International Journal of Botany Studies
www.botanyjournals.com
ISSN: 2455-541X
Received: 09-01-2026, Accepted: 08-02-2026, Published: 23-02-2026
Volume 11, Issue 1, 2026, Page No. 220-224

Morpho-anatomical and molecular characterization of *Desmodium Alysicarpoides* van Meeuwen: A bridging leguminous species

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Abstract
Background: *Desmodium alysicarpoides* Van Meeuwen, a member of Fabaceae, is an herbaceous species showing traits intermediate between *Alysicarpus* and *Desmodium*, raising taxonomic ambiguity.
Methods: Morphological, anatomical, micromorphological, seed-coat (spermoderma), and molecular (DNA barcoding with *rbcl*) approaches were employed on field-collected and herbarium-confirmed specimens. Transverse sections, epidermal peels, maceration studies, and SEM analyses were performed. Molecular sequencing followed PCR amplification, alignment, and phylogenetic analysis.
Results: Morphological features include erect to branched stems (30–60 cm), trifoliate leaves, bright red flowers, and falcate pods with 4–6 joints. Anatomically, stems are circular with conjoint, collateral vascular bundles; leaves are dorsiventral with amphistomatic paracytic stomata; maceration showed four vessel types. SEM revealed rugose-reticulate testa and distinct hilum. Molecular phylogeny grouped *D. Alysicarpoides* with *Alysicarpus* (bootstrap 99%), supporting closer affinity.
Conclusions: Integration of morphology, anatomy, and molecular data supports taxonomic realignment of *D. Alysicarpoides* with *Alysicarpus parviflorus*. This highlights its transitional traits and the significance of integrative taxonomy in resolving complex legume systematics.

Keywords: *Desmodium alysicarpoides*, *alysicarpus*, morphology, DNA barcoding

Introduction

The genus *Desmodium* DC. (Fabaceae: Desmodieae) represents a diverse assemblage of herbaceous and shrubby legumes widely distributed across tropical and subtropical regions of the world. Species of *Desmodium* are used in traditional medicine, pasture improvement, and as cover crops to prevent soil erosion, underscoring their multifaceted value in both natural and managed ecosystems.

Despite their importance, the taxonomy of *Desmodium* has remained complex and often controversial. Earlier classifications treated the genus as a large, heterogeneous assemblage comprising more than 350 species worldwide. However, advances in morphological and molecular systematics have revealed that *Desmodium* in its broad sense is polyphyletic, encompassing several distinct evolutionary lineages. Consequently, numerous species previously assigned to *Desmodium* have been transferred to closely related genera such as *Alysicarpus*, *Hylodesmum*, *Desmodiastrum*, and *Codariocalyx* (Ohashi, 2018; Ohashi, 2021) [7, 8]. These taxonomic revisions reflect the ongoing efforts to achieve monophyly and phylogenetic consistency within the tribe Desmodieae.

Among the taxa illustrating this taxonomic ambiguity is *Desmodium alysicarpoides* Van Meeuwen, a species native to India and predominantly distributed in Maharashtra and adjacent regions. Morphologically, *D. Alysicarpoides* exhibits a suite of intermediate characteristics between *Desmodium* and *Alysicarpus*, particularly in its floral and fruit morphology, seed structure, and vegetative features. Such intermediate traits have led to considerable uncertainty regarding its correct generic placement. Some researchers have suggested its affinity with *Alysicarpus* due to similarities in pod segmentation and calyx morphology, whereas others have maintained its inclusion within *Desmodium* based on certain stipule and leaf features. However, these conclusions have largely been drawn from external morphology, with limited emphasis on anatomical or molecular corroboration.

Previous morpho-taxonomic treatments have acknowledged the ambiguous position of *D. Alysicarpoides* but have been insufficient to resolve its phylogenetic status conclusively. The absence of integrated studies combining macro- and micromorphological, anatomical, and molecular data has hindered a clear understanding of its systematic placement. In recent years, modern tools such as scanning electron microscopy (SEM) of the Spermoderma and DNA barcoding using plastid and nuclear markers have proven invaluable in resolving taxonomic complexities within Fabaceae and other plant groups. Such multidisciplinary approaches enable more robust phylogenetic inference and facilitate the reassessment of long-standing taxonomic inconsistencies.

The present study aims to address the taxonomic uncertainty surrounding *Desmodium alysicarpoides* through a comprehensive investigation that integrates morphological, anatomical, and molecular datasets. Specifically, this work employs macro- and micromorphological characterization, detailed anatomical sectioning, SEM analysis of seed coat patterns, and DNA barcoding to elucidate the phylogenetic relationships of the species. By combining traditional taxonomic methods with modern molecular tools, the study seeks to clarify the systematic position of *D. Alysicarpoides* and contribute to a more accurate and stable classification within the tribe Desmodieae. The findings are expected to enhance our understanding of evolutionary relationships among allied genera and provide valuable insights into the taxonomic realignments necessary for the group's phylogenetic coherence.

Materials and Methods

1. Field Sampling and Morphological Studies

Field explorations were conducted between 2023 and 2025 across different eco-geographical zones of Maharashtra, India, including Ahmednagar, Pune, and Kolhapur districts. Live specimens of *Desmodium alysicarpoides* Van Meeuwen were collected during the flowering and fruiting seasons (August–December).

Fresh plant materials were photographed in situ, focusing on habit, leaf architecture, floral morphology, and fruit characters. Diagnostic morphological features—including stem indumentum, stipule shape, leaf arrangement, inflorescence type, corolla coloration, pod segmentation, and seed characteristics—were recorded following standard herbarium protocols (Jain & Rao, 1977) [2]. Voucher specimens were prepared, pressed, and deposited in the BAMU Herbarium (Dr. Babasaheb Ambedkar Marathwada University, Aurangabad) and the VH Herbarium (Pune) for reference. Identification was confirmed by comparison with authentic herbarium specimens and relevant taxonomic keys and floras (Cooke, 1903; Sharma *et al*., 1996) [1, 9].

2. Anatomical and Micromorphological Analysis

Transverse sections of stem, leaf, and petiole were prepared from fresh specimens using free-hand sectioning with sharp blades. Sections were fixed in FAA, dehydrated through graded alcohol series, and double-stained with safranin and fast green. After clearing in xylene, permanent slides were mounted in DPX and examined under a compound microscope. Photomicrographs were taken for documentation.

Maceration studies were performed using Jeffrey's fluid to dissociate individual elements of vascular tissues. Quantitative anatomical parameters such as xylem vessel diameter, cortical thickness, and stomatal frequency were recorded using an ocular micrometer.

For micromorphological characterization, both adaxial and abaxial leaf epidermal peels were prepared by mechanical stripping and staining with 1% safranin. Observations were made to determine the stomatal type (anomocytic, paracytic, or diacytic) and trichome diversity (glandular, non-glandular, unicellular, or multicellular). Stomatal index (SI) was calculated using the formula:

$$SI = S / S+E \times 100$$

Where S = number of stomata per unit area and E = number of epidermal cells per unit area. Trichome density was expressed as the mean number of trichomes per mm² of leaf surface. Microscopic images were captured using a digital imaging system attached to the microscope and analyzed with imagej software.

3. Spermoderma (Seed Coat) Analysis

Mature, healthy seeds were air-dried and cleaned to remove adhering debris. For scanning electron microscopy (SEM), seeds were first sonicated in distilled water for 10 minutes to remove surface contaminants, followed by air drying at room temperature. Dried seeds were mounted on aluminum stubs using double-sided carbon tape and sputter-coated with gold using a JEOL JFC-1600 coater.

The gold-coated specimens were examined under a JEOL JSM-IT200 scanning electron microscope at an accelerating voltage of 10–15 kv. Micrographs were captured at various magnifications to examine testa cell patterns, surface ornamentation, hilum and micropyle morphology, and seed dimensions. Seed coat surface terminology follows the guidelines proposed by Barthlott (1981) [4] and Boesewinkel & Bouman (1984) [5]. The spermoderma characters were compared with those of *Desmodium* and *Alysicarpus* species available in reference literature and herbarium collections to assess diagnostic differences.

4. Molecular Characterization and Phylogenetic Analysis

Genomic DNA was extracted from young leaves using the NucleoSpin Plant II Kit (Macherey-Nagel, Germany). DNA quality and quantity were verified by Nanodrop spectrophotometry and 0.8% agarose gel electrophoresis. PCR amplification of *rbcL* and *matK* loci was performed using universal primers in 25 µl reactions, followed by sequencing on an ABI 3730XL sequencer. Purified amplicons (~800 bp) were aligned using ClustalW in MEGA 6.0, and phylogenetic trees were constructed via the Maximum Likelihood method with 1000 bootstrap replicates. Sequences of *Desmodium* and *Alysicarpus* species from GenBank were compared, with *Medicago sativa* and *Glycine max* as outgroups, confirming close genetic affinity of *D. alysicarpoides* with *Alysicarpus parviflorus*.

Results and Discussion

1. Morphological Characteristics

Desmodium alysicarpoides Van Meeuwen (Fig. A) is an erect to sub-diffuse herb, 30–60 cm tall, covered with greyish pubescence. The stem is terete and finely hairy, and the root system is tap-rooted with lateral branches. Leaves are alternate and trifoliolate; the terminal leaflet (2.5–4.5 × 1.5–2.5 cm) is larger and elliptic-obovate, with entire margins, rounded apex, and cuneate base. Stipules are lanceolate and ciliate, persisting at the base of petioles (1–2 cm). The adaxial leaflet surface is glabrous while the abaxial side bears dense, appressed hairs.

Inflorescences are terminal racemes (5–15 cm), bearing bright red to purplish-pink papilionaceous flowers. The calyx is campanulate and 5-lobed; the corolla has a broad reflexed standard, oblong wings, and a curved keel enclosing the diadelphous (9 + 1) staminal column. Pods are falcate, 4–6-jointed, distinctly reticulate, and constricted between the seeds; mature pods are brownish and dehiscent. Seeds are small, reniform, and non-arillate. The combination of *Desmodium*-like floral features and *Alysicarpus*-type pod segmentation suggests intermediate evolutionary traits between the two genera.

Fig A - *Desmodium alysicarpoides Meeuwen*

2. Anatomical Features (Fig. B)

The stem shows a circular outline with a single-layered epidermis and uniseriate, multicellular trichomes. The cortex is 2–3 layers thick, parenchymatous, and followed by a discontinuous pericycle. Vascular bundles are conjoint, collateral, and open, forming a complete ring. Resin ducts are present near the phloem, while the pith consists of large, thin-walled parenchyma cells with intercellular spaces.

Roots exhibit a multilayered epiblema and a broad cortex containing starch grains and resinous material. The endodermis bears prominent Casparian strips; the pericycle is single-layered. Xylem and phloem are radially arranged, and xylem rays are well developed.

Petiole sections are semicircular, with a uniseriate epidermis, hypodermal collenchyma (2–3 layers), and parenchymatous ground tissue. The vascular bundle is crescentic with distinct xylem and phloem zones.

Leaves are dorsiventral with a single-layered palisade and a spongy mesophyll of irregular parenchyma. The upper and lower epidermal layers are covered by cuticle and possess paracytic stomata on both surfaces (amphistomatic condition). The midrib region shows a large, crescent-shaped vascular bundle enclosed by bundle sheath extensions.

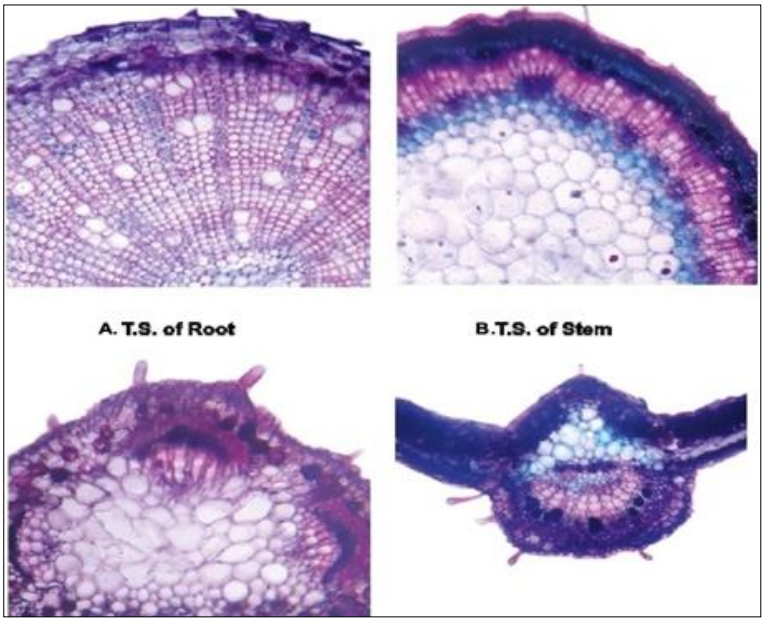

Fig B: Anatomy of *Desmodium alysicarpoides Meeuwen*

3. Micromorphological Observations (Fig. C–D)

Trichomes are uniseriate, curved, non-glandular, and measure 57–106 µm in length. They are more abundant on the abaxial leaf surface. Epidermal cells are irregularly polygonal with undulate anticlinal walls, 7.5–11.25 × 2.5–8.75 µm. Stomata are paracytic, amphistomatic, with kidney-shaped guard cells. The stomatal index ranges from 28.57 to 35.85 (mean 30.76). The predominance of paracytic stomata and amphistomatic leaves indicates closer affinity to *Alysicarpus*, which typically shares these traits.

Fig C: Trichome *D. Alysicarpoides Meeuwen*

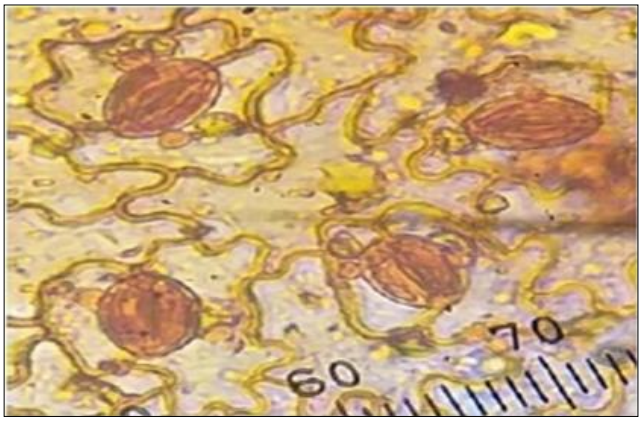

Fig D: Stomata *D. Alysicarpoides Meeuwen*

4. Maceration Studies (Fig. E)

Macerated stem and root elements revealed four vessel types: simple pitted, bordered pitted, spiral, and scalariform.

- Simple pitted vessels were tubular, thin-walled, 43.75–118 µm long (mean 90 µm), with alternate pits.
- Bordered pitted vessels were 43.75–56.25 µm (mean 50 µm), thick-walled, and with opposite bordered pits.
- Spiral vessels measured 21.25–96.25 µm (mean 51.25 µm), showing distinct lignified spiral thickenings.
- Scalariform vessels were shortest (28.75–36.25 µm; mean 31.87 µm), drum-shaped with elongated pits.

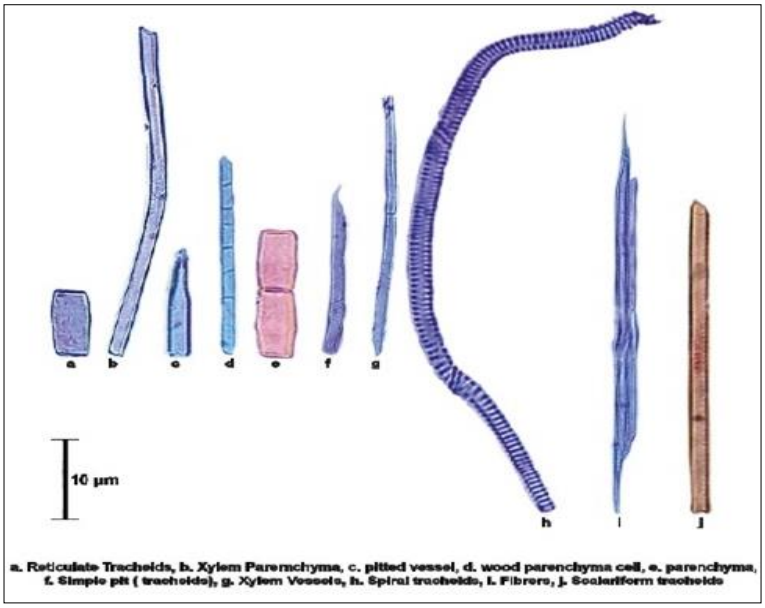

Fig E: Maceration. *D. Alysicarpoides Meeuwen*

5. Spermoderma (Seed Coat) Micromorphology (Fig. F)

SEM analysis revealed seeds 1300–1350 µm long and 950–1000 µm wide, reniform and flattened. The seed surface is rough, with interwoven ridges forming a distinct reticulate testa. Testa cells are polygonal with elevated anticlinal walls and shallowly depressed periclinal regions. The hilum is centrally located, slightly recessed, and lacks an aril. The coarse reticulate ornamentation resembles that of *Alysicarpus rugosus*, differing from the smoother testa typical of *Desmodium*.

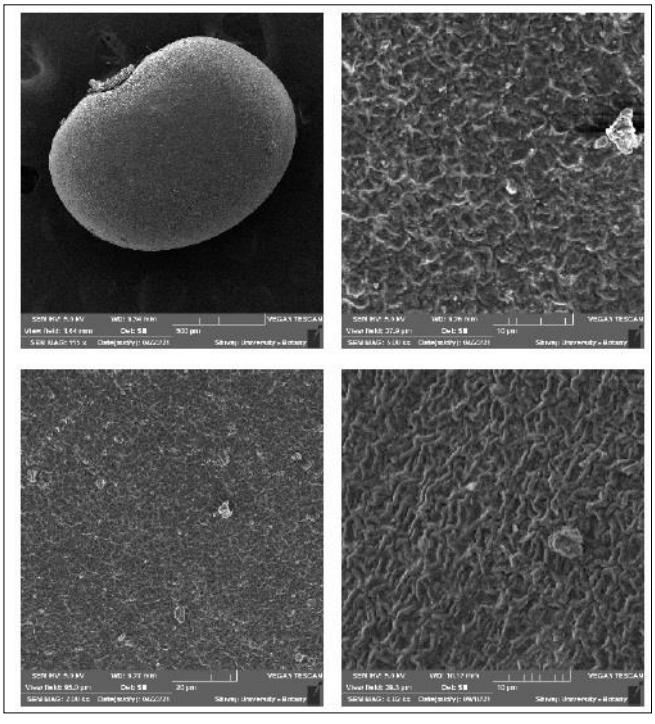

Fig F: SEM. *D. Alysicarpoides Meeuwen*

6. Molecular Phylogeny (Fig. G)

The chloroplast *rbcl* gene (~810 bp) was successfully amplified and sequenced. The representative sequence fragment is as follows:

TTGACTTATTATACTCCTGAGTATGAAACCAAAGA
TACTGATAACAAGGCAGCATTCCGAGTAACTCCTC
AACCGGGAG..

BLAST analysis indicated highest sequence similarity (≥99%) with Alysicarpus vaginalis and A. Rugosus, whereas similarity with Desmodium species was 95–96%. Phylogenetic reconstruction using the Maximum Likelihood method (MEGA 6; 1000 bootstrap replicates) grouped D. Alysicarpoides within the Alysicarpus clade, supported by a 99% bootstrap value. The Desmodium taxa formed a separate, well-supported cluster (bootstrap 97%), with Medicago sativa and Glycine max used as outgroups.

The close clustering of D. Alysicarpoides with Alysicarpus species corroborates morphological and micromorphological evidence and suggests that this taxon represents a transitional lineage between the two genera. The molecular data strongly support its reassignment to Alysicarpus, pending further multilocus analyses (matk, ITS) for confirmation.

Discussion

The morpho-anatomical, micromorphological, and molecular findings obtained in this study collectively reaffirm the complex and hybrid nature of *Desmodium alysicarpoides* (syn. *Alysicarpus parviflorus* Dalzell). Historically, the taxonomic status of this taxon has been debated due to its morphological intermediacy between *Desmodium* and *Alysicarpus*. The present investigation provides comprehensive evidence integrating classical and molecular parameters to clarify its systematic position.

Morphological and Anatomical Interpretation

The external morphology of *D. Alysicarpoides*—an erect to sub-diffuse herb with trifoliolate leaves and falcate, multi-jointed pods—shares affinities with both genera. The floral characters, including the small papilionaceous corolla and pod segmentation, show resemblance to *Alysicarpus*, whereas the general habit and leaf arrangement retain similarities with *Desmodium*. Such intermediacy supports earlier suppositions that this species represents an evolutionary link or a transitional form between the two genera.

Anatomically, the presence of unicellular to multicellular uniseriate trichomes, conjoint collateral vascular bundles, and the occurrence of resin ducts are notable. The dorsiventral leaf structure with paracytic stomata and well-differentiated palisade and spongy mesophyll indicates adaptive features typical of *Alysicarpus*. The root anatomy, showing a multilayered epiblema and Casparian strips in the endodermis, reflects xerophytic adaptations, perhaps linked to its distribution in semi-arid regions of Maharashtra. The comparative anatomy thus demonstrates that while *Desmodium* species generally possess glandular trichomes and a less differentiated cortex, *D. Alysicarpoides* exhibits features aligning more with *Alysicarpus*.

Micromorphological and Spermoderma Insights

The micromorphological analysis provided valuable diagnostic characteristics. The predominance of curved, non-glandular, uniseriate trichomes and amphistomatic leaves with paracytic stomata support its placement closer to *Alysicarpus*. The measured stomatal index (28.57–35.85) corresponds well with reported values for *Alysicarpus* species, confirming consistency in epidermal traits.

Seed coat micromorphology, as revealed by Scanning Electron Microscopy (SEM), offered some of the most decisive evidence. The seeds are kidney-shaped with distinctly reticulate and rugose testa, lacking an aril, and possess a centrally recessed hilum. These characters are in agreement with those of *Alysicarpus parviflorus* and differ significantly from the smooth to finely striate testa commonly seen in *Desmodium*. The reticulate seed surface, with raised ridges forming polygonal patterns, thus serves as a robust diagnostic trait in support of its reassignment.

Molecular Phylogenetic Evidence

Molecular characterization using the chloroplast *rbcl* region further validated the morphological interpretations. BLAST analysis indicated the highest sequence similarity with *Alysicarpus* species, and phylogenetic tree reconstruction grouped *D. Alysicarpoides* with *Alysicarpus* clade with strong bootstrap support (99%). This molecular evidence corroborates previous suspicions of misplacement within *Desmodium* and strongly advocates for its inclusion under *Alysicarpus*.

The congruence between morphological, anatomical, and molecular datasets demonstrates the effectiveness of integrative taxonomy in resolving long-standing classification ambiguities. The study not only provides a clearer understanding of species boundaries but also contributes to refining legume taxonomy, where convergent evolution often complicates generic delimitations.

Conclusion

The present investigation clearly establishes the systematic identity of *Desmodium alysicarpoides* through an integrative taxonomic approach encompassing morphology, anatomy, micromorphology, and molecular phylogeny. The combined evidence demonstrates that this species exhibits greater morphological and anatomical affinity with the genus *Alysicarpus* than with *Desmodium*.

Molecular analysis using the *rbcL* gene region strongly supports this conclusion, revealing a close phylogenetic relationship between *D. alysicarpoides* and *Alysicarpus parviflorus* Dalzell. Based on these integrative findings, *Desmodium alysicarpoides* is reinstated and recognized as *Alysicarpus parviflorus* Dalzell.

This study highlights the significance of employing a multidisciplinary framework in resolving complex taxonomic ambiguities within the tribe Desmodieae (family Fabaceae). The successful synthesis of traditional morphological data with molecular evidence provides not only greater taxonomic clarity but also deeper insights into evolutionary divergence and species delimitation in leguminous plants.

Overall, the study reinforces the vital role of morpho-molecular integration as a modern and reliable approach for accurate species identification and systematic classification in plant taxonomy.

Acknowledgements

The authors thank Jijamata College of Science and Arts and Vivekanand College for laboratory facilities, and BAMU/VH herbaria for specimen access.

Ethical Issues

- **Compliance with Ethical Guidelines:** No human or animal experiments were conducted. Standard ethical practices in plant research were followed.
- **Funding:** The authors did not receive any financial support for this research, authorship, or publication.
- **Authors' Contributions:**
- **Conceptualization:** NRV, ASS, PSS
- **Methodology & Investigation:** NRV, ASS
- **Data Analysis:** PSS
- **Writing – Original Draft:** NRV
- **Writing – Review & Editing:** PSS, ASS
- **Conflict of Interest:** The authors declare no conflict of interest.

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