

Morphological Variations in *Marsilea* Species across Aquatic and Terrestrial Habitats of Kota District, Rajasthan, India

Research Article

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Abstract

The genus *Marsilea* comprises amphibious ferns known for their remarkable adaptability to varied aquatic and semi-aquatic habitats. Numerous species of *Marsilea* exhibit morphological changes depending on their habitat or geographic location. This study investigates morphological and ecological variation across eight natural populations of *Marsilea* from distinct habitat types within Kota District, Rajasthan, India. Extensive field surveys were conducted across diverse habitats, including wetlands, canal systems, agricultural fields, and temporary water bodies, during different seasons. A total of 80 specimens, comprising aquatic, semi-aquatic, and terrestrial forms, were assessed for key morphological traits, including leaflet length and width, petiole length, sporocarp characteristics, and growth patterns. Environmental parameters, including soil type, water availability, and anthropogenic pressures, were also recorded to understand habitat influences. The findings reveal significant intra and interspecific variations in response to local ecological conditions, highlighting the phenotypic plasticity of *Marsilea* populations. Additionally, the study underscores the role of habitat disturbance and climatic factors in shaping the distribution and vitality of these species. The results contribute valuable insights into the adaptive strategies of *Marsilea* spp., and they show that *Marsilea minuta* is more ecologically versatile and stress-tolerant, making it more adaptable to different environmental conditions than *Marsilea cf. coromandelina* in the semi-arid landscape of Kota district, and provide a baseline for future conservation and ecological studies..

Keywords: *Marsilea*; Comparative Analysis; Morphological Variation; Habitat Ecology; Kota District; Rajasthan; Phenotypic Plasticity

Introduction

The genus *Marsilea*, commonly known as water clovers or pepperworts, comprises small aquatic ferns belonging to the family *Marsileaceae* [1]. In India, several species of *Marsilea* are distributed across diverse habitats, with notable occurrences in the Kota District of the Hadoti Plateau, southeastern Rajasthan. Among these, *Marsilea minuta*, a cosmopolitan species, is frequently observed thriving in ditches and ponds throughout the year in the Kota region, whereas *Marsilea cf. coromandelina* has been recorded only during the monsoon season near Borabas village, growing in limited terrestrial habitats [2]. Notably, the genus is well recognized for its

high degree of phenotypic plasticity, which enables individuals to modify their morphology in response to varying environmental conditions [3]. This characteristic is particularly significant in the Kota region, which experiences pronounced wet-dry seasonal cycles and supports a mosaic of aquatic, amphibious, and terrestrial microhabitats. Furthermore, previous studies have indicated that the genetic diversity of *Marsilea* plays a crucial role in promoting these plastic traits, thereby enhancing the species' ability to adapt across heterogeneous environments [4]. Therefore, investigating the morphological variation of *Marsilea* species across such contrasting habitats provides valuable insight into their habitat-specific adaptations. In addition, the seasonal wetlands in this region harbour

ecologically sensitive and endemic flora, which are increasingly under threat due to anthropogenic land-use changes. Importantly, compared to aquatic environments, terrestrial habitats generally expose plants to more fluctuating environmental factors, further influencing morphological differentiation [5]. In this context, Baruah and Sarma have emphasized the significance of morpho-ecological variation and phenotypic plasticity in *Marsilea*, particularly in regions experiencing ecological disturbances or anthropogenic pressures (IUCN, 2022) [6, 7]. Consequently, the Hadoti Plateau especially the Kota District emerges as an ideal landscape to study these dynamics, given its diverse aquatic and terrestrial systems, including permanent ponds, riverbanks, seasonal wetlands, and dry agricultural patches. Nevertheless, despite its ecological richness, this region remains understudied in terms of pteridophyte diversity and morphological adaptation. This is especially true for genera like *Marsilea*, which are highly responsive to subtle microenvironmental variables such as soil moisture, water depth, and human disturbance [8]. Hence, the present study focuses on easily quantifiable morphological traits such as leaflet length and width, petiole length, and sporocarp size in order to assess environmental influences on morphological differentiation. Ultimately, this research contributes not only to a better understanding of ecological adaptation and phenotypic plasticity in *Marsilea* but also to the growing need for region-specific baseline data that can inform both taxonomic resolution and conservation planning amid on-going habitat degradation and climate variability.

Materials and Methods

Study Area and Sampling Sites

The study was conducted in Kota District, Rajasthan (23°45'–25°53' N, 75°09'–77°26' E), located on the Hadoti plateau, Rajasthan in the Chambal River basin. Based on preliminary surveys, eight representative populations of *Marsilea* were selected from distinct habitat types (Table no.1). The dominant species at each site was identified: *M. minutain* the aquatic, *Marsilea minuta* hybrid in amphibiotic sites, and *M. cf. coromandelina* in the purely terrestrial site. Each site was geo-referenced using GPS, and dominant habitat features were recorded.

Table 1: Site-wise details of collection, habitat types, and dominant *Marsilea* species

S.no.	<i>Marsilea</i> population	Sampling Sites	Habitat characteristics
1.	<i>Marsilea minuta</i>	Kanwas	Perennial river edge, with semi-aquatic submerged zones
2.	<i>Marsilea minuta</i>	Abheda pond	Rain-fed seasonal pond with marshy edges
3.	<i>Marsilea minuta</i>	Borabas pond	Shallow seasonal pond, surrounded by shrubs
4.	<i>Marsilea</i> hybrid spp.	Kewal nagar	Temporary wetland within village outskirts
5.	<i>Marsilea</i> hybrid spp.	Morukalan	Agricultural fields
6.	<i>Marsilea</i> hybrid spp.	Kolani	Perennial pond with stable water levels
7.	<i>M.cf. coromandelina</i>	Borabas	Water-accumulating depressions along roadsides
8.	<i>M.cf. coromandelina</i>	Abhera bio logical park	Temporary water bodies

Sampling and Identifications: A total of 10 individuals were sampled per site (n = 80 in total). Plants were collected during the active growing season and analysed fresh for morphological traits. Parameters recorded included leaflet length and width, petiole length, number of sporocarps per plant, sporocarp shape, and size. Standard botanical keys, Flora of Rajasthan – Vol 1 and 2 and regional floras (Khullar, 1994; Chandra, 2000;Fraser-Jenkins, C. R. (2008) were used for preliminary identification.

Morphological measurements: Morphological analysis was conducted to evaluate species-specific and habitat-induced variations among *Marsilea* populations collected from aquatic, amphibious, and terrestrial habitats in Kota District, Rajasthan. In the laboratory, each specimen was carefully washed and assessed for the following key morphological traits:

- **Leaflet length (cm):** Measured from the base to the tip of the leaflet using a digital Vernier calliper.
- **Petiole length (cm):** Distance from the rhizome node to the base of the first leaflet.
- **Sporocarp size (length × breadth in cm):** Measured using a calliper, focusing on mature sporocarps only.
- **Sporocarp shape and surface features:** Observed under a stereomicroscope for ribbing, hairiness, and structural characteristics.
- **Number and position of sporocarps:** The number and Position of sporocarps were visually recorded, with the petiole’s orientation and attachment points noted (horn).

The traits were chosen based on their ecological relevance and established use in previous *Marsilea* studies (Mangestuti et al., 2017; Sharma &Bhardwaja, 2019). [2, 9]

Identification of Hybrids: The leaf of hybrid *Marsilea* is a compound, heteromorphic structure with four leaflets arranged in a clover-like pattern, exhibiting intermediate size, texture, and venation between *M. minuta* and *M. cf. coromandelina*, and often marked by variability in petiole length, leaflet symmetry, marginal curvature and sporocarps arrangements. This key is based solely on morphological observations, and further anatomical or molecular analysis is recommended to validate hybrid status.

Statistical analysis: Mean trait values were compared across habitat groups. One-way ANOVA tests ($\alpha = 0.05$) assessed whether leaflet length, petiole length, or sporocarp size differed significantly among the three habitat categories (aquatic, amphibious, terrestrial). Descriptive statistics (mean ± standard deviation) were tabulated, and a bar chart was prepared to illustrate leaflet length differences. [4]

All measurements were conducted using standard protocols for aquatic Pteridophytes (Khullar, 2000; Pérez-Harguindeguy et al., 2013) [13, 15].

Results

A comparative analysis was conducted on *Marsilea* taxa, including *Marsilea minuta*, *M. cf. coromandelina* and *Marsilea minuta* hybrid, from 8 distinct habitats in the Kota region (Kanwas, Abheda

Table 2: Habitat assessment of different areas of Kota district, Rajasthan

Specimens collected from	Kanwas	Abhedha pond	Borabas pond	Kewal nagar	Moru kalan	Kolani	Borabas	Abhera biological park
Habitat type	River side	Seasonal pond	Seasonal pond	Temporary wetland	Agricultural Fields	Permanent pond	Road side patches	Temporary water body
Soil type	Loam -sandy loam	Silty clay loam	Clayey-loam	Heavy clay	Sandy-Loam	Clayey - silty clay	Loam with gravel content	Loam-clay loam
Soil pH	~7.5-8.2	~7.0-7.2	~7.0-7.5	~7.8-8.4	~7.2-7.8	~7.2-8.0	~6.8-7.2	~6.8-7.5
Water Availability	permanent	Seasonal	Seasonal	Temporary	Temporary	Permanent	Temporary	Temporary
Anthropogenic disturbance	Low	Medium	High	Medium	Low	Low	High	High

Table 3: Morphological Characteristics of *Marsilea* Populations in Different Areas of Kota District

<i>Marsilea</i> Spp.	<i>Marsilea minuta</i>			<i>Marsilea hybrid</i>			<i>M.cf. coromendelina</i>	
Specimens collected from	Kanwas	Abhedha pond	Borabas pond	Kewal nagar	Moru kalan	Kolani	Borabas	Abhera biological park
Leaflet Length (cm) (Mean ± SD)	1.6± 0.22	1.2± 0.41	1.3± 0.20	2.33±0.51	1.73±0.4	1.86±0.35	0.8±0.21	1.1±0.3
Leaflet margin	Entire	almost entire	Almost entire	Slightly crenate	Almost crenate	Slightly crenate	Crenate	Crenate
Leaflet Shape	obdeltoidwith rounded tips, thin and membranous	rounded tips, thin and membranous	Often narrow, rounded tips, thin and membranous	Rounded tips to obtriangular, not thicker than <i>M. coromendelina</i>	Slightly obdeltoid, thin and more membranous	broadly obtriangular; margins often less regularly curved	Obtriangular or more angular, thicker and leathery	Obtriangular, thicker and leathery
Petiole Length (cm) (Mean ± SD)	12.2 ± 0.32	6.86 ± 0.35	6.82 ± 0.30	6.93 ± 0.52	6.88 ± 0.25	10.5 ± 1.68	4.2 ± 0.40	5.21 ± 0.35
Sporocarp Size (cm) (LxB)	0.17x0.2	0.2x0.32	0.22x0.3	0.31x0.42	0.27x0.32	0.25x0.32	0.42x0.21	0.38x0.22
No.& position of sporocarp	2-3 Arising Often horizontally aligned from near base of the leaf stalk	2-3 Arising from near base of the leaf stalk	3-4 sporocarps attached to the base of petiole	2 or paired sporocarps arising straight from each base of petiole	3-4 clustered, arising from single node of rhizome	6-8 or in groups arising from single node of rhizome	One on distinct stalks arising obliquely directed upward from near or above rhizome nodes	1-2 Erect, angled or obliquely directed upward from near rhizome node
Shape of sporocarp	Unribbed, obliquely ovoid, Upper and lower both obtuse horn	Unribbed, Oval, two procumbents	Unribbed, oval, upper prominent and lower blunt horn	Ribbed, bean like, flattened, Upper prominent and lower obtuse horn	Ribbed, slightly ovoid, upper prominent and lower blunt horn	Slightly hairy, Ovoid, flattened, upper prominent and lower blunt horn	Rigid, quadrilateral with perceived ridges, Upper blunt horn and lower obtuse horn	Hairy, rigid, square shape with perceived ridges
Growth Habit	Prostrate and spreading across moist soil or shallow water margins, delicate fronds	Semi-erect to creeping habit along pond fringes; slender petioles and membranous leaflets	Creeping to slightly tufted growth; thrives in shallow, stagnant water.	Erect to sub-erect, arising from petiole base anchored in semi-permanent water bodies; robust fronds	Creeping habit with semi-erect fronds; moderately robust fronds suggest seasonal inundation.	Semi-erect to tufted, growing in moist depressions and paddy fields; broader leaflets and elongated petioles	Compact, erect tufts; thick, leathery fronds and rigid sporocarps suggest adaptation to shallow,	Erect to sub-erect, forming small clumps; thrives in seasonally flooded areas near perennial water bodies.

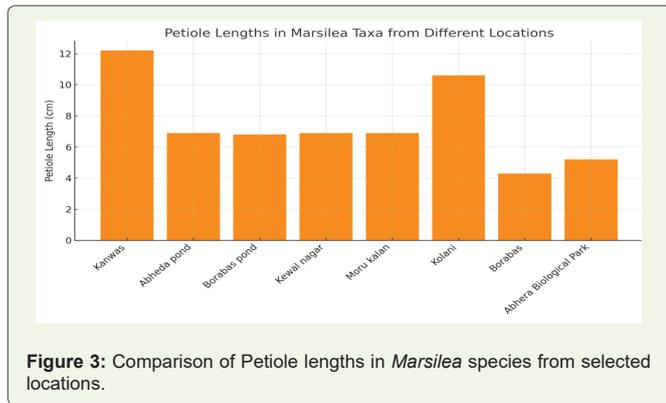
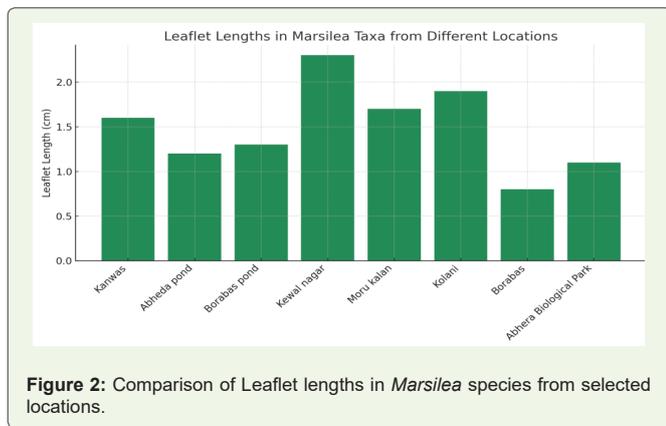
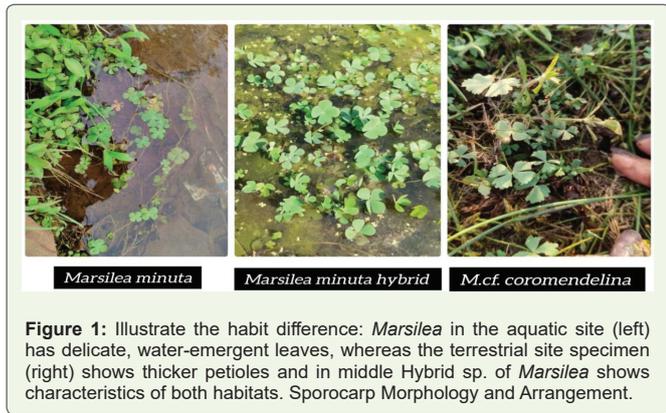
pond, Borabas pond, Kewal Nagar, Moru Kalan, Kolani, Borabas and Abhera Biological Park).

Morphological Assessment: Key morphological characters such as leaflet size, leaflet shape and margin, petiole length, sporocarp size, number and position of sporocarps, and sporocarp shape showed significant interspecific and intraspecific variation:

Leaflet length ranged from 0.8 ± 0.21 cm in *Marsilea* sp. from Borabas to 2.33 ± 0.51 cm from Kewal Nagar. Margins varied from entire (*M. minuta*) to crenate (Borabas and Abhera samples). Leaflet

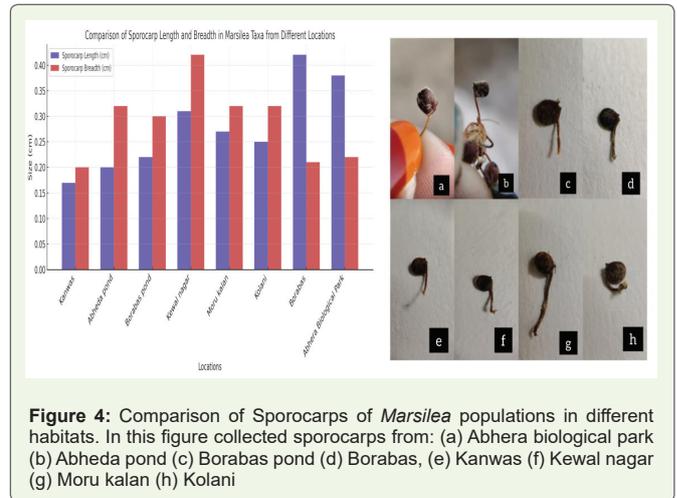
shape ranged from obdeltoid and membranous (*M. minuta*) to rigid, leathery obtriangular forms (*M. cf. coromendelina*). It was observed that leaflet of *Marsilea minuta* hybrid spp. is larger than *M. minuta* and *M. cf. coromendelina* due to amphibious characteristics, more water availability and Heavy clay type of soil is favourable for Broad leaves of *Marsilea* spp.

Petiole length showed considerable variation, with the longest observed in *M. minuta* from kanwas (12.2 ± 0.32 cm) and the shortest in *Marsilea cf. coromendelina* from Borabas (4.2 ± 0.40 cm), indicating



different growth strategies and ecological adaptations. It was observed that Permanent water availability, low anthropogenic disturbance and Loam to sandy loam soil also called alluvial soil is favourable for *Marsilea* petiole length.

Sporocarp size ranged from 0.17 x 0.2 cm (*M. minuta*) to 0.42 x 0.21 cm (*Marsilea* sp. from Borabas). *Marsilea* hybrid spp. displayed ribbed, bean-like sporocarps, while the Borabas and Abhera samples had rigid, quadrilateral or square sporocarps with distinct ridges and hairs. The number and positioning of sporocarps also varied, with *M. hybrid* bearing 3–4 attached at the petiole base and the Kolani form producing clusters of 6–8 per node.



Discussion

The results clearly demonstrate pronounced morphological variation in *Marsilea* across different habitat types, reflecting the genus's inherent phenotypic plasticity. Specifically, aquatic and amphibious individuals exhibited significantly larger leaflets and longer petioles compared to their terrestrial counterparts. This enlargement of leaf structures in semi-aquatic conditions may serve to maximize photosynthetic capacity under higher light availability and simultaneously reduce the impact of anthropogenic disturbances. In contrast, the relatively smaller leaf form observed in submerged conditions might be an adaptive strategy to minimize water drag. These external morphological traits are consistent with the findings of Sharma et al. (2019), who reported that *Marsilea* specimens from flooded environments developed extensive aerenchyma and thinner stele, while those growing on land possessed denser internal tissues [2]. Although internal anatomy was not examined in the present study, the distinct external features likely result from similar physiological responses to varying hydric regimes.

Moreover, the relative stability in sporocarp size across habitats suggests that reproductive organs may exhibit lower plasticity than vegetative parts. In line with previous observations of *M. minuta*, all studied populations consistently produced small, mature sporocarps. This uniformity implies that reproductive development in *Marsilea* may be governed by a conserved genetic program, relatively unaffected by environmental variability. However, it is important to note that sporocarp viability and production rates factors that could also be influenced by habitat quality were not assessed in this study. Therefore, future research should address these parameters to gain deeper insight into the reproductive ecology of the genus.

These findings resonate with other studies on *Marsilea*. For instance, Mangestuti et al. (2017) [9] demonstrated that *M. crenata* individuals cultivated in soil differed markedly in leaflet thickness and stomatal density from those grown in water, highlighting habitat-driven morphological differentiation. Similarly, anatomical investigations conducted in Kota by Sharma et al. (2019) supported population-level variation correlated with habitat history.

Broadening the scope, similar trends have been documented across fern taxa. For example, Farrar (1974) [10] observed that ferns from open, drier habitats produced thicker fronds compared to those adapted to shaded, humid environments, illustrating phenotypic modulation of gametophytes in response to ecological pressures. Such parallels underscore that *Marsilea*, like other ferns, possesses a high degree of plasticity, enabling it to adjust morphologically to variations in light and moisture availability. Additionally, these observations are congruent with earlier reports by Smith et al. (2006) and Khullar (1994) [12, 13], which emphasize the moisture-responsive plasticity in *Marsilea* morphology.

Furthermore, similar patterns of morphological flexibility have been noted by Khullar (1994) and Chandra (2000) [14], who documented extensive trait variability among Indian pteridophytes. The present study builds upon these findings by further confirming the role of habitat heterogeneity in shaping phenotypic expression. For example, wetlands such as Kewal Nagar and permanent ponds like Kolani supported robust individuals with larger leaflets and longer petioles. These habitats likely provide stable moisture availability, higher organic content, and microclimatic consistency conducive to optimal growth. In contrast, *Marsilea* populations inhabiting more ephemeral or disturbed environments such as Borabas roadside and Abhera Biological Park tended to exhibit stunted growth or greater morphological variability, likely resulting from stress-induced adaptations or developmental limitations.

Interestingly, transitional zones and anthropogenically impacted habitats harbored individuals exhibiting intermediate morphological traits that bridged characteristics of *M. minuta* and other closely related species. This phenomenon may suggest ongoing hybridization, introgression, or ecotypic divergence. Notably, this hypothesis finds support in the broader literature, including studies by Walker (1961) and Schneider et al. (2004) [18, 19], which discuss the evolutionary implications of such intermediate forms in fern taxa. These patterns may reflect ongoing speciation events or rapid evolutionary responses to complex and fluctuating environmental conditions.

Looking ahead, future investigations should incorporate quantitative measurements of environmental variables such as water depth, soil moisture, and organic content to strengthen the ecological interpretation of the observed trends. Preliminary observations already indicate that aquatic sites maintained standing water throughout the year, while terrestrial sites underwent complete desiccation during summer months. Therefore, it is reasonable to infer that the observed morphological variations arise not only from plastic responses but also potentially from local adaptations to differing moisture regimes.

Conclusion

This study provides comprehensive evidence of significant morphological variability among *Marsilea* species across distinct aquatic, amphibious, and terrestrial habitats in Kota District, Rajasthan. The observed differences in key vegetative and reproductive traits, *Marsilea minuta* was commonly found in permanent aquatic environments and exhibited delicate, membranous fronds with longer petioles, reflecting adaptation to submerged conditions.

In contrast, *M. cf. coromandelina*, primarily found in terrestrial or seasonally dry habitats, showed thicker, leathery leaflets and compact growth forms, indicating adaptations to water stress and fluctuating soil moisture. Notably, hybrid populations displayed intermediate morphological traits, suggesting possible natural hybridization events and highlighting the genus's phenotypic plasticity. Environmental factors such as soil type, water availability, and anthropogenic disturbances played a critical role in shaping the observed morphological differences. The study reveals that permanent water bodies with loamy soils promote larger vegetative structures, while temporary and disturbed sites lead to more compact growth forms and altered sporocarp features.

These findings underscore the adaptive strategies of *Marsilea* species in response to ecological gradients and highlight the need for further integrative taxonomic work, including molecular analyses, to clarify species boundaries and evolutionary relationships. Conservation attention is also warranted for morphologically distinct or potentially endemic populations, particularly those in ecologically vulnerable areas like Borabas, Kolani, and Abhera Biological Park, where habitat degradation poses a risk to genetic and ecological diversity. The study suggests additional molecular and physiological research to help clarify taxonomic ambiguities, evaluate adaptive responses, and inform successful biodiversity management strategies for the genus *Marsilea* in the Hadoti region and beyond. This will help advance species delimitation and conservation planning.

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