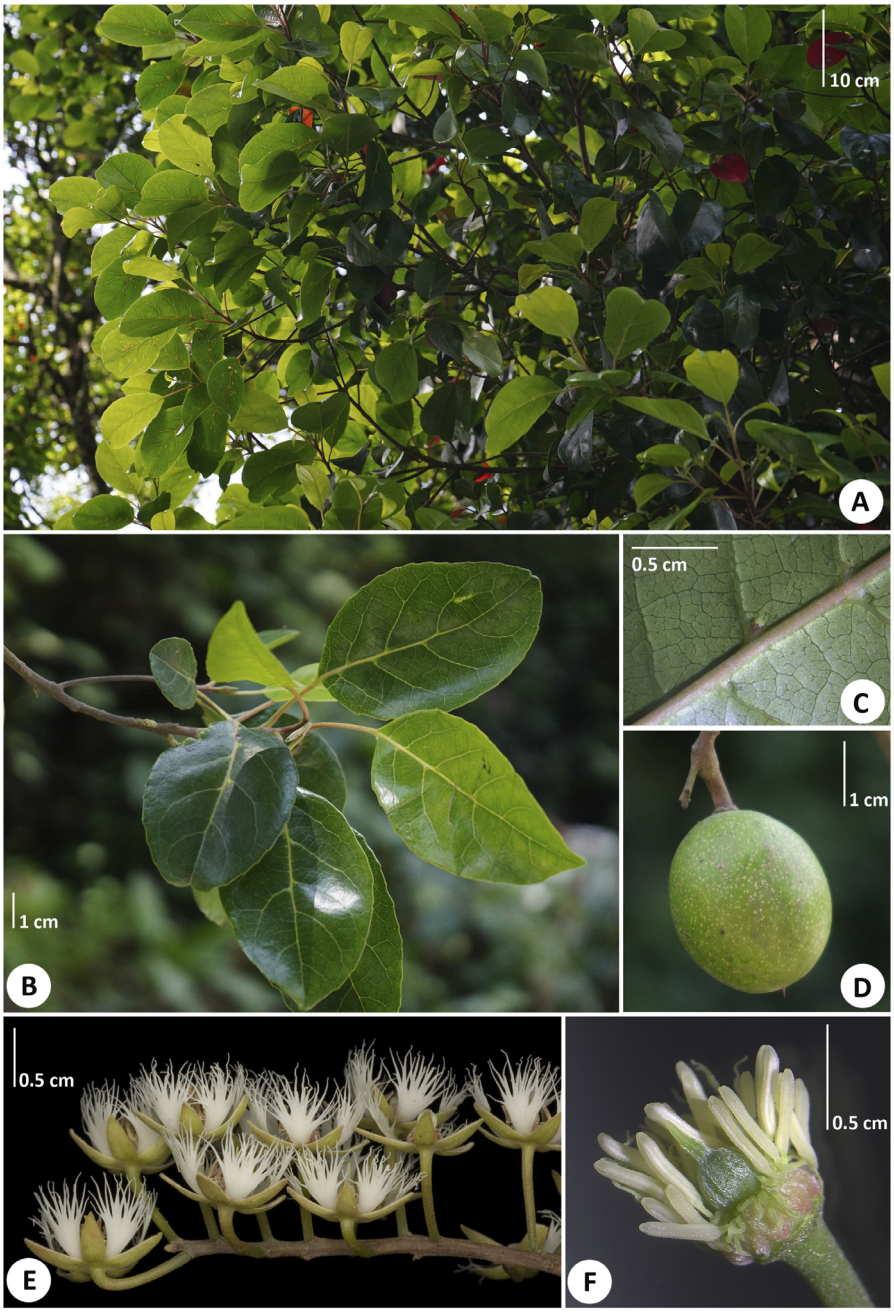

Figure 4. *Elaeocarpus pulneyensis* Gole *et al.*, sp. nov. A, Branches, showing leaves with domatia on the abaxial side; B, leaves, showing blunt apex; C, abaxial surface of leaf, showing domatia near primary vein; D, fruit; E, raceme, showing flowers with laciniate petals; F, dissected young flower, showing anthers without awns or hairs surrounding gynoecium. Photographs: Charuta Gole and Navendu Page.

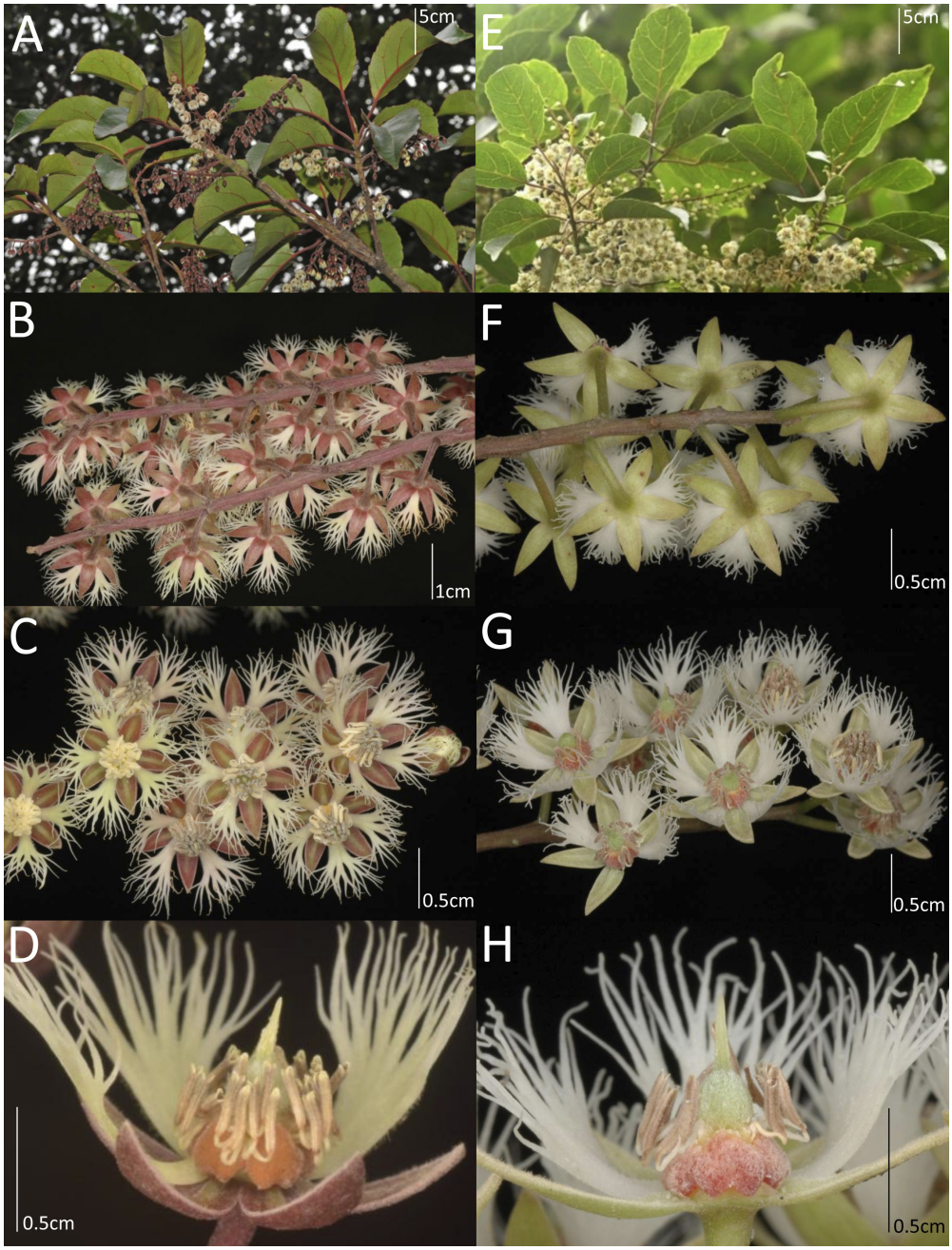

Figure 5. Comparative floral morphology of *Elaeocarpus variabilis* and *E. pulneyensis*. A–D, *Elaeocarpus variabilis*: A, flowering branch; B, dorsal view of the raceme; C, ventral view of the raceme; D, close-up of flower. E–H, *E. pulneyensis*: E, flowering branch; F, dorsal view of the raceme; G, ventral view of the raceme; H, close-up of flower. Photographs: Navendu Page.

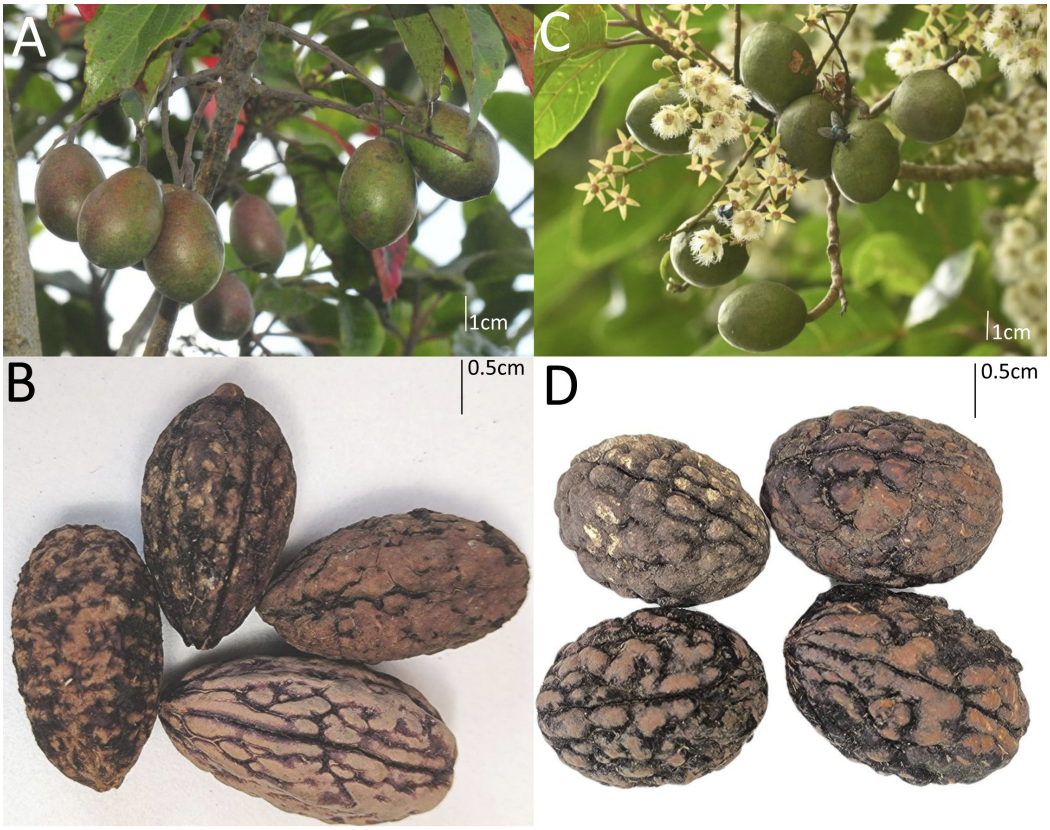

Figure 6. Comparative fruit and pyrene morphology of *Elaeocarpus variabilis* and *E. pulneyensis*. A and B, *Elaeocarpus variabilis*: A, fruits; B, pyrenes. C and D, *Elaeocarpus pulneyensis*; C, fruits; D, pyrenes. Photographs: Charuta Gole and Navendu Page.

Key to the species of Elaeocarpus *sect.* Elaeocarpus *from peninsular India*

1a. Anther tips with setae or tuft of hairs with varying length _____ 2
1b. Anther tips without setae or tuft of hairs _____ 5

2a. Pyrenes ellipsoid or globose, if ellipsoid then bluntly rounded at both ends _____ 3
2b. Pyrenes oblong, ovoid or obovoid _____ 4

3a. Leaf elliptic-orbicular; apex obtuse or rounded. Sepals scarlet red _____ *E. gaussenii*
3b. Leaf elliptic, lanceolate, apex shortly acuminate. Sepals green _____ *E. weibelii*

4a. Pyrenes obovoid, never ovoid or ellipsoid, cuneate (narrowing abruptly below the bulge), acute at the base _____ *E. gadgilii*

4b Pyrenes obovoid or oblong, narrowing gradually below the bulge, rounded or obtuse at the base _____ *E. serratus*

5a. Fruit ellipsoid; pyrenes ellipsoid, rounded at both ends. Sepals light green **E. pulneyensis**

5b. Fruit obovoid; pyrenes oblong, obovoid, tapering towards base, rounded at apex. Sepals scarlet red _____ *E. variabilis*

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Data availability statement

The relevant DNA sequences are deposited in GenBank, with accession numbers as given in the **Appendix table**.

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Appendix

Appendix table. Details of genetic sequences of species used in the present study

Species	Collector	Collection no.	Herbarium	Locality	ITS GenBank no.	*trn*L–F GenBank no.
Aceratium concinnum	D. M. Crayn	858	NSW	Australia	DQ448684	DQ444678
Elaeocarpus angustifolius	D. M. Crayn	572	NSW	New Guinea	KJ675645	KJ631297
Elaeocarpus angustifolius	Not available	NSW 710750	NSW	India	KJ675646	KJ631298
Elaeocarpus dongnaiensis	Nguyen Van Du	HNK 1118	K	Vietnam	KJ675653	KJ675690
Elaeocarpus gadgilii	C. N. Gole	CNG 350	SPPU	Nelliampathy, Kerala	OR116444	OR059200
Elaeocarpus gadgilii	C. N. Gole	CNG 352	SPPU	Nelliampathy, Kerala	OR116436	OR059201
Elaeocarpus gaussenii	C. N. Gole	CNG 347	SPPU	Meghamalai, Tamil Nadu	OR077105	OR059196
Elaeocarpus gaussenii	C. N. Gole	CNG 348	SPPU	Meghamalai, Tamil Nadu	OR098634	OR059199
Elaeocarpus glaber	Not available	VIC 179A	Not available	Not available	KJ675654	KJ675696
Elaeocarpus grandis	P. I. Ford	NSW 27569	BRI	Australia	KJ675655	KJ675700
Elaeocarpus nouhuysii	D. M. Crayn	530	NSW	New Guinea	KJ675659	KJ675712
Elaeocarpus nouhuysii	D. M. Crayn	533	NSW	New Guinea	KJ675660	KJ675713
Elaeocarpus obovatus	M. Rossetto & D. M. Crayn	*s.n.*	No voucher?	Australia	KJ675661	KJ675714
Elaeocarpus polydactylus	D. M. Crayn	577	NSW	New Guinea	KJ675682	KJ675715
Elaeocarpus ptilanthus	D. M. Crayn	554	NSW	New Guinea	KJ675683	KJ675716
Elaeocarpus pulneyensis	C. N. Gole	CNG 359	SPPU	Kodaikanal, Tamil Nadu	OR116435	OR059202
Elaeocarpus pulneyensis	C. N. Gole	CNG 360	SPPU	Kodaikanal, Tamil Nadu	OR116434	OR059203
Elaeocarpus ruminatus	Y. Baba	446	CNS	Australia	KJ675662	KJ675719
Elaeocarpus serratus	C. N. Gole	CNG 319	SPPU	Kudrevetti, Tamil Nadu	OR098633	OR146954
Elaeocarpus serratus	C. N. Gole	CNG 320	SPPU	Kakachi, Tamil Nadu	OR098632	OR146955
Elaeocarpus serratus	C. N. Gole	CNG 349	SPPU	Gersoppa, Karnataka	OR098634	OR146956
Elaeocarpus sphaericus	Not available	NSW 710753	NSW	India	KJ675679	KJ631299
Elaeocarpus stellaris	C. Costion	3531	CNS	Not available	KJ675665	KJ675726
Elaeocarpus stipularis	Not available	NSW 710749	Not available	Not available	KJ675666	KJ675727
Elaeocarpus tectorius	C. N. Gole	CNG 354	SPPU	UBK, Simlipal TR, Odisha	OR104998	OR059204
Elaeocarpus tectorius	C. N. Gole	CNG 357	SPPU	UBK, Simlipal TR, Odisha	OR105740	OR146957
Elaeocarpus variabilis	C. N. Gole	CNG 318	SPPU	Poopara, Kerala	OR130933	OR059198
Elaeocarpus variabilis	C. N. Gole	CNG 323	SPPU	Gureghar, Maharashtra	OR098634	OR032600
Elaeocarpus variabilis	C. N. Gole	CNG 329	SPPU	Lingmala, Maharashtra	OR098634	OR032601
Elaeocarpus variabilis	C. N. Gole	CNG 337	SPPU	Kothagiri, TamilNadu	OR054158	OR059195
Elaeocarpus variabilis	C. N. Gole	CNG 338	SPPU	Kothagiri, TamilNadu	OQ685021	OQ689748
Elaeocarpus variabilis	N. V. Page	NVP 305	SPPU	Periya RF, Kerala	OR130934	OR059197
Elaeocarpus weibelii	C. N. Gole	CNG 366	SPPU	Makutta Ghat, Karnataka	NA	NA
Elaeocarpus weibelii	C. N. Gole	CNG 370	SPPU	Valparai, Tamil Nadu	NA	NA

NA, not applicable.