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Molecular systematics, DNA barcoding and conservation genetics of *Anogeissus* Guill. (DC.) & Perr. in the expanded genus *Terminalia* L. A review with special reference to Indian Arid-Zone taxa (Combretaceae)

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Abstract
This review summarises recent advances on molecular systematics, DNA barcoding and conservation genetics of *Anogeissus* (DC.) Guill. & Perr. in the extended genus *Terminalia* L. (Combretaceae). Recent phylogenetic and phylogenomic studies have consistently placed genus *Anogeissus* within *Terminalia* resulting in a major taxonomic rearrangement of the group. The molecular basis of this reclassification is reviewed and its implications for species delimitation and conservation of Indian arid zone taxa are discussed. Methodological aspects of molecular studies in the Combretaceae with special reference to Phylogeny of *Anogeissus* and *Terminalia* and DNA extraction from polyphenol- and polysaccharide-rich tissues. Studies have demonstrated that recovery of PCR-amplifiable DNA from mature tree tissues requires an optimised three-step extraction protocol comprising a pre-washing with Triton X-100, modified CTAB extraction with polyvinylpyrrolidone and ascorbic acid, and spin-column purification. Comparative analysis of the barcode loci (*rbc*L, *mat*K, ITS and *psb*A-*trn*H) suggests that matK provides higher species level resolution and that multilocus approaches are more powerful than single marker analysis. *Anogeissus* species are ecologically and medicinally important but we have very little knowledge about the genetic diversity and population structure of these species. The review suggests to combine multilocus DNA barcoding with highly polymorphic markers (e.g. ISSRs and microsatellites) for better conservation genetics and species delimitation studies.

Keywords: *Anogeissus*, *Terminalia*, Combretaceae, DNA barcoding, *mat*K, *rbc*L

Introduction

The family Combretaceae R.Br. (Order Myrtales, Berger *et al*., 2016) [34] includes more than 600 species of trees, shrubs, and lianas that are distributed throughout tropical and subtropical climates around the world. Fossil evidence suggests the family is of Gondwanan origin (Ullah *et al*., 2025) [32] and has experienced significant intercontinental dispersal events, largely dictated by fruit form and seed dispersal strategies (Jurd & Pole, 2017) [12]. The family has been split into two tribes, Laguncularieae and Combreteae, with about 10 taxa distributed in several tropical environments (Maurin *et al*., 2023) [18].

The genus *Anogeissus* (DC.) Guill. & Perr. has been extensively revised taxonomically. Molecular phylogenetic analyses based on plastid and nuclear DNA markers have consistently shown that *Anogeissus* is phylogenetically nested within *Terminalia*, supporting its inclusion in a broad circumscription of *Terminalia* (Maurin *et al*., 2017, 2023) [18, 19]. The reclassification is one of the largest systematic changes in the Combretaceae in recent years.

In arid and semi-arid regions of India, species of *Anogeissus* have immense ecological, economic and ethnomedicinal importance (Mann *et al*., 2009) [16]. The major Indian taxa are *Anogeissus acuminata* (Roxb. ex DC.) Wall. ex Guill. & Perr., *Anogeissus latifolia* (Roxb. ex DC.) Wall. ex Guill. & Perr., *Anogeissus pendula* Edgew., *Anogeissus sericea* var. *nummularia* King ex Duthie and *Anogeissus sericea* Brandis var. sericea (Bhandari, 1978; Meena, 2014). These species are widely used for timber, charcoal, fodder and gum by local communities living in dryland habitats. *A. latifolia* ("Dhaora") is the most important commercial source of gum ghatti, a non-starch polysaccharide used as an emulsifying agent in pharmaceutical and food industries (Dadhich *et al*., 2022) [8]. Different parts of plant have been used in traditional medicine for the treatment of wounds, fever and snakebites (Kumar *et al*., 2025) [25].

Recent phytochemical and pharmacological studies have revealed the therapeutic potential of the genus. Dadhich *et al*. (2022) [8] reported the conventional therapeutic importance of *Anogeissus* species particularly in wound healing, fever therapy and infection control. Yadav *et al*. (2026) [35] reported that *A. latifolia* has major amounts of flavonoids, tannins, phenols and other bioactive chemicals associated with significant antioxidant activity.

Conservation status and threats

Genetic diversity is a core aspect of biodiversity and is critical for species to adapt to changes in their environment and prolonged ecological pressures. Thus, the knowledge of genetic variability is essential for the formulation of effective conservation strategies (Hasnain & Mehvish, 2020) [10].

Several *Anogeissus* species are currently under serious threat of conservation concern by reason of their ecological and economic importance in Rajasthan and surrounding areas. *Anogeissus sericea* var. *nummularia* is endangered

and *A. latifolia* is threatened due to overexploitation for timber and fuelwood, low seed viability, anthropogenic disturbances and low natural regeneration (Kumar *et al*., 2010) [14]. *A. acuminata* is considered endangered in parts of China, but a complete assessment of its broader distribution across Asia has not been performed (Yu *et al*., 2020) [10].

A major concern in the way of population recovery is the very low seed viability reported in several species. More than 95% of the seeds harvested in *A. latifolia* may be nonviable, with germination rates as low as 1–2%, severely limiting the natural regeneration potential (Dadhich *et al*., 2022) [8]. Population decline in dry areas has been exacerbated by habitat fragmentation, overexploitation and increasing anthropogenic pressures (Mohammed *et al*. 2023) [21].

Despite acknowledged conservation concerns, the molecular-level comprehension of genetic diversity, population structure, and gene flow within Indian populations of *Anogeissus* remains significantly insufficient. Specifically, scattered populations along the Aravalli area have not been comprehensively examined utilising molecular markers. The lack of baseline genetic data constitutes a significant constraint for the development of scientifically informed conservation and restoration initiatives.

Taxonomic Complexity

The taxonomic history of *Anogeissus* in the Combretaceae family has been complex and controversial (Barrett *et al*., 2015). The conventional classifications, which are based mainly on morphology, frequently led to indistinct generic limits, as there is a substantial overlap of diagnostic characters between related genera (Tan *et al*., 2002) [31]. Over the last two decades, molecular phylogenetic studies have greatly clarified these relationships and revolutionised systematic understanding of the group.

Preliminary phylogenetic analyses based on plastid genes (e.g. *rbc*L, *mat*K, and *psa*A-*ycf*3) in combination with nuclear ITS sequences showed that *Anogeissus* is phylogenetically nested within a paraphyletic *Terminalia* (Maurin *et al*., 2017) [19]. The results cast doubt on traditional taxonomic limits in the Combretaceae and provide strong molecular evidence to support inclusion of *Anogeissus* within an enlarged *Terminalia*. So, in a complete systematic review of the family, Maurin *et al*. (2017) [19] reclassified all the species of *Anogeissus* into *Terminalia*.

Additional phylogenomic analyses using the Angiosperms353 universal probe set corroborated this updated classification. Using multiple low-copy nuclear loci, Maurin *et al*. (2021) [17] showed that the previously recognised genera *Anogeissus*, *Buchenavia*, *Bucida*, and *Pteleopsis* are within a strongly supported monophyletic clade of *Terminalia*, sister to *Conocarpus* in the subtribe Terminaliinae. The results provided genome-wide support for previous multilocus phylogenetic results and greatly reinforced the revised circumscription of *Terminalia*.

Multilocus DNA barcoding studies at regional level on Indian *Terminalia* taxa from Rajasthan have further highlighted the systematic complexity of the group. Kumar and Kataria (2026) [15] analysed nuclear ITS and plastid markers (*mat*K, *rbc*L and *psb*A-*trn*H) in several taxa including *T. coronata* L., *T. coronata* var. *parvifolia* (C.B. Clarke) Chakrab. & Anand Kumar, *T. anogeissiana* Gere & Boatwr., *T. pendula* (Edgew.) Gere & Boatwr. and *T. pendula* var. *pendula*. Their results confirmed that species-level delimitation within the group remains difficult due to morphological convergence, phenotypic plasticity and the relatively conserved nature of several barcode loci.

These studies reveal a major systematic problem. The evolutionary placement of *Anogeissus* within *Terminalia* is now well established; however, the precise molecular delimitation of closely related species and varietal taxa is still poorly resolved. In addition, intraspecific genetic variation across geographically dispersed populations has been poorly studied especially in Indian arid-zone environments. Thus, significant gaps persist in the molecular taxonomy and conservation genetics of these ecologically important organisms.

Molecular approaches and scope of the review

Environmental variation also drives extensive phenotypic plasticity in Anogeissus and related *Terminalia* taxa and further complicates species delimitation (Gere *et al*., 2013). Morphological characteristics are often changed in response to habitat conditions, which reduces the reliability of classical taxonomic methods based on external characters (Nicotra *et al*., 2010) [22]. Thus, integrative approaches with molecular data are increasingly important for accurate identification, phylogenetic reconstruction, and evolutionary interpretation of these taxa (Kress *et al*. 2014) [13].

Molecular markers offer much higher resolution than morphology-based methods, as it directly measures genetic variation and evolutionary relationships (Singh *et al*., 2017) [30]. DNA barcoding is one of the most successful approaches among these for species identification and phylogenetic inference. DNA barcoding uses short and standardised regions of the genome, which can discriminate taxa at various hierarchical levels and can also help resolve taxonomic ambiguities in morphologically complex groups (Roy, 2014) [28].

The Consortium for the Barcode of Life (CBOL) recommended the plastid loci rbcL and matK as the standard two-locus core barcode system for land plants. Later studies, however, have shown that nuclear ribosomal regions, including ITS and ITS2, can provide greater discriminative power at lower taxonomic levels, especially among closely related angiosperm species (CBOL Plant Working Group, 2009; China Plant BOL Group, 2011) [6, 7]. Each marker has a different contribution to phylogenetic resolution due to variation in evolutionary rates. The rbcL gene is highly conserved and useful for genus- and family-level relationships, while matK generally provides better resolution at the species level due to its higher substitution rate. In contrast, the rapidly evolving nuclear ITS region is particularly useful for resolving recent divergences and closely related taxa (Kumar & Kataria, 2026) [15].

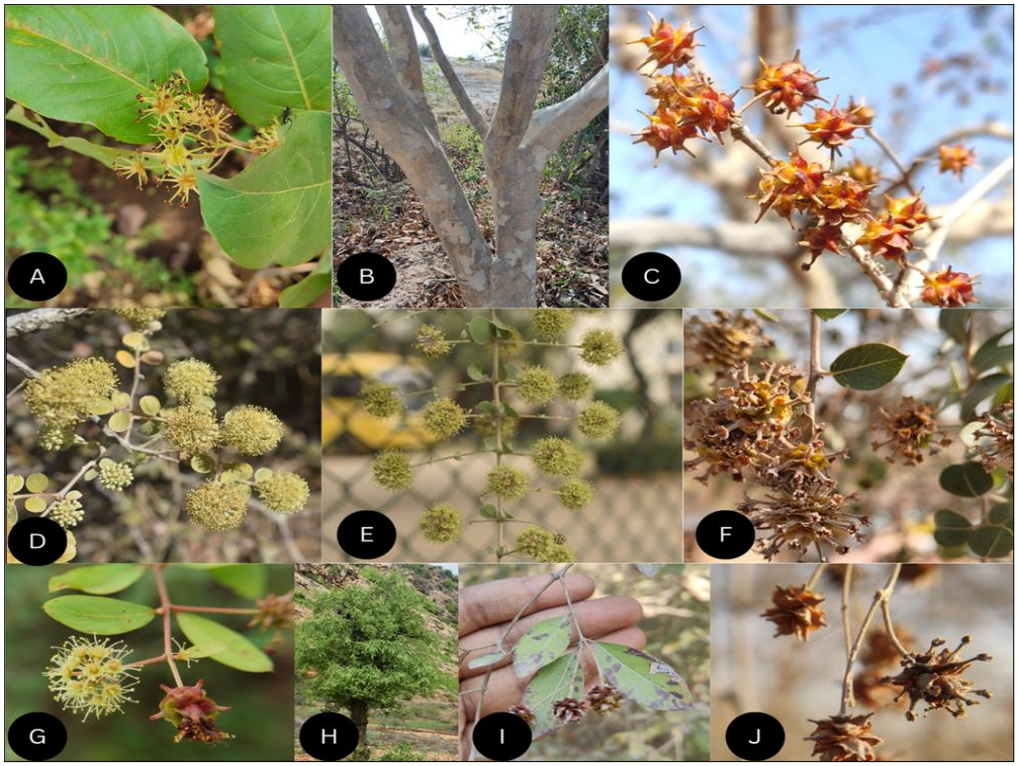

Fig 1: Variations in Habit: (A) Anogeissus latifolia; Leaves and Head, (B). Bark of A. latifolia(C). Fruits of A. latifolia (D). A. sericea var. sericea, Head (E). A. sericea var. nummularia, Heads, (F). Mature fruits of A. sericea var. nummularia, (G). A. pendula, Leaves and Head, (H) Habit of A. pendula, (I). Head of A. pendula, (J). Mature Head of A. pendula

Multilocus approaches have consistently shown better performance than single marker analyses for species delimitation and phylogenetic reconstruction in *Terminalia* studies. This pattern is consistent over the entire geographic range of the family. Gere *et al*. (2013) showed that evaluation of four barcode markers in southern African Combretaceae indicated that the core barcodes alone were less effective than multilocus combinations. Addition of *trn*H-*psb*A to the core system significantly improved the species level discrimination at both subgeneric and sectional levels.

Molecular data interpretation based on a single molecular marker might be misleading, because different genomic regions may have different selective pressures and evolutionary histories (Avise *et al*., 2012) [3]. The combination of multiple plastid and nuclear loci is required for robust phylogenetic inferences in taxonomically difficult groups (Roy, 2014) [28].

The taxonomic uncertainties, molecular studies of *Anogeissus* are faced with significant methodological difficulties in DNA extraction from tissues rich in polyphenols and polysaccharides. Population level genetic studies are highly limited across the Indian distribution range of these taxa. The present review aims to: Based on these gaps,

1. synthesize current knowledge regarding the molecular systematics, phylogenetics, and genetic diversity of *Anogeissus* within the expanded *Terminalia* framework;
2. critically evaluate methodological approaches employed in previous molecular investigations, particularly those related to DNA extraction and marker selection;
3. identify major gaps in current understanding of species delimitation and conservation genetics; and
4. Propose a future molecular research framework to support evidence-based conservation and management of ecologically and economically important arid-zone taxa in India.

Comparative Methodology and Challenges in Molecular Systematics of Combretaceae

1. Early multilocus parsimony frameworks

The molecular systematics of Combretaceae has been greatly advanced by the progressive incorporation of plastid, nuclear and phylogenomic data sets. The foundation was laid by Maurin (2010) who based on his doctoral work mainly on African representatives provided a robust subgeneric and sectional classification framework through a combined analysis of *rbc*L, ITS, *trn*H-*psb*A and *psa*A-*ycf*3 markers. Importantly, this study was the first to provide compelling molecular evidence that *Terminalia* in its traditional sense was paraphyletic, including genera such as *Anogeissus*, *Buchenavia* and *Pteleopsis* – setting the stage for their later taxonomic transfer to an expanded *Terminalia*. Bayesian molecular dating methods, and in particular BEAST, have offered a complementary phylogenetic framework, by estimating divergence times and placing patterns of diversification in the context of environmental and evolutionary history (Reis *et al*., 2015) [26].

Building on this foundation, Maurin *et al*. (2017) [19] expanded the phylogenetic sampling to over 114 species across 14 Combretaceae genera using a five-locus framework (*rbcL*, *matK*, *trnH-psbA*, *psaA-ycf3*, and *ITS*), achieving the first comprehensive resolution of relationships within Combretinae and Terminaliinae. Analytical congruence across Maximum Parsimony (PAUP*), Bayesian inference (MrBayes), and Maximum Likelihood (RAxML) approaches confirmed the stability of inferred topologies. The inclusion of *Anogeissus*, *Buchenavia*, and

Pteleopsis within *Terminalia* received consistently high support across all methods. Marker-level evaluation revealed considerable variation in phylogenetic informativeness: *rbcL* contributed the fewest parsimony-informative sites among plastid loci, while nuclear *ITS* showed the highest sequence variability; *matK*, *trnH-psbA*, and *psaA-ycf3* occupied intermediate positions, underscoring the advantage of multilocus over single-marker approaches. The discriminatory utility of *trnH-psbA* has been independently confirmed in southern African Combretaceae, where its addition to core barcode datasets markedly improved species-level resolution (Gere *et al*., 2013). Biogeographic structuring was also evident, with geographically coherent clades identified within Terminaliinae, and *Conocarpus* consistently resolved as sister to the expanded *Terminalia*.

A subsequent step-change in resolution was achieved by Yu *et al*. (2020) through complete chloroplast genome sequencing of *Anogeissus acuminata*. Whole-plastome Maximum Likelihood analysis placed *A. acuminata* as sister to *Terminalia guyanensis* with full bootstrap support, providing plastome-scale corroboration for the inclusion of *Anogeissus* within *Terminalia*. The dataset additionally resolved deeper familial relationships, revealing a well-supported affinity between the *Anogeissus–Terminalia* lineage and mangrove-associated genera *Laguncularia* and *Lumnitzera*. The markedly higher node support throughout the plastome-based topology, relative to earlier single-locus analyses, highlights the superior resolving power of whole-genome approaches for phylogenetic inference within Combretaceae.

2. Phylogenomic resolution through target capture sequencing

One of the most significant methodological advances in Combretaceae systematics has been the use of target capture phylogenomics with the universal probe set Angiosperms353. This approach allowed for the simultaneous recovery of hundreds of low-copy nuclear loci across many taxa and greatly increased phylogenetic resolution throughout the family (Johnson *et al*., 2018) [11].

Maurin *et al*. (2023) [18] used this phylogenomic framework to sample approximately 30% of Combretaceae diversity, by analysing 353 nuclear loci from 200 representative taxa. In contrast to previous multilocus studies limited to a handful of plastid and nuclear markers, this genome-scale dataset provided unprecedented resolution of deep and shallow evolutionary relationships within the family.

The analytical framework combined concatenated maximum likelihood analyses with RAxML and coalescent-based species tree estimation implemented in ASTRAL-III (Mirarab and Warnow, 2015). Quartet-based analyses were used to assess gene tree discordance, and TreeShrink (Mai and Mirarab, 2018) was applied to identify and remove anomalous long branches before final phylogenomic reconstruction. The reliability of the nodes was evaluated by several statistics, including quartet support values, local posterior probabilities, and bootstrap percentages, thus providing a stringent test of the phylogenetic confidence (Maurin *et al*., 2023) [18].

3. Regional DNA barcoding of Indian *Terminalia*

Multilocus DNA barcoding has increasingly been used to resolve species-level ambiguities in Indian *Terminalia*, with regional studies providing valuable molecular frameworks for identification. The discriminatory power of four barcode markers, ITS, *mat*K, *rbc*L and *psb*A-*trn*H was evaluated among *Terminalia* taxa from Rajasthan and the advantages and disadvantages of individual loci were discussed by Kumar and Kataria (2026) [15]. Nucleotide composition analysis revealed a consistent dichotomy between genome compartments: plastid markers were characteristically AT-rich, the nuclear ITS region showed elevated GC content, a hallmark of divergent evolutionary constraints on the two genomes.

Among the markers studied, *mat*K was the most useful for species discrimination, showing the highest number of parsimony-informative sites, larger interspecific genetic distances and a clear placement of *T. anogeissiana* as a distinct lineage. Conversely, ITS failed to resolve closely related varietal taxa, despite overall sequence variability, suggesting insufficient resolution for recently diverged lineages or those with overlapping morphology. These results agree with limitations observed in previous studies of the systematics of Combretaceae (Maurin *et al*., 2017) [19] and support the need for multilocus approaches in taxonomically complex groups of the family.

Concatenated phylogenetic analyses integrating all four loci generated comparatively well-supported clades and produced improved species-level resolution relative to single-marker analyses. The study therefore reinforced the growing consensus that combined plastid and nuclear barcode frameworks provide more reliable molecular identification and phylogenetic reconstruction within *Terminalia* and allied taxa.

DNA Extraction from Recalcitrant Combretaceae Taxa

The availability of high-quality genomic DNA is crucial for accurate molecular characterisation and for conservation genetic analysis. One of the most persistent methodological challenges in molecular studies of the Combretaceae, however, is the extraction of amplifiable DNA from mature tissues rich in secondary metabolites (Singh *et al*., 2023) [29]. Species of *Anogeissus* and *Terminalia*, particularly those adapted to arid and semi-arid environments, accumulate large amounts of tannins, polyphenols and polysaccharides within leaf tissues. These compounds often co-precipitate with nucleic acids during extraction procedures and strongly inhibit downstream enzymatic reactions such as PCR amplification (Rezadoost *et al*. 2016) [27].

Commonly used commercial spin-column kits and conventional CTAB extraction protocols are often not sufficient for these chemically recalcitrant taxa as contaminating secondary metabolites interfere with DNA purity and integrity (Rana *et al*., 2025) [25]. Hence, optimisation of extraction method is necessary for successful molecular analysis of Combretaceae species.

A major methodological advance was made by Gupta *et al*. (2011) [9] who designed a standardised three-step sequential extraction protocol specifically for mature arid-zone Combretaceae taxa. The protocol was validated in seven ecologically and medicinally important species, namely *Anogeissus sericea* var. *nummularia*, *A. pendula*, *A. latifolia*, *Terminalia arjuna*, *T. bellirica*, *T. catappa* and *T. chebula*.

The initial step involved washing of the frozen leaf powder with 2% Triton X-100 prior to cell lysis to eliminate a substantial amount of interfering secondary metabolites

(Gupta *et al*., 2011) [9]. This wash pre-treatment was important to minimise contamination by polyphenols and polyssacharides. The second step was a modified CTAB extraction, where polyvinylpyrrolidone (PVP) and ascorbic acid were added as phenolic-binding and antioxidant agents respectively, to reduce oxidative browning and DNA degradation. Finally, the extracted DNA was purified with a commercial spin-column system to further improve the template quality. The need for PVP supplementation during CTAB extraction has also been reported in DNA barcoding studies of southern African Combretaceae using silica gel-dried and herbarium material (Couvreur *et al*. Polysaccharide interference is a common methodological problem across the whole family, and not a region-specific problem as shown by Gere *et al*. (2013).

Experimental evaluation demonstrated that without the washing step with Triton X-100, poor or failed PCR amplification was observed for all tested species regardless of downstream purification processes (Wu *et al*., 2016) [34]. Similarly, spin-column purification alone was not enough without prior modified CTAB extraction. These observations validated that all the three stages of the protocol were essential to get high quality PCR amplifiable DNA from mature Combretaceae tissues (Gupta *et al*., 2011) [9].

Successful RAPD amplification and clear sequencing of the rbcL barcode locus for all the taxa tested confirmed the efficiency of the protocol. Further quality-control criteria were applied in subsequent DNA barcoding studies on Indian *Terminalia* species which included optimal A260/280 and A260/230 absorbance ratios prior to PCR amplification and bidirectional sequencing (Kumar & Kataria, 2026) [15].

Table 1: Summary of experimental observations and validation outcomes demonstrating the necessity and effectiveness of the optimized DNA extraction and purification protocol for PCR amplification and DNA barcoding in Combretaceae species

Step / Parameter	Observation	Outcome / Significance
Triton X-100 washing omitted	Poor/failed PCR amplification (Wu *et al*., 2016) [34]	Washing step essential for inhibitor removal
Spin-column purification alone	Ineffective DNA amplification (Gupta *et al*., 2011) [9]	Modified CTAB extraction required before purification
DNA quality-control measures	Optimal A260/280 and A260/230 ratios used (Kumar & Kataria, 2026) [15]	Improved sequencing accuracy and PCR success

The methodological relevance of optimised DNA extraction goes far beyond technical efficiency. Reliable extraction protocols are essential for all downstream applications in molecular taxonomy, phylogenetics, population genetics and conservation biology. The quality of extraction is critical for the accuracy and reproducibility of phylogenetic inference and genetic diversity assessment in chemically challenging taxa like *Anogeissus*. Standardised extraction procedures should be considered as a prerequisite for future molecular investigations of Indian Combretaceae.

Molecular Marker Selection for Population Genetics and Conservation

The successful isolation of high-quality genomic DNA and the choice of appropriate molecular markers are crucial factors that affect the accuracy and biological significance of genetic analyses. Marker systems differ widely in their level of polymorphism, reproducibility, genome coverage, technical requirements and cost effectiveness (Ramesh *et al*., 2020) [24]. Therefore, the marker chosen should be suitable for the specific aims of the study whether they are related to phylogenetic reconstruction, species delimitation, population structure or conservation genetics (Al-Samarai & Al-Kazaz, 2015) [1].

In plant genetics, molecular markers are usually divided into hybridization-based and PCR-based systems (Barman & Kundu, 2019) [4]. The classical hybridization-based approach is represented by Restriction Fragment Length Polymorphism (RFLP) and the PCR-dependent systems include RAPD, AFLP, ISSR, SSR (microsatellites), SNP, SRAP, SCoT and iPBS markers. PCR based markers are generally preferred because they require small amount of DNA, no radioactive materials, relatively high reproducibility and rapid detection of polymorphism (Amom *et al*., 2017) [2].

Of these systems, ISSR markers have been particularly useful for population genetic studies of arid-zone plants as they are simple, reproducible and can reveal high levels of polymorphism without the need for prior genomic information. Their utility has been especially evident in conservation orientated studies of fragmented dryland tree populations (Meena & Kant, 2022) [20]. On the other hand, SNP markers are an advanced generation of molecular markers that can detect the single nucleotide variation across the genome and are increasingly linked to economically important traits and fine-scale genetic analysis (Amom *et al*., 2017) [2].

The potential application of ISSR markers for threatened desert trees was successfully demonstrated in another ecologically important species of Rajasthan's dry forests, Tecomella undulata. ISSR analysis of twelve fragmented populations revealed the presence of two admixed gene pools where the majority of the genetic variation was found within rather than between populations (Meena & Kant, 2022) [20]. These patterns are typical for long-lived outcrossing tree species and provide valuable information for the identification of conservation units, the prioritisation of populations for in situ protection, and the selection of genetically representative material for ex situ conservation programs (Upendra *et al*., 2022) [33].

RAPD markers have also been employed in *Anogeissus* research, but their reproducibility is highly dependent on the strict optimisation of experimental conditions. Parameters such as template DNA concentration, primer choice,

magnesium ion concentration, annealing temperature and Taq polymerase amount need to be carefully standardised for each species (Devpura & Purohit, 2012). Singh *et al*. (2017) [30] studied genetic variation among eight genotypes in A. acuminata using 22 RAPD primers. The study produced 166 amplification products of which approximately 64% were polymorphic. RAPD-based analyses showed substantial variation in primers' capability to detect polymorphism, highlighting the significance of primer selection.

Amplified fragments were analysed with POPGENE software to estimate genetic distances and to build dendrograms based on genetic distance of Nei (Gere *et al*., 2013). The resulting clustering patterns gave preliminary insights into inter-genotypic relationships within the species and demonstrated the usefulness of RAPD markers for rapid assessment of genetic diversity.

AFLP markers have been used to characterise population structure of *Anogeissus*. Oberprieler *et al*. (2009) [23] examined the genetic diversity and the phylogeographic history of A. dhofarica, an endemic species confined to the monsoonal fog oases of the southern Arabian Peninsula. In the study, 212 individuals yielded 97 polymorphic fragments with four selective primer combinations. Genetic diversity was estimated according to the number of polymorphic loci, Shannon diversity indices and the partitioning of the variation by AMOVA. The results demonstrated the great utility of AFLP fingerprinting for the assessment of the genetic consequences of habitat fragmentation in geographically isolated tree populations.

Table 2: Comparative overview of major molecular marker systems and their reported applications in genetic diversity, phylogeographic, and conservation studies of dryland tree taxa

Molecular Marker	Observation from Studies	Major Application / Significance	Citation
RFLP	Classical hybridization-based marker system	Early genetic analysis and genome studies	Al-Samarai & Al-Kazaz (2015) [1]
RAPD	Generated 166 bands with ~64% polymorphism in *A. acuminata*; reproducibility depends on optimization	Rapid assessment of genetic diversity	Devpura & Purohit (2012); Singh *et al*. (2017) [30]
AFLP	Produced 97 polymorphic fragments in *A. dhofarica* populations	Population structure and phylogeographic analysis	Oberprieler *et al*. (2009) [23]
ISSR	Detected substantial polymorphism without prior genomic data	Conservation genetics and fragmented population studies	Meena & Kant (2022) [20]
SSR (Microsatellites)	Highly polymorphic multilocus marker system	Population-level diversity and gene flow studies	Amom *et al*. (2017) [2]
SNP	Detects single nucleotide variations across the genome	Fine-scale genetic and trait-based analyses	
SRAP	PCR-based marker targeting coding regions	Functional and breeding-related studies	
SCoT	Gene-targeted polymorphism detection	Genetic diversity and phylogenetic studies	
iPBS	Retrotransposon-based polymorphism analysis	Evolutionary and genomic diversity assessment	

These studies demonstrate that no single marker system is universally optimal for all applications. Instead, integrated molecular approaches combining multilocus DNA barcoding for species-level identification with highly polymorphic markers such as ISSRs, AFLPs, microsatellites, or SNPs for population-level analyses are likely to provide the most comprehensive understanding of genetic diversity and evolutionary structure within *Anogeissus* and related *Terminalia* taxa.

Phylogenetic Analysis of Ncbi-Retrieved Sequence Data

The availability of publicly accessible DNA sequence repositories such as GenBank has greatly accelerated progress in phylogenetic research in plants. However, the incomplete representation of *Anogeissus* and *Terminalia* taxa in these databases is a major limitation for the comprehensive molecular systematic studies. Although *rbc*L and *mat*K are used as standard plant barcode loci, sequence availability for many South Asian taxa is sparse, which limits the phylogenetic resolution and reliability of comparative analyses.

The present synthesis highlights phylogenetic analyses conducted using *matK* and *rbcL* sequences retrieved from NCBI GenBank in order to evaluate evolutionary relationships among selected representatives of *Anogeissus* and *Terminalia*.

1. *matK* Gene Analysis

Phylogenetic reconstruction based on chloroplast matK sequences was carried out using thirteen taxa representing both *Anogeissus* and *Terminalia*. MEGA12 was used to construct phylogenetic trees with the Neighbor-Joining (NJ) method and 1,000 bootstrap replicates to estimate node support under the Kimura 2-parameter substitution model. The outgroup taxon was Combretum indicum.

The resulting phylogeny produced a well-resolved topology that generally conformed to recognised generic boundaries within the Combretaceae. Two strongly supported monophyletic clades representing *Anogeissus* and *Terminalia* were recovered and these two groups together formed a larger highly supported Combretaceae lineage.

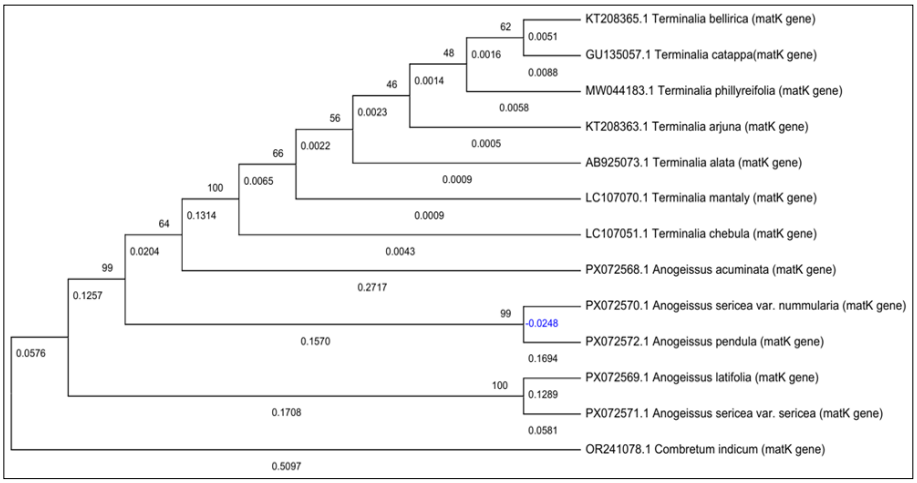

Fig 2: Neighbour-Joining (NJ) phylogenetic tree based on matK chloroplast gene sequences of Anogeissus taxa and selected Combretaceae members, constructed under the Kimura 2-parameter (K2P) substitution model. Bootstrap support values (≥50%) from 1000 replicates are shown at internal nodes. Branch lengths are proportional to nucleotide substitutions per site. Combretum indicum (OR241078.1) served as the outgroup

Within the *Terminalia* clade, bootstrap support was exceptionally strong and the topology reflected a gradual hierarchical divergence pattern. *Terminalia chebula* occupied the basal position, followed sequentially by *T. mantaly*, *T. alata*, *T. arjuna*, and *T. phillyreifolia*. A closely related sister pair consisting of *T. bellirica* and *T. catappa* formed the terminal branch of the clade. These relationships collectively suggest progressive diversification within the genus and indicate that *T. chebula* may represent one of the earliest diverging lineages among the sampled taxa.

The *Anogeissus* clade was well supported and divided into two major subclades, the first of which included the well-supported sister pair of *A. sericea* var. *nummularia* and *A. pendula*, with low molecular divergence. *Anogeissus acuminata* was the sister to this pair and had the greatest branch length of any sampled *Anogeissus* taxon, indicating relatively higher molecular divergence within the genus.

The second subclade comprised *A. latifolia* and *A. sericea* var. *sericea*, which formed another strongly supported sister relationship. Overall, the *matK*-based phylogeny successfully resolved major lineages and provided clear molecular evidence supporting close evolutionary affinities among morphologically related taxa.

Importantly, the analysis demonstrated the comparatively high discriminatory power of *matK* relative to more conserved plastid loci. The marker effectively resolved both interspecific relationships and broader generic boundaries, reinforcing its value as one of the most informative barcode loci for molecular systematic studies within Combretaceae.

2. rbcL Gene Analysis

Phylogenetic analysis based on chloroplast *rbcL* sequences was conducted using thirteen taxa representing *Anogeissus*, *Terminalia*, and *Combretum indicum* as the outgroup. Evolutionary relationships were reconstructed using the Neighbor-Joining method with evolutionary distances calculated through the Maximum Composite Likelihood model implemented in MEGA12. The final alignment comprised 1,265 nucleotide positions.

Phylogeny based on *rbc*L showed lesser sequence divergence and lower resolution at deeper taxonomic levels as compared to *mat*K based analysis. However, the analysis recovered a strongly supported *Anogeissus* clade where all sampled taxa clustered together with little differentiation in branch-length. Species such as *A. pendula*, *A. sericea*, *A. latifolia* and *A. sericea* var. *nummularia* showed nearly the same sequence profiles as indicated by very small or zero branch lengths.

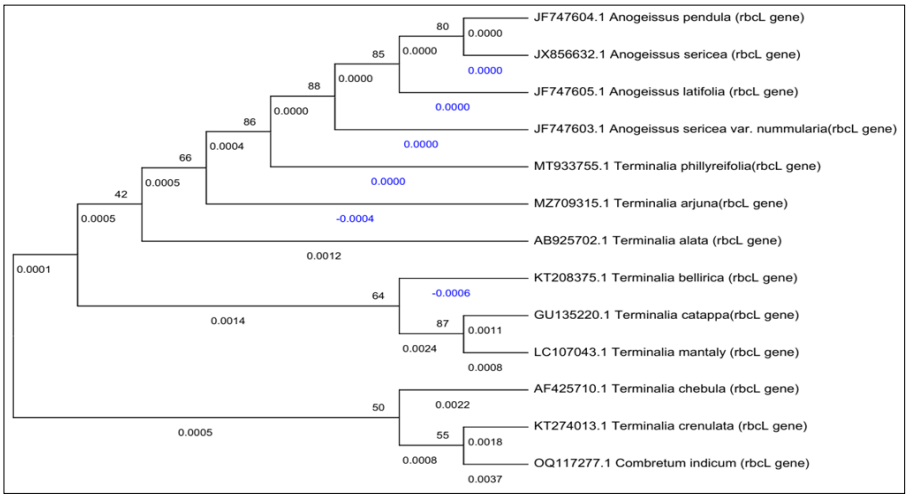

Fig 3: Neighbor-Joining phylogenetic tree reconstructed using rbcL gene sequences retrieved from NCBI GenBank. Bootstrap support values (1,000 replicates) are indicated at internal nodes; branch lengths represent the number of substitutions per site. Branch lengths shown in blue indicate negative values, which are computational artefacts of the Neighbor-Joining algorithm and should be interpreted as zero divergence

This high sequence conservation suggests a low discriminatory power of *rbc*L at the intrageneric level in *Anogeissus*. The marker is useful for broader phylogenetic placement and generic affiliation, but its low rate of substitution limits its utility for distinguishing closely related species or varietal taxa. The *rbc*L gene has similar limitations in many angiosperm groups particularly when recent diversification or morphological convergence is involved.

Terminalia taxa were relatively more topologically complex, although support values at some deeper nodes remained relatively weak. In one major subclade, *T. phillyreifolia* and *T. arjuna* formed a very close pair, with negligible sequence divergence, and *T. alata* was resolved as sister to this group. However, the node that connects this assemblage to the *Anogeissus* clade was supported by relatively low bootstrap support (BS). This indicates a lack of phylogenetic signal to confidently resolve deeper relationships using *rbc*L alone.

A second *Terminalia* lineage included *T. bellirica*, *T. catappa*, and *T. mantaly*. Within this group, *T. catappa* and *T. mantaly* formed a well-supported sister pair, whereas *T. bellirica* occupied a comparatively basal position. Another lineage containing *T. chebula* and *T. crenulata* demonstrated moderate support and grouped unexpectedly close to the outgroup taxon *Combretum indicum*.

The placement of *Combretum indicum* within proximity to certain *Terminalia* taxa is consistent with earlier molecular studies indicating historical paraphyly within Combretaceae. Such patterns further highlight the limitations of single-locus analyses and reinforce the necessity of multilocus or phylogenomic approaches for robust generic delimitation and evolutionary interpretation.

In conclusion, the *rbc*L analysis confirmed the utility of the marker for higher taxonomic level placement; however, at the same time, it showed the limited power of *rbc*L for species discrimination within *Anogeissus* and *Terminalia*. The phylogenetic resolution of the locus was much lower than that of *mat*K, supporting previous recommendations that *rbc*L should be used primarily in combination with more variable plastid or nuclear markers in DNA barcoding studies of Combretaceae.

Identified Knowledge Gaps and Future Research Priorities

The methodological trajectory of molecular systematics in Combretaceae highlights that the improvements in phylogenetic resolution were not accomplished by a single technological breakthrough, but rather by gradual integration of optimised DNA extraction protocols, multilocus sequencing strategies, advanced analytical frameworks, and phylogenomic datasets. These developments together have greatly clarified the evolutionary position of *Anogeissus* in *Terminalia*. However, there are still several important systematic, methodological and conservation-related gaps.

A major limitation is the poor representation of South Asian and Australian *Terminalia* diversity in international sequence repositories such as GenBank. The absence of complete regional barcode datasets restricts the accuracy of BLAST-based species identification and can artificially inflate estimates of genetic divergence for conserved loci such as rbcL. The lack of sequence data and the low confidence in taxonomic assignment of regional taxa hinder large-scale comparative phylogenetic studies.

Another problem that is not solved yet is the delimitation of closely related species and varietal forms. Despite the great increase in phylogenetic resolution provided by multilocus DNA barcoding, current barcode markers are insufficient to resolve recent radiations and morphologically-overlapping taxa in some cases. Despite its relatively high variability, the ITS region has failed to consistently discriminate some varietal forms within *Terminalia*, while conserved plastid markers often show insufficient sequence divergence for resolving species. These observations imply that additional rapidly evolving nuclear loci or genome-wide approaches may be required for the accurate delimitation of problematic taxa.

More studies are warranted to increase the geographic and taxonomic sampling of the seven major clades recognised within the expanded *Terminalia*. More extensive sampling would allow for a more complete infrageneric classification and better understanding of regional diversification patterns throughout the pan-tropical distribution of the genus.

The arguably most serious gap is the near-absence of population genetic data for the Indian species of *Anogeissus*. Despite their ecological significance and conservation concern, there has been no systematic molecular assessment of intraspecific genetic diversity, population structure or gene flow across fragmented populations in the Aravallis or other dryland ecosystems of India. This information is critical for identification of genetically distinct conservation units, assessment of the effect of habitat fragmentation and design of effective restoration strategies.

Comparative studies on other threatened arid-zone trees suggest that such investigations are urgently needed. For example, ISSR-based studies on *Tecomella undulata* revealed important patterns of admixture and genetic partitioning which provided direct inputs for conservation planning (Meena & Kant, 2022) [20]. Population-level studies are urgently required for threatened *Anogeissus* taxa, especially those classified as endangered or vulnerable.

Future studies should therefore aim at integrative molecular frameworks using multilocus DNA barcoding, hypervariable population genetic markers (e.g. ISSRs and microsatellites), and phylogenomic sequencing approaches. These integrated strategies would greatly improve the delineation of species, elucidate evolutionary relationships, and provide the genetic basis for evidence-based conservation and sustainable management of these ecologically important arid-zone tree species.

Limitations

This study relies entirely on a review and synthesis of previously published literature and publicly available molecular datasets; no primary field surveys or population-level genetic analyses were conducted. The review consolidates the extant molecular knowledge on Anogeissus and allied *Terminalia* taxa. There are a number of key limitations that remain inherent to both the literature available and the molecular resources currently available for the group.

One of the biggest constraints is lack of comprehensive phylogenetic and population level studies on Indian *Anogeissus* taxa. Although broad phylogenetic relationships among Combretaceae have been resolved with multilocus and phylogenomic approaches, detailed studies at the interspecific and intraspecific levels are still scarce. This gap is of particular importance for populations distributed

over the Indian arid zone where fragmentation and environmental heterogeneity could have a strong impact on the genetic structure.

The high degree of phenotypic plasticity in *Anogeissus* species complicates taxonomic interpretation. Morphological characters are often highly plastic under ecological conditions, which increases the chance of misidentification during specimen collection and molecular analysis. This variability introduces uncertainty into morphology-based classifications and may impact the accuracy of publicly available sequence data generated from mis-identified specimens

Phylogenetic reconstructions generated from GenBank sequences also revealed inconsistencies in sequence divergence among some taxa when analysed. High intraspecific divergence was observed at the same barcode loci in several cases. Such anomalies could result from sampling errors, misidentification of species, sequencing artefacts, or poor quality of DNA templates used in previous studies. In many cases, the voucher validation of publicly deposited sequences has not been rigorously performed, and the interpretation of phylogenetic relationships based solely on database-generated datasets should therefore be taken with caution.

Methodological limitations related to tissue sampling are also a significant limitation in molecular studies of tropical and subtropical Combretaceae taxa. Most species are deciduous, showing strong seasonal variation in leaf availability, thus restricting optimal sampling periods for molecular work. In *Anogeissus*, high levels of secondary metabolites such as tannins and polyphenols are accumulated by mature tissues, which often interfere with the processes of DNA extraction and downstream amplification. Even with optimised extraction protocols, degraded DNA or DNA contaminated with inhibitors can impair the sequence quality and decrease the analytical reliability.

Finally, while multilocus barcoding approaches have significantly improved species level resolution, several closely related taxa and varietal forms remain unresolved with current markers. The persistent dependence on a limited number of plastid and nuclear loci restricts our capacity to identify recent divergence events and subtle evolutionary differentiation. Future genome-scale approaches and more extensive geographic sampling will therefore be necessary to resolve these remaining systematic uncertainties.

Conclusions

The genus *Anogeissus* (DC.) Guill. & Perr., now generally placed in the expanded genus *Terminalia* L. based on strong multilocus and phylogenomic evidence, is an ecologically important and conservation-priority group of arid-zone tree taxa in the Indian subcontinent. Molecular systematic studies in Combretaceae have progressed over the past two decades from initial parsimony-based phylogenetic studies to complex multilocus and coalescent-based phylogenomic frameworks, thereby greatly advancing the knowledge on evolutionary relationships within the family.

The present review illustrates how a series of methodological advances, such as optimised DNA extraction procedures, multilocus DNA barcoding strategies, complete plastome analyses, and target-capture phylogenomics, have collectively resolved many long-standing taxonomic ambiguities surrounding *Anogeissus*. The results of these studies consistently place *Anogeissus* phylogenetically nested within *Terminalia*, thus confirming its revised taxonomic placement. They also show that species-level delimitations and intraspecific evolutionary structure are still poorly understood, especially in closely related taxa and geographically fragmented populations.

A major methodological conclusion that stems from this synthesis is that the successful molecular investigation of Indian Combretaceae critically depends on the use of optimised extraction protocols developed specifically for tissues rich in polyphenols. Thus, the validated three-step sequential extraction approach, including Triton X-100 pre-washing, modified CTAB extraction with PVP and ascorbic acid, and spin-column purification, should be considered essential for the retrieval of PCR-amplifiable DNA from mature arid-zone taxa.

The review also emphasises the need for systematic population genetic studies of *Anogeissus* from the Aravalli region and other fragmented dryland landscapes of India. Despite increasing conservation concern, molecular data on genetic diversity, gene flow and population structure are almost entirely absent for these taxa. Future conservation genetics research will probably best be served by integrative approaches that combine multilocus DNA barcoding with highly polymorphic markers like ISSRs, microsatellites and genome-scale sequencing technologies.

In general, enhancing the molecular resources for *Anogeissus* and related *Terminalia* taxa is imperative not only to address the remaining systematic ambiguities but also to formulate science-based conservation and management plans. Given the ecological importance, economic value and medicinal potential of these dryland tree species, comprehensive molecular characterisation should be a high research priority for conservation of arid-zone biodiversity in India.

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