

Variations in external and pollen morphological structures among four species of *Terminalia* L. (Combretaceae) from Terai-Dooars region of West Bengal, India

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Abstract

Four economically important species of *Terminalia* L. (Combretaceae) were studied for the main macro-morphological interspecific distinctions using mostly lamina. Their structure of bark, shape of lamina and type of fruits are quite distinct and characteristic. Though basically uniform but, there are some recognisable differences in their pollen morphology. The grains were prolate-spheroidal to sub-prolate and with prominent pseudo-colpi. The main differences among the grains were with the exine sculpturing that ranged from microrugulate in *T. catappa* L. to perforate-microrugulate in *T. bellirica* (Gaertn.) Roxb. and *T. chebula* Retz. and also striate in case of *T. arjuna* (Roxb. ex DC.) Wight & Arn. Two Keys, one with macro-morphology and the other with pollen morphology has been provided in the article.

Key words: *Terminalia*, Lamina structure, Pollen morphology

INTRODUCTION

Terminalia L. is a pantropical genus of Combretaceae with about 150 species of large trees (Mabberley 2005). The generic name '*Terminalia*' comes from the Latin word 'terminus' or 'terminalis' (i.e. ending), that refers to the formation of leaves near tips of the shoots (www.worldagroforestry.org/treedb2/speciesprofile.php?Spid=18136). Most of the species of *Terminalia* are distributed in tropical regions and are economically important in different ways including medicinal, ornamental, soil stabilizer, edible fruits and for quality timber (Pearson & Brown, 1932; Hill, 1952). Clarke (1879) recorded 12 species from British India and Clement (1991) recorded six species for the *Bhutan flora* that also covered Terai-Dooars and Darjeeling hills of West Bengal and Sikkim. Cytological studies on this genus revealed the variations in base, haploid, diploid and polyploid numbers at inter- and intra-specific levels (Sen 1955; Janaki Ammal & Sobti 1962; Mehra & Khosla 1972; Gill *et al.* 1982).

Conventionally, a plant is generally identified from its leaf structure, but in many species of *Terminalia* it is much overlapping. So a detailed study to characterize the lamina is essential for the genus.

Use of pollen morphological characters as a tool for the identification of plants and to understand their taxonomy is well known. Establishment of phylogeny among different taxa also can be drawn using such characters (Chanda *et al.* 1988).

In the present work four species of the genus *Terminalia* L. [*T. arjuna*, *T. bellirica*, *T. chebula* and *T. catappa*] was studied for their external structure and pollen morphology aiming for their effective identification. All the studied species are economically very important. While all the four species are medicinally important, they also produce good planking quality timber. In Indian Ayurvedic system ‘*trifala*’ (three fruits) is one important medicine in which two species of *Terminalia* (*T. bellirica* and *T. chebula*) are used (Kirtikar & Basu 1935). *T. catappa* also produce edible nuts and oil. However, all these plants are growing in Terai and Dooars. Being very tall trees, collection of flowering shoots become difficult that, quite often, create difficulty in their identification.

Previously, Krachai and Pornpongrueng (2015) worked on the pollen morphology of some species of Combretaceae from Thailand. However, no investigation has yet been made to understand its generic delimitation using pollen morphological characters.

In the present study an attempt has been made to investigate and to understand the variations and relation among the four species of *Terminalia* occurring in the Terai and Dooars regions of West Bengal using palynological and external morphological data.

MATERIALS AND METHODS

Well grown vegetative parts of four species of *Terminalia* L. (Combretaceae) were collected for external morphological works. This include mature leaves, bark, flowers and fruits. For, pollen morphological works anthers were collected from mature flower buds or from partially opened flowers to avoid contamination. Voucher specimens were processed into mounted herbarium sheets following conventional methods (Jain & Rao 1977). Verification of nomenclature was done by visiting www.theplantlist.org. Polliniferous materials were acetolysed following Erdtman (1952, 1960, 1969) and were mounted in unstained Keisser glycerin jelly (Keisser 1935). Voucher specimens and slides will be deposited at the NBU-Herbarium after the works are over. Light microscopical work was done under a Magnus Trinocular Microscope.

For descriptive terminology Erdtman (1952, 1960, 1969), Chanda (1966), Kremp (1965) and Faegri & Iversen (1964) were followed.

Details of the voucher specimens has been provided in Table 1.

Table 1. Details of the voucher specimens used for the study [All voucher specimens are the numbers of ‘Baishakhi & AP Das’. MWLS = Mahananda Wildlife Sanctuary]

Taxa Names	Voucher specimen	Local name	Place of collection	Occurrence
<i>Terminalia arjuna</i> (Roxb. ex DC.) Wight & Arn.	0400 dtd. 26.05.2015	<i>Arjun</i>	Sukna	Wild
<i>Terminalia bellirica</i> (Gaertn.) Roxb.	0427 dtd. 04.10.2015	<i>Baherha</i>	MWLS	Wild
<i>Terminalia chebula</i> Retz.	0104 dtd. 16.04.2013	<i>Haritaki</i>	MWLS	Wild
<i>Terminalia catappa</i> L.	0289 dtd. 24.09.2014	<i>Kath-badam</i>	Siliguri	Planted

RESULTS

External morphology

The external morphology of four species *Terminalia* L. is given below:

***Terminalia arjuna*:** Medium trees, 6 – 15 m high with large buttresses, bark pale greenish to whitish grey. Leaves usually sub-opposite, oblong-elliptic, 5 – 25 x 4 – 9 cm, entire, obtuse, base cordate; petioles 5 – 10 mm long with 2 glands at the summit. Inflorescence short axillary spikes or small terminal panicles; flowers sessile, yellowish white, stamens much exerted. Fruits ovoid-oblong, 2.5 – 5 cm long, brown when ripe, 5-winged. [Plate 1: 1]

***Terminalia bellirica*:** Large trees upto 35 m high with large buttresses; bark grey and longitudinally ridged. Leaves clustered at the end of branches, elliptic to ovoid, 4 – 24 x 2 – 11 cm, subentire, acute, base cuneate; petiole 2 – 8 cm, with 2 glands above the middle. Inflorescence axillary, simple spikes, 4 – 12 cm long; flowers solitary, small, 3 – 15 cm, calyx 4.5 - 5.5 mm, broadly cup-shaped, 5-lobed, pubescent, disc densely hairy. Drupes sub-globose to obovoid, weakly to strongly 5-ridged, 2 – 3 cm, pubescent. [Plate 1: 2]

***Terminalia catappa*:** Trees to 20 m high; bark brownish black with longitudinal peelings. Leaves clustered at the end of the branches; lamina obovate, 15 – 36 x 8 – 24 cm, subentire, obtuse or acute, base cordate; petioles 0 - 2.5 cm. Inflorescence axillary, simple, slender spikes, 8 – 15 cm long, numerous flowered; flowers fragrant, off-white; calyx 4.5 cm, pubescent to sub-glabrous, 5-lobed; stamens 10, exerted. Drupes ovoid or ellipsoid, slightly or strongly compressed, 2-ridged, 3.5 – 5 x 2 - 3.5 cm, glabrous when mature. [Plate 1: 3]

***Terminalia chebula*:** Trees to 30 m high; bark greyish black to grey and coarsely split and thick. Leaves ovate-oblong or elliptic, 7 – 18 x 4.5 – 10 cm, entire, acute, base rounded to cuneate; petiole 2 – 3 cm with 2 inconspicuous glands near apex. Inflorescence axillary or terminal, spikes simple, 5 – 14 cm; calyx 3 – 3.5 cm, glabrous, 5-lobed; stamens 10, exerted. Drupes sub-globose, smooth or with 5-ridges which becomes deeply wrinkled when dry, glabrous. [Plate 1: 4]

Pollen morphology

External morphology of pollen grains has been studied under the light microscope and were characterized as follows:

Terminalia arjuna

3-colporate with prominent psdeudo-colpi; isopolar; radiosymmetric; small; EV: prolate spheroidal; PV: circular; colpi linear with acute ends and broad at middle; pseudo-colpi almost the size of colpi; ora lalongate; exine: striate, 1.07 μm ; PA: 14 μm , ED: 15.9 μm . [Plate 1: 5 & 6]

Terminalia bellirica

3-colporate with prominent psdeudo-colpi; isopolar; radiosymmetric; small; EV: prolate spheroidal; PV: circular; colpi linear with acute ends and broad at middle; pseudo-colpi almost the size of colpi; ora lalongate; exine: perforate-microrugulate, 2.25 μm ; PA: 17 μm , ED: 17.9 μm . [Plate 1: 7 & 8]

Terminalia chebula

3-colporate with prominent psdeudo-colpi; isopolar; radiosymmetric; small; EV: prolate-spheroidal; PV: circular; colpi linear with acute ends and broad at middle; pseudo-colpi almost

the size of colpi; ora lalongate; exine: perforate-microrugulate, 1.07 μm ; PA: 15.13 μm , ED: 13.3 μm . [Plate 1: 9 & 10]

Terminalia catappa

3-colporate with prominent psuedo-colpi; isopolar; radiosymmetric; small; EV: prolate-spheroidal to sub-prolate; PV: circular; colpi linear with acute ends and broad at middle; pseudo-colpi almost the size of colpi, fused at apocolpia; ora lalongate; exine: microrugulate, 2.25 μm ; PA: 25 μm , ED: 26 μm . [Plate 1: 11 & 12]

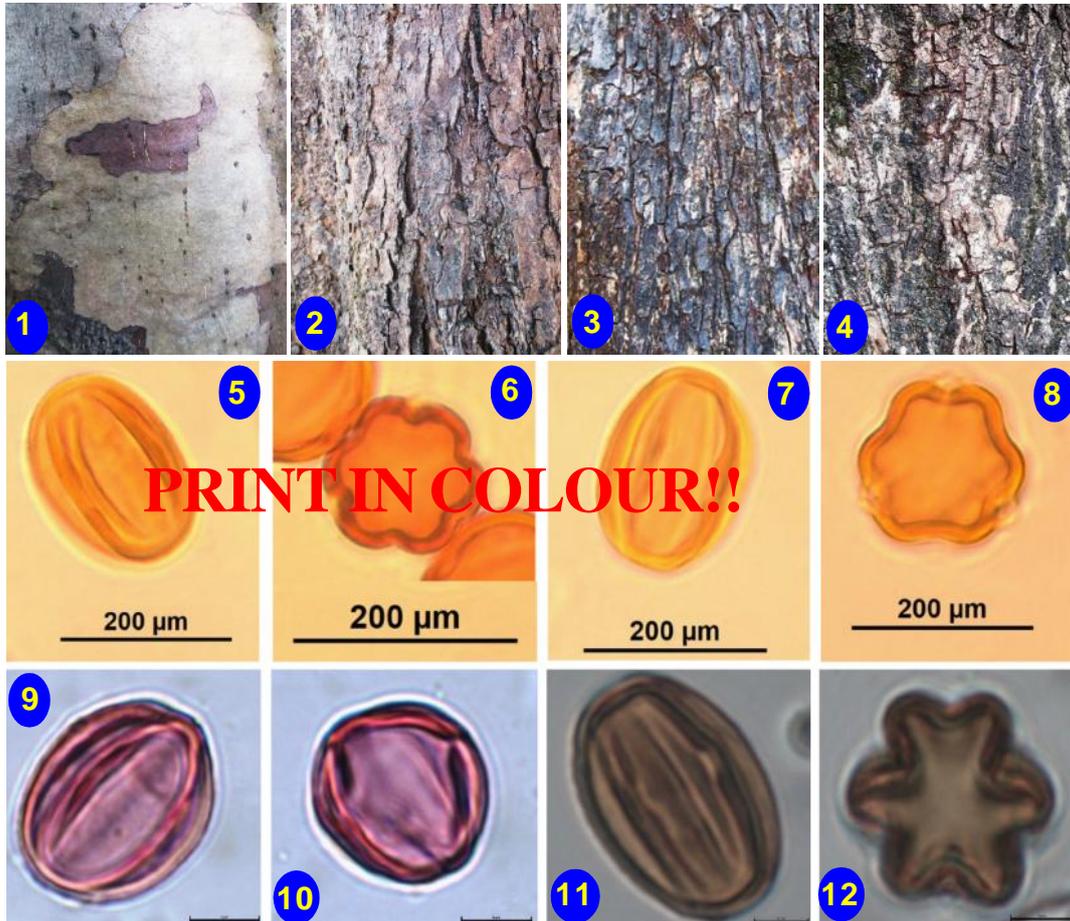


PLATE – I: 1. Bark of *T. arjuna*; 2. Bark of *T. bellirica*; 3. Bark of *T. catappa*; 4. Bark of *T. chebula*; 5. EV of *T. arjuna*; 6. PV of *T. arjuna*; 7. EV of *T. bellirica*; 8. PV of *T. bellirica*; 9. EV of *T. chebula*; 10. PV of *T. chebula*; 11. EV of *T. catappa*; 12. PV of *T. catappa* [EV = Equatorial View; PV = Polar View]

DISCUSSION AND CONCLUSION

The main macro-morphological distinction among the species can be done by the shape and size of their bark, lamina, and inflorescence and fruit structures. The sizes of these four tree-species were quite different. It varied from 15 m in *T. arjuna* to 35 m in *T. bellirica*, 20 m in *T. catappa* and 30 m in *T. chebula*. The primary distinction between the species using the macro-morphology can be done by the shape and size of the lamina. Leaves are clustered towards the apex of the branches in *T. bellirica* and *T. catappa*. In *T. arjuna* the leaves are oblong-elliptic, 5 – 25 x 4 –

9 cm, whereas in *T. bellirica* the leaves are elliptic to ovoid, 4 – 24 x 2 – 11 cm. In *T. catappa* the leaves are obovate, 15 – 36 x 8 – 24 cm, but in *T. chebula* they are ovate-oblong or elliptic, 7 – 18 x 4.5 – 10 cm. The presence and position of glands also quite characteristic.

The fruit structure also helped in identification of the species. Fruits of *T. arjuna* are obovoid-oblong, woody reddish-brown, winged; in *T. bellirica* they are sub-globose or broadly ellipsoid, light yellow, weakly 5-ridged; in *T. catappa* they are oval, rounded to flattened, green- red, two ridged; in *T. chebula* sub-globose, brownish black, five ridged, which become deeply wrinkled when dry.

An artificial key has been prepared using the morphological characters.

KEY TO THE SPECIES BASED ON EXTERNAL MORPHOLOGY

- 1a. Petiole without glands at the summit; calyx lobes pubescence *T. bellirica*
- 1b. Petioles with 2 glands at summit 2
- 2a. Fruits winged; young stem and leaves not blackish hairy 3
- 2b. Fruits not winged; young stem blackish hairy *T. chebula*
- 3a. Fruits 2- winged; pericarp fleshy, senescent leaves red *T. catappa*
- 3b. Fruits 5- winged; pericarp fibrous; senescent leaves not red *T. arjuna*

In their pollen morphology, though they are basically uniform but there are some distinct recognisable differences. All the grains were with tricolporate aperture. Although Krachai and Pornpongrungrueng (2015) reported pseudo-colpi only in *T. catappa*, but from the present study pseudo-colpi were noticed in all the four species. Pseudo-colpi fused at the polar area only in *T. catappa*. Exine ornamentation and size showed little difference between the grains. It is striate in *T. arjuna*, perforate -microrugulate in *T. bellirica* and *T. chebula*, and

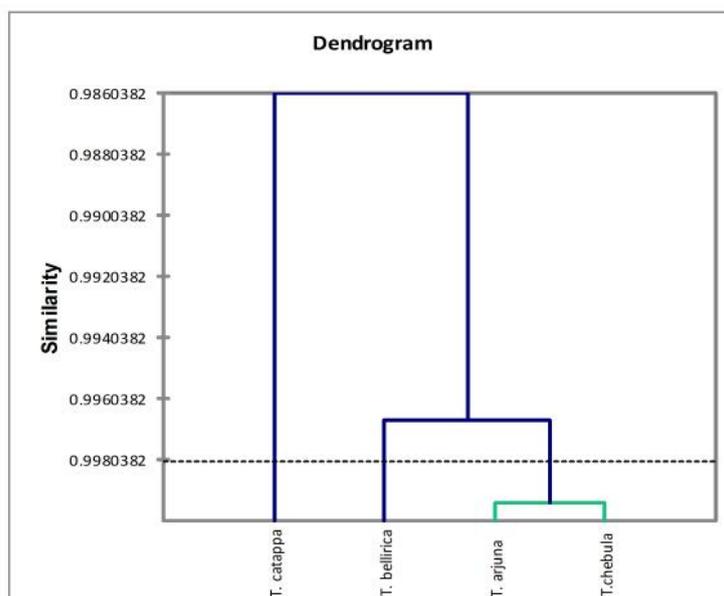


Figure 1. Dendrogram based on morphological data on four species of *Terminalia* L.

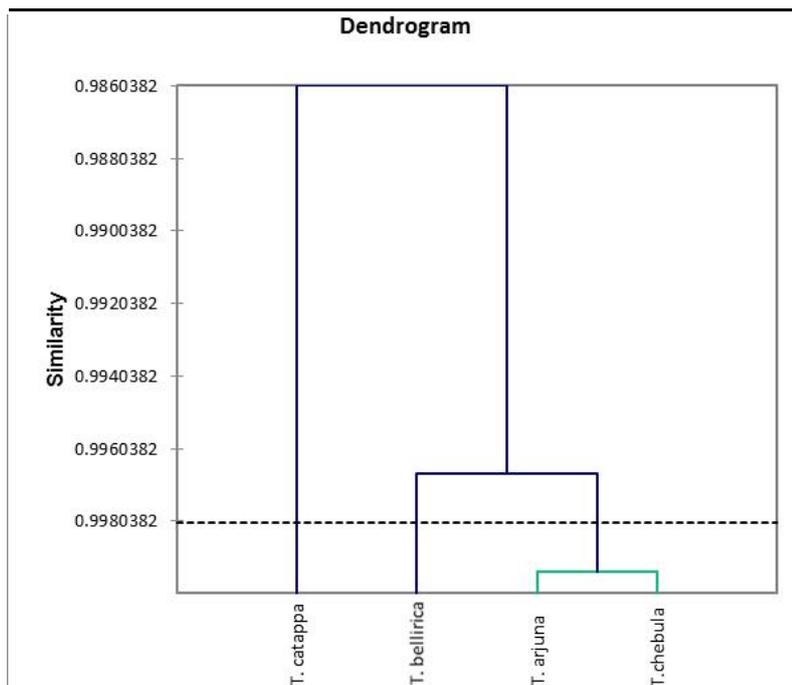


Figure 2. Dendrogram based on palynological data on four species of *Terminalia* L.

microrugulate in *T. catappa*. The size varied from 14 μm in *T. arjuna* to 25 μm in *T. catappa*. The shape of the grains and the apertures showed no variation. These observations indicate the remoteness of *T. catappa* from the remaining three species in pollen morphology and that is also reflected in both the dendrograms (Figures 1 & 2). The perforate-microrugulate exine also express the closeness of *T. bellirica* and *T. chebula*. On the other hand apertural types of all the species are very similar and that expresses their integrity at the generic level.

An artificial key has been constructed using the pollen morphological characters.

KEY TO THE SPECIES BASED ON POLLEN MORPHOLOGY

- 1a. Pseudo-colpi fused at apocolpia *T. catappa*
- 1b. Pseudo-colpi not fused at apocolpia 2
- 2a. Exine striate *T. arjuna*
- 2b. Exine perforate-microrugulate 3
- 3a. Exine 2.25 μm thick *T. bellirica*
- 3b. Exine 1.07 μm thick *T. chebula*

Two dendrograms were prepared using the macro-morphological data (Figure 1) and the pollen morphological data (Figure 2) as well. Interestingly, both the dendrograms came out exactly similar. Here also isolation of *T. catappa* from three other species is quite prominent. Only deviation, interestingly, *T. chebula* is placed apart from *T. bellirica* and, probably, needs further investigation.

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LITERATURE CITED

- Chanda, S. 1966. On the pollen morphology of Centrolepidaceae, Restionaceae and Flagellariaceae with special reference to taxonomy. *Grana Palynol.* 6(3): 365 – 415.
- Chanda, S.; Nilsson, S. & Blackemore, S. 1988. Phylogenetic trends in the Alismatales with reference to pollen grains. *Grana.* 27: 257 – 272.
- Clarke, C.B. 1879. *Terminalia* in Hooker, J.D. (ed.), *The Flora of British India*, vol. 2. L. Reeve & Co., Ashford, Kent, London
- Clement. 1991. *Terminalia* L. in Grierson, A.J.C. & Long, D.G. (eds.), *Flora of Bhutan*, Vol. 2, Pts. 1. Royal Botanic Garden, Edinburgh
- Erdtman, G. 1952. *Pollen morphology and plant taxonomy: Angiosperms*. Almqvist and Wiksell, Stockholm.
- Erdtman, G. 1960. The acetolysis method. A revised description. *Svensk Botanisk Tidskrift*, Bd. 54, H4: 561 – 564.
- Erdtman 1969. *Handbook of Palynology*. Munksgaard, Copenhagen.
- Faegrie, K. & Iversen, J. 1964. *Text book of pollen analysis* (2nd. Ed.). Oxford.
- Gill, B.S.; Bir, S.S. & Singhal, V.K. 1982. Cytogenetics of some timber species of *Terminalia* Linn. (Combretaceae). *Proc. Indian Natn. Sci. Acad.* B48 (6): 773 – 790.
- Hill, A.F. 1952. *Economic Botany*. McGraw Hill Brook Co. Inc., New York, Toronto, London.
- Jain, S.K. & Rao, R.R. 1977. *A Handbook of Field and Herbarium Methods*. Today & Tomorrow's Printers and Publishers, New Delhi.
- Janaki Ammal, E.K. & Sobti, S.N. 1962. Polyploidy in the genus *Terminalia*. *Sci. Cult.* 28: 378 – 380.
- Kirtikar, R. B. & Basu, B. D. 1935. *Indian Medicinal Plants*, Vol. I - IV. Lalit Mohan Basu, Allahabad.
- Kisser, J. 1935. *Bemerkungen zum Einschluss in Glycerin-Gelatine*. *Z. Wiss. Mikr.*
- Krachai & Pornpongrungrueng. 2015. Pollen morphology of Combretaceae from Thailand and its taxonomic significance. *Thai For. Bull. (Bot.)* 43:4 – 14.
- Kremp, G.O.W. 1965. *Morphologic encyclopedia of palynology*. Univ. Arizona Press, Tucson.
- Mabberley, D.J. 2005. *The Plant Book – A portable dictionary of the vascular plants*. First Asian Edition. Cambridge University Press, U.K.
- Mehra, P.N. & Khosla, P.K. 1972. Cytogenetical studies of Himalayan Hemamelidaceae, Combretaceae and Myrtaceae. *Silv. Genet.* 21: 186 – 190.
- Pearson, R.S. & Brown, H.P. 1932. *Commercial Timbers of India*. Vols. I & II. Reprinted by: A.J. Reprints Agency, New Delhi.
- Sen, S. 1955. Chromosome numbers in the family Combretaceae. *Curr. Sci.* 12: 422 – 423.
- www.theplantlist.org
- www.worldagroforestry.org/treedb2/speciesprofile.php?Spid=18136