CELL BIOLOGY & DEVELOPMENT
Volume 9, Number 2, December 2025
Pages: 99-112
E-ISSN: 2580-4499
DOI: 10.13057/cellbioldev/v090204

Stomatal anatomical variation of water hyacinth (*Pontederia crassipes*) across Bengawan Solo Oxbow Environments, Indonesia

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Manuscript received: 21 April 2025. Revision accepted: 12 November 2025.

Abstract. *Zagoto JR, Febrisa KN, Mahmudah IR, Qanita IM, Rifki AN, Setyawan AD. 2025. Stomatal anatomical variation of water hyacinth (*Pontederia crassipes*) across Bengawan Solo Oxbow Environments, Indonesia. Cell Biol Dev 9: 99-112.* Oxbow lakes formed along large tropical rivers represent heterogeneous lentic habitats that can impose contrasting environmental conditions on aquatic macrophytes. This study examined stomatal anatomical variation of water hyacinth (*Pontederia crassipes*) across three Bengawan Solo Oxbow Environments in Central Java, Indonesia, and evaluated how these anatomical traits relate to supporting physiological and growth indicators. Stomatal characteristics were quantified using a leaf-impression method on adaxial and abaxial leaf surfaces, while environmental parameters were measured in situ. Chlorophyll status was assessed using a Soil Plant Analysis Development (SPAD) Index, and plant performance was evaluated through dry biomass measurement. Across all oxbows, *P. crassipes* exhibited an amphistomatous leaf condition with anomocytic stomatal complexes, indicating a conserved stomatal structural pattern consistent with its free-floating growth form. Within this shared framework, quantitative variation in stomatal size and density distinguished oxbow environments. Tangkisan showed the highest stomatal densities on both leaf surfaces, whereas Kadokan exhibited the lowest values, with Sidowarno occupying an intermediate position. SPAD chlorophyll index and dry weight also differed among oxbows but did not correspond directly with stomatal traits, demonstrating that physiological status and biomass accumulation are influenced by multiple interacting environmental factors rather than stomatal anatomy alone. By integrating stomatal anatomy with SPAD and biomass measurements, this study highlights stomatal traits as robust anatomical indicators of environmental context in oxbow habitats, while emphasizing the role of physiological and growth metrics as supporting, not primary, indicators. The findings provide an anatomically grounded framework for comparative assessment of plant-habitat interactions in heterogeneous oxbow environments and underscore the potential of stomatal anatomy as a practical, field-relevant tool for evaluating habitat assessment in tropical river systems.

Keywords: Aquatic macrophytes, oxbow lakes, *Pontederia crassipes*, stomatal anatomy, tropical rivers

INTRODUCTION

Aquatic ecosystems represent complex lentic and lotic systems where biological communities interact closely with physicochemical conditions to regulate ecosystem structure and function. Within lentic environments, aquatic macrophytes play a central role by contributing to primary productivity, stabilizing sediments, regulating nutrient dynamics, and providing habitat for diverse aquatic organisms (Gultom et al. 2023). Their morphological and physiological traits often respond sensitively to environmental variation, making aquatic plants useful biological indicators for assessing habitat condition and stress gradients in freshwater systems.

Oxbow lakes are distinctive lentic habitats formed when river meanders are cut off from the main channel through natural erosion-sedimentation processes or deliberate river-course modification (Ayunin and Mulyani 2023; Izzati and Rustandi 2025). Once isolated, oxbows experience reduced hydrological connectivity and limited water exchange, which can generate pronounced spatial heterogeneity in water chemistry, microclimate, and biological communities. Such heterogeneity is often amplified in tropical river systems that have undergone channel straightening and flood-control interventions, where former river segments persist as stagnant or semi-stagnant waterbodies. Consequently, oxbows provide natural environmental gradients suitable for examining plant structural responses under contrasting but spatially proximate conditions.

The Bengawan Solo River, the longest river in Java, Indonesia, has produced numerous oxbow environments along its course as a result of long-term geomorphological dynamics and river-management activities (Saputra 2020). These oxbows, locally referred to as *kali mati*, vary in water circulation, vegetation cover, and physicochemical conditions, despite their shared riverine origin. Such variation creates an opportunity to evaluate how aquatic plants adjust structurally and physiologically across different oxbow environments within the same watershed context.

Water hyacinth (*Pontederia crassipes* (Mart.) Solms) is a free-floating aquatic macrophyte widely distributed in tropical and subtropical regions and commonly found in oxbow habitats. Ecologically, *P. crassipes* is recognized for its rapid growth, high biomass production, and capacity to respond to changes in water quality. The species has been described as a biological indicator and, under controlled conditions, as a potential phytoremediator capable of reducing organic pollutants and dissolved substances in freshwater systems (Suraya 2019; Ningrum et al. 2020; Ajithram et al. 2021). Because its occurrence and physiological condition often mirror environmental conditions, *P. crassipes* has been widely used in studies of aquatic ecosystem health and disturbance.

At the anatomical level, stomata represent a critical interface between plant tissues and the external environment. Stomata are epidermal pores flanked by guard cells that regulate gas exchange and transpiration, thereby linking environmental exposure to photosynthetic performance and water relations. Variation in stomatal traits, including size, density, and distribution pattern, is widely recognized as a structural manifestation of plant adjustment to environmental conditions (Hetherington and Woodward 2003; Lawson and Vialet-Chabrand 2019). In aquatic and semi-aquatic plants, stomatal anatomy is particularly informative because it integrates both atmospheric and aquatic habitat influences into measurable epidermal features (Driesen et al. 2020).

Several environmental factors are known to influence stomatal differentiation during leaf development. Atmospheric conditions such as air temperature, relative humidity, and light intensity shape evaporative demand and carbon assimilation potential, which can be reflected in stomatal size and density patterns (Hetherington and Woodward 2003; Sumadji et al. 2023). In aquatic macrophytes, water-related parameters such as Dissolved Oxygen (DO), Total Dissolved Solids (TDS), and pH provide important habitat context by influencing plant vigor and metabolic balance, although their effects on stomatal traits are often indirect and mediated through overall plant physiological status (Wetzel 2001; Munns and Tester 2008). Physiological proxies such as chlorophyll-related indices measured by Soil Plant Analysis Development (SPAD) can capture changes in pigment status associated with environmental stress, while biomass accumulation reflects the integrated outcome of carbon gain and stress costs (Uddling et al. 2007; Lambers et al. 2008).

Despite extensive research on *P. crassipes* focusing on distribution, water quality, and biomass production, field-based studies that explicitly integrate stomatal anatomical variation with environmental context in oxbow systems remain limited. In particular, there is a lack of comparative evidence linking stomatal traits of *P. crassipes* to contrasting oxbow environments within the Bengawan Solo River system in Central Java. Most existing studies emphasize physiological or ecological outcomes without examining epidermal-level anatomical indicators that can provide mechanistic insight into plant-environment interactions (Villamagna and Murphy 2010).

Therefore, this study aims to analyze stomatal anatomical variation of water hyacinth (*P. crassipes*) across three Bengawan Solo Oxbow Environments with contrasting abiotic conditions. Specifically, we compare stomatal size, density, and distribution patterns between adaxial and abaxial leaf surfaces and relate these anatomical traits to measured environmental parameters. SPAD chlorophyll index and dry biomass are included as supporting physiological indicators to contextualize stomatal anatomy within overall plant status and performance. We hypothesize that differences in oxbow environmental conditions are associated with consistent variation in stomatal anatomical traits, while SPAD and biomass reflect complementary physiological responses rather than serving as primary drivers.

MATERIALS AND METHODS

Study area and sampling design

This study was conducted in three oxbow waterbodies formed along the Bengawan Solo River in Sukoharjo District, Central Java, Indonesia, namely Oxbow Sidowarno, Oxbow Kadokan, and Oxbow Tangkisan. The Bengawan Solo is the longest river on Java and has undergone substantial geomorphological modification due to long-term natural meandering processes and river-management interventions aimed at flood control and channel stabilization (Saputra 2020). These processes have resulted in the formation of multiple oxbow environments, locally referred to as *kali mati*, which function as partially or fully isolated lentic habitats with limited hydrological connectivity to the main river channel (Figure 1).

The three study sites are located within the same river basin but exhibit contrasting local environmental settings. Oxbow Sidowarno is situated at approximately 7.6495° S and 110.7942° E, Oxbow Kadokan at approximately 7.6212° S and 110.8140° E, and Oxbow Tangkisan at approximately 7°41′59.6″ S and 110°47′57.8″ E. All sites lie at comparable elevations (approximately 98-99 m above sea level), minimizing the influence of altitudinal variation and allowing site-level differences to be interpreted primarily in relation to local habitat conditions rather than broad topographic gradients.

Oxbow lakes are characteristically U-shaped waterbodies formed when a river meander is cut off from the active channel through erosion-sedimentation processes or deliberate river-course modification, resulting in a transition from flowing (lotic) to stagnant or semi-stagnant (lentic) conditions (Ayunin and Mulyani 2023; Izzati and Rustandi 2025). Following isolation, oxbows often experience reduced water exchange, increased residence time, and spatial heterogeneity in physicochemical conditions. In tropical river systems such as Bengawan Solo, these features can be further influenced by surrounding land use, vegetation cover, and episodic flooding, producing distinct microhabitats within relatively short spatial distances.

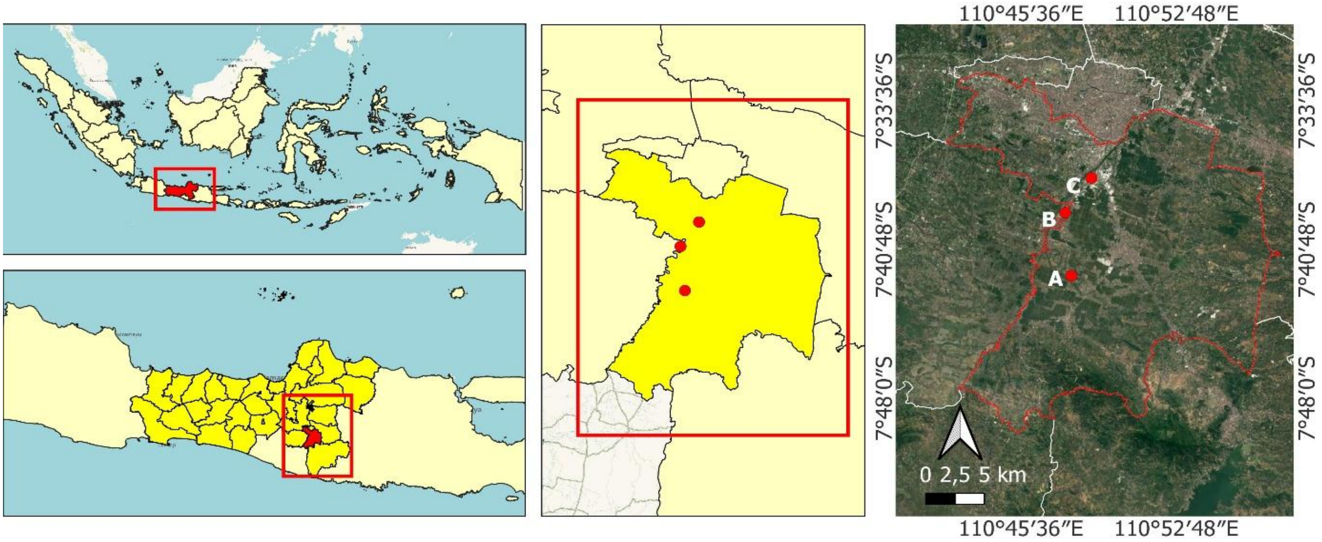

Figure 1. Map of the study area showing the location of three Bengawan Solo Oxbows in Sukoharjo District, Central Java, Indonesia. A. Sidowarno, B. Kadokan, and C. Tangkisan

Sampling was designed as a comparative field study across the three oxbow environments to evaluate variation in stomatal anatomical traits of water hyacinth (*P. crassipes*) under contrasting habitat conditions. The experimental unit was defined as the individual plant. Within each oxbow, nine individual plants were sampled, yielding a total of 27 plants across the three sites. Individual plants were selected randomly from accessible water hyacinth stands at each oxbow, without preference for plant size or apparent vigor, while excluding individuals that were visibly damaged or senescent. This approach was intended to capture representative within-site variability while maintaining comparability among sites.

To ensure consistency in anatomical comparisons, only mature, fully expanded, and intact leaves were used for stomatal observations from each sampled plant. By standardizing the developmental stage of sampled leaves and treating individual plants as independent biological replicates, the sampling design allowed among-oxbow comparisons of stomatal traits to be evaluated without confounding effects of leaf age or pseudoreplication. The shared riverine origin of the three oxbows provides an appropriate spatial framework for assessing how localized environmental differences among oxbow environments are reflected in stomatal anatomical variation of *P. crassipes*.

Sampling time and abiotic measurements

Field sampling and abiotic measurements were conducted during the rainy season, between January and March 2025. This period was selected because oxbow waterbodies in the Bengawan Solo system typically exhibit stable surface-water availability and active growth of aquatic macrophytes during the wet season, enabling consistent sampling of water hyacinth populations under comparable hydrological conditions. Because all measurements were carried out within a single sampling campaign, the resulting dataset represents a snapshot of environmental conditions and does not capture diel or seasonal variability beyond the sampling window.

A set of key abiotic parameters was measured at each oxbow to characterize local habitat conditions and provide environmental context for interpreting stomatal anatomical variation. Atmospheric variables included air temperature and relative humidity, measured at the sampling site near the plant canopy. Aquatic variables included water temperature, water pH, Dissolved Oxygen (DO), and Total Dissolved Solids (TDS), which together describe the thermal, chemical, and metabolic state of the oxbow water. In addition, substrate pH was measured to characterize the physicochemical condition of the underlying sediment, which can influence nutrient availability and overall plant performance in aquatic macrophytes (Wetzel 2001). Light intensity was recorded as photosynthetically active irradiance expressed in lux to capture differences in the local radiative environment among oxbows.

All abiotic measurements were obtained in situ using calibrated digital instruments. Water temperature, water pH, DO, and TDS were measured directly in the field by immersing the probe of a multiparameter water-quality meter at a consistent depth below the water surface. Readings were recorded after stabilization of the displayed values to ensure measurement reliability. Air temperature and relative humidity were measured at approximately plant-canopy height using a digital thermo-hygrometer, while light intensity was measured using a portable lux meter positioned above the leaf surface to minimize shading effects. Substrate pH was measured by inserting a pH probe into the upper sediment layer adjacent to sampled plants.

Spatial replication was applied within each oxbow to account for within-site environmental heterogeneity. Abiotic parameters were measured at three sampling points per oxbow, distributed across accessible areas of the water hyacinth stands. At each point, measurements were repeated three times, resulting in nine readings per abiotic variable for each oxbow. Point-level means were first calculated, and these values were then averaged to obtain oxbow-level summaries used in subsequent analysis. This design provided a balanced comparison among oxbows while avoiding overrepresentation of localized microhabitat conditions.

Because abiotic measurements and plant sampling were conducted concurrently, the environmental data represent the conditions experienced by the sampled plants at the time of collection. As measurements were not repeated across different seasons or hydrological states, abiotic variables are interpreted as descriptive indicators of habitat context rather than as definitive drivers of long-term anatomical differentiation.

Stomatal imprint and microscopic observation

Stomatal anatomical characters of *P. crassipes* were examined using the leaf impression (replica) technique, which is widely applied for epidermal observation because it preserves stomatal structure and allows repeated measurements without damaging the original tissue (Windarsih et al. 2022). Prior to imprinting, selected leaves were gently cleaned using tissue paper to remove surface debris and water droplets that could interfere with image clarity. For each sampled individual, impressions were prepared from both the adaxial and abaxial leaf surfaces to enable paired comparison of stomatal traits between surfaces.

Imprints were obtained from mature, fully expanded, and intact leaves selected from a comparable developmental position on each plant. A flat mid-lamina region was chosen while avoiding major veins to minimize within-leaf structural variation. A thin, even layer of transparent nail polish was applied to the leaf surface and allowed to dry completely until a clear film formed. Transparent adhesive tape was then carefully pressed onto the dried film and peeled off to lift the nail-polish replica. The tape carrying the epidermal imprint was mounted on a labeled microscope slide. The same procedure was repeated on the opposite leaf surface using a comparable lamina region, and each surface was mounted on a separate slide.

Prepared slides were observed using an Olympus CX31 light microscope at 400× magnification. This magnification was selected to ensure clear visualization of guard cells, stomatal pores, and surrounding epidermal cells while maintaining a sufficiently large field of view for density estimation. Digital micrographs were captured from each slide for subsequent measurement and analysis. To account for within-leaf variability without inflating replication, three non-overlapping fields of view were systematically selected from each leaf surface by shifting the microscope stage across comparable regions of the lamina (Qodriyah et al. 2021).

Quantitative stomatal measurements were conducted using ImageJ software after appropriate calibration of the scale (Schneider et al. 2012). Stomatal length and width were measured in micrometers (μm) on clearly identifiable, intact stomata, following consistent measurement criteria across all samples (Febjislami and Hasibuan 2023). Stomatal length was defined as the maximum distance between the ends of the guard cells along the longitudinal axis of the pore, while stomatal width was measured perpendicular to this axis at the widest point of the guard-cell pair.

The number of stomata within each microscopic field of view was recorded to estimate stomatal density. Density values were calculated by dividing the number of stomata counted in a field of view by the known area of that field at 400× magnification (0.1734 mm^2), yielding stomatal density expressed as stomata per mm^2. To avoid pseudoreplication, measurements from the three fields of view for each leaf surface were averaged to obtain plant-level means for stomatal length, stomatal width, and stomatal density. These plant-level values were used in all subsequent statistical comparisons among oxbow sites, ensuring that individual plants, rather than microscopic subsamples, constituted the unit of replication.

SPAD chlorophyll measurement

Leaf chlorophyll status of *P. crassipes* was estimated using a portable SPAD meter (SPAD-520 Plus, Konica Minolta, Japan). The SPAD meter provides a rapid and non-destructive measurement of relative chlorophyll content based on leaf light transmittance and is widely used as a physiological proxy for photosynthetic capacity and pigment status in field studies (Uddling et al. 2007; Hanafiyanto and Wahyono 2021). Although SPAD values do not represent absolute chlorophyll concentration, they offer a reliable comparative index for assessing differences in chlorophyll status among plants and habitats when measured consistently.

SPAD measurements were conducted on the same mature, fully expanded leaves used for stomatal observations to maintain correspondence between anatomical and physiological data. For each individual plant, the SPAD sensor was positioned on the central portion of the leaf lamina, avoiding major veins to reduce measurement variability. Care was taken to ensure that the leaf surface was clean and free from excessive moisture before measurement.

To account for small-scale heterogeneity within the leaf, SPAD readings were taken at three nearby points on the lamina of each leaf. These readings were recorded sequentially and averaged to obtain a single plant-level SPAD value. By treating the averaged value as the unit of analysis, within-leaf variation was incorporated without inflating the number of independent observations. The resulting SPAD values were used as supporting physiological indicators to contextualize stomatal anatomical variation and biomass differences among oxbow environments.

Dry weight measurement

Plant dry weight was measured as an indicator of biomass accumulation and overall growth performance of *P. crassipes* across the different oxbow environments. Dry weight provides an integrated measure of plant carbon gain after the removal of tissue water and is commonly used to assess comparative growth responses under contrasting environmental conditions (Lambers et al. 2008; Sopiana et al. 2022).

A total of 27 individual plants were included in the dry weight analysis, consisting of nine individuals from each oxbow site. After collection, excess surface water was gently removed from each plant using tissue paper to minimize weighing error. Whole plants were then placed in a drying oven (Memmert UF30) and dried at 65°C for three days, a temperature and duration sufficient to achieve constant mass without causing thermal degradation of plant tissues (Fahruddin et al. 2023). Following oven drying, samples were cooled in a desiccator to prevent moisture reabsorption prior to weighing.

Dried samples were weighed using an analytical balance with appropriate precision, and dry weight was recorded in grams for each individual plant. The individual plant was treated as the experimental unit, and the resulting dry weight values were used directly in statistical analyses. By standardizing drying conditions and measurement procedures across all samples, dry weight data provided a consistent basis for comparing biomass accumulation of *P. crassipes* among the three Bengawan Solo Oxbow Environments.

Data analysis

All quantitative data were analyzed using the individual plant as the experimental unit to ensure statistical independence among observations. For stomatal anatomical traits, measurements obtained from three non-overlapping microscopic fields of view per leaf surface (adaxial and abaxial) were treated as subsamples and averaged to generate plant-level means for stomatal length, stomatal width, and stomatal density. Similarly, SPAD values obtained from three readings per leaf were averaged to produce a single plant-level SPAD value, and dry weight was recorded once per individual plant. These plant-level means were used consistently in all subsequent analyses.

Differences among oxbow sites were tested using one-way Analysis of Variance (ANOVA), with oxbow location as the categorical factor. When the ANOVA indicated significant differences among sites, pairwise comparisons were conducted using Tukey's Honestly Significant Difference (Tukey HSD) post hoc test at a significance level of $\alpha = 0.05$. This approach allowed identification of specific site-to-site differences while controlling for multiple comparisons.

Subsampling and replication were explicitly distinguished to prevent pseudoreplication, which can occur when multiple measurements from the same experimental unit are incorrectly treated as independent observations (Hurlbert 1984). In this study, repeated microscopic fields of view and repeated SPAD readings were considered technical subsamples used to improve measurement precision, not independent biological replicates. By summarizing these subsamples at the plant level prior to statistical testing, the analysis maintained a clear separation between biological replication (individual plants) and technical replication (within-plant measurements). This procedure ensured that statistical inferences regarding differences among oxbow environments were based on appropriate degrees of freedom and reflected true between-site variation rather than within-sample measurement repetition.

RESULTS AND DISCUSSION

Abiotic characteristics of oxbow environments

The three Bengawan Solo Oxbows exhibited clear differences in both microclimatic and aquatic conditions, despite their close geographic proximity and similar elevation (Table 1). Air temperature varied among sites, with Kadokan recording the highest mean air temperature (36.95°C), followed by Sidowarno (35.50°C), while Tangkisan showed the lowest value (33.86°C). In contrast, relative humidity displayed an inverse pattern, with Sidowarno exhibiting the highest humidity (71.22%), followed by Tangkisan (67.00%), and Kadokan the lowest (64.63%). This temperature-humidity contrast indicates differing atmospheric evaporative regimes among oxbows.

Light intensity differed markedly across sites. Tangkisan experienced substantially higher irradiance (85,436.67 lux) than Sidowarno (30,655.71 lux) and Kadokan (34,065.56 lux), which showed relatively comparable values. These differences highlight strong site-level variation in the radiative environment experienced by *P. crassipes* stands and suggest that light availability is a major distinguishing factor among the three oxbows.

Aquatic parameters also varied among locations. Dissolved Oxygen (DO) was highest in Tangkisan (11.2 mg L^{-1}), intermediate in Kadokan (9.53 mg L^{-1}), and lowest in Sidowarno (8.1 mg L^{-1}). Total Dissolved Solids (TDS) followed a consistent gradient, with the highest values recorded in Tangkisan (3,203.33 mg L^{-1}), followed by Sidowarno (2,774.44 mg L^{-1}), and the lowest in Kadokan (2,426.25 mg L^{-1}). Water pH showed relatively narrow variation among oxbows (7.37-7.68), whereas substrate pH exhibited greater differences, with Sidowarno having the highest value.

Taken together, these results indicate that each oxbow represents a distinct combination of atmospheric, radiative, and aquatic conditions. This environmental heterogeneity provides a contrasting habitat framework for evaluating site-level variation in stomatal anatomy, physiological status, and biomass of *P. crassipes*.

Table 1. Average values of abiotic parameters measured in three Bengawan Solo Oxbow Environments in Central Java, Indonesia

Abiotic factor	Location		
	Tangkisan	Sidowarno	Kadokan
Water pH	7.37	7.68	7.60
DO (mg/L)	11.20	8.10	9.53
TDS (mg/L)	3,203.33	2,774.44	2,426.25
Air temperature (°C)	33.86	35.50	36.95
Humidity (%)	67.00	71.22	64.63
Water temperature (°C)	34.23	33.99	32.80
Substrate pH	5.00	6.06	5.90
Soil temperature (°C)	31.11	31.22	31.75
Salinity (%)	0	0	0
Elevation (masl)	99.00	98.00	99.00
Light intensity (lux)	85,436.67	30,655.71	340,65.56

Stomatal type and distribution pattern

Microscopic observations of *P. crassipes* leaves revealed a consistent stomatal structural pattern across all three Bengawan Solo Oxbows. Stomata were observed on both adaxial and abaxial leaf surfaces, indicating that the leaves of *P. crassipes* are amphistomatous. This distribution pattern was consistent among sampled individuals from Sidowarno, Kadokan, and Tangkisan and reflects the emergent-free-floating habit of the species, in which both leaf surfaces can participate in gas exchange.

At the cellular level, stomata were distributed among epidermal cells without a fixed arrangement of specialized subsidiary cells. Guard cells were kidney-shaped, which is typical of many angiosperms. Based on the arrangement and number of surrounding epidermal cells, the stomatal complex was classified as anomocytic, characterized by the absence of morphologically distinct subsidiary cells and the presence of several epidermal cells of similar size and shape encircling each stoma. In the present study, each stomatal complex was typically surrounded by four to six epidermal cells, supporting the anomocytic classification.

The amphistomatous condition observed in *P. crassipes* is ecologically consistent with its leaf orientation and habitat. Unlike many true floating-leaf hydrophytes, in which the abaxial surface remains persistently submerged, and stomata are restricted to the adaxial surface, water hyacinth leaves are held above the water surface by inflated petioles. As a result, both leaf surfaces are intermittently exposed to the atmosphere, although the abaxial surface often experiences a more humid and shaded microenvironment. This ecological context provides a plausible basis for the presence of stomata on both surfaces while allowing for potential differences in stomatal size and density between adaxial and abaxial sides, which are examined in subsequent sections.

Qualitative comparison of micrographs showed that stomatal pores, guard cells, subsidiary epidermal cells, and surrounding epidermal tissue were clearly identifiable on both leaf surfaces across all oxbows. These observations confirm that the same anatomical features were consistently captured and analyzed across sites, providing a reliable morphological foundation for quantitative comparisons of stomatal size and density among oxbow environments.

Stomatal size variation among oxbows

Stomatal size of *P. crassipes* varied among oxbow environments and between leaf surfaces (Table 2). On the adaxial surface, stomatal length ranged from 26.85 ± 4.4 µm in Sidowarno to 30.89 ± 4.7 µm in Tangkisan, with Kadokan showing intermediate values (28.11 ± 5.2 µm). Adaxial stomatal width also differed among sites, with the greatest mean width recorded in Tangkisan (14.19 ± 6.9 µm) and the smallest in Kadokan (9.11 ± 3.1 µm), while Sidowarno exhibited intermediate values.

On the abaxial surface, stomata were generally longer than those on the adaxial surface across all sites. Abaxial stomatal length was highest in Tangkisan (34.30 ± 3.3 µm), followed by Sidowarno (30.67 ± 3.4 µm) and Kadokan (30.33 ± 4.3 µm). A similar pattern was observed for abaxial stomatal width, with Tangkisan exhibiting the largest mean width (12.41 ± 1.5 µm), whereas Sidowarno (8.78 ± 1.5 µm) and Kadokan (8.96 ± 2.5 µm) showed smaller and comparable values.

Comparison between leaf surfaces indicated consistent surface-related differences in stomatal geometry. Across oxbows, abaxial stomata tended to be longer, whereas adaxial stomata tended to be wider. These surface-specific patterns were observed at all three sites, although the magnitude of the differences varied among oxbows.

Results of the Tukey HSD post hoc test indicated that not all pairwise comparisons among oxbows were statistically significant. Stomatal length and width in Tangkisan were frequently assigned to distinct letter groups relative to Sidowarno and Kadokan, whereas several measurements in Sidowarno and Kadokan shared letter groupings, indicating no significant difference at the 5% level. Accordingly, differences in stomatal size should be interpreted as site-specific patterns with varying degrees of statistical separation rather than as uniform contrasts across all oxbows.

Stomatal density variation among oxbows

Stomatal density of *P. crassipes* showed clear and consistent variation among oxbow environments and between leaf surfaces (Table 3). Across all sites, stomata were present on both adaxial and abaxial surfaces, confirming the amphistomatous leaf condition. However, the degree of stomatal deployment per unit leaf area differed markedly among oxbows.

Table 2. Mean stomatal length and width (± SD) on adaxial and abaxial leaf surfaces of *Pontederia crassipes* in three Bengawan Solo Oxbow Environments in Central Java, Indonesia, with different letters indicating significant differences according to Tukey HSD test (α = 0.05)

Oxbow	Adaxial		Abaxial		Average
	Length ± SD (µm)	Width ± SD (µm)	Length ± SD (µm)	Width ± SD (µm)	
Tangkisan	30.89^{b} ± 4.7	14.19^{b} ± 6.9	34.3^{b} ± 3.3	12.41^{b} ± 1.5	22.95
Sidowarno	26.85^{a} ± 4.4	11.52^{ab} ± 1.9	30.67^{a} ± 3.4	8.78^{a} ± 1.5	19.45
Kadokan	28.11^{ab} ± 5.2	9.11^{a} ± 3.1	30.33^{a} ± 4.3	8.96^{a} ± 2.5	19.13
Average	28.62	11.61	31.76	10.05	

Note: Average represents the arithmetic mean of adaxial and abaxial stomatal length and width values and is provided for descriptive comparison only

Table 3. Number and density of stomata (mean ± SD) on adaxial and abaxial leaf surfaces of *Pontederia crassipes* across three Bengawan Solo Oxbow Environments, in Central Java, Indonesia, with different letters indicating significant differences according to Tukey HSD test (α = 0.05)

Oxbow	Total of stomata		Stomatal density (/mm^2)		Average
	Adaxial	Abaxial	Adaxial	Abaxial	
Tangkisan	17.48^c ± 1.6	16.89^c ± 4.0	101.05^c ± 9.3	97.62^c ± 23.3	58.26
Sidowarno	14.89^b ±1.3	14.89^b ± 1.4	86.06^b ± 8.0	86.06^b ± 8.3	50.48
Kadokan	13.67^a ±1.7	11.22^a ± 2.0	78.99^a ± 10.1	64.86^a ± 11.9	42.19
Average	15.35	14.33	88.7	82.85	

Note: Average values represent descriptive arithmetic means of stomatal counts per field and stomatal densities and are not used for statistical inference

On the adaxial surface, the highest stomatal density was recorded in Tangkisan (101.05 ± 9.3 stomata mm^{-2}), followed by Sidowarno (86.06 ± 8.0 stomata mm^{-2}), while Kadokan exhibited the lowest density (78.99 ± 10.1 stomata mm^{-2}). A similar ranking was observed on the abaxial surface, with Tangkisan again showing the highest density (97.62 ± 23.3 stomata mm^{-2}), Sidowarno intermediate (86.06 ± 8.3 stomata mm^{-2}), and Kadokan the lowest (64.86 ± 11.9 stomata mm^{-2}). These values indicate a consistent site-level gradient in stomatal density across both leaf surfaces.

Comparison between leaf surfaces revealed differences in adaxial-abaxial allocation of stomata that varied among sites. In Tangkisan, stomatal density was relatively balanced between surfaces, with slightly higher values on the adaxial surface. Sidowarno exhibited nearly identical densities on both surfaces, whereas Kadokan showed a pronounced reduction in abaxial stomatal density relative to the adaxial surface. This surface-specific pattern indicates that while amphistomy is maintained across oxbows, the proportional distribution of stomata between leaf surfaces differs among locations.

Statistical analysis using the Tukey HSD test demonstrated significant differences in stomatal density among oxbows. Tangkisan was consistently assigned to the highest letter group for both adaxial and abaxial densities, whereas Kadokan was assigned to the lowest group, and Sidowarno occupied an intermediate position. These results indicate that the observed differences represent robust between-site variation rather than random within-site variability.

The quantitative patterns in Table 3 are visually supported by representative micrographs, which show shorter inter-stomatal spacing in Tangkisan compared with Sidowarno and Kadokan, particularly on the abaxial surface (Figure 2). Although these micrographs are not used as a source of independent quantitative data, they provide qualitative confirmation that the observed differences in stomatal density reflect genuine anatomical variation captured at the same magnification and under comparable imaging conditions.

SPAD chlorophyll index

SPAD chlorophyll index of *P. crassipes* differed significantly among the three Bengawan Solo Oxbow Environments (Table 4). Sidowarno exhibited the highest mean SPAD value (50.26 ± 1.4), followed by Tangkisan (45.99 ± 1.7), while Kadokan showed the lowest value (37.57 ± 0.8). The magnitude of separation among sites was substantial, with Sidowarno exceeding Kadokan by more than one-third of the latter's SPAD value, indicating marked differences in chlorophyll status among oxbows.

Statistical analysis using one-way ANOVA followed by Tukey HSD test confirmed that SPAD values differed significantly among all three oxbows at the 5% significance level. Sidowarno, Tangkisan, and Kadokan were assigned to distinct letter groups, indicating that none of the pairwise comparisons shared overlapping statistical groupings. These results demonstrate that variation in SPAD chlorophyll index among oxbows was consistent and robust across sampled individuals.

The ranking pattern of SPAD values indicates that chlorophyll status was highest in Sidowarno, intermediate in Tangkisan, and lowest in Kadokan. This pattern did not mirror stomatal density exactly, as Tangkisan, despite exhibiting the highest stomatal densities, did not show the highest SPAD values. Instead, Sidowarno achieved the greatest chlorophyll index, suggesting that chlorophyll status reflects the combined influence of multiple environmental factors rather than a direct correspondence with stomatal anatomical traits alone.

To provide an integrative framework for interpreting SPAD variation in relation to the measured abiotic context, a conceptual model was developed linking atmospheric, radiative, and aquatic factors to chlorophyll status (Figure 3). In this model, air temperature, relative humidity, and light intensity are positioned as primary drivers influencing evaporative demand, photoprotection costs, and pigment turnover, while aquatic descriptors such as dissolved oxygen, total dissolved solids, and pH are treated as contextual factors affecting overall plant vigor. This conceptualization emphasizes that SPAD values represent an integrated physiological outcome rather than a response to a single environmental variable.

The SPAD results demonstrate clear site-level differentiation in chlorophyll status of *P. crassipes* across Bengawan Solo Oxbows and provide a physiological complement to the observed anatomical variation. These findings establish SPAD as a supporting indicator that contextualizes stomatal traits within broader patterns of plant physiological condition across contrasting oxbow environments.

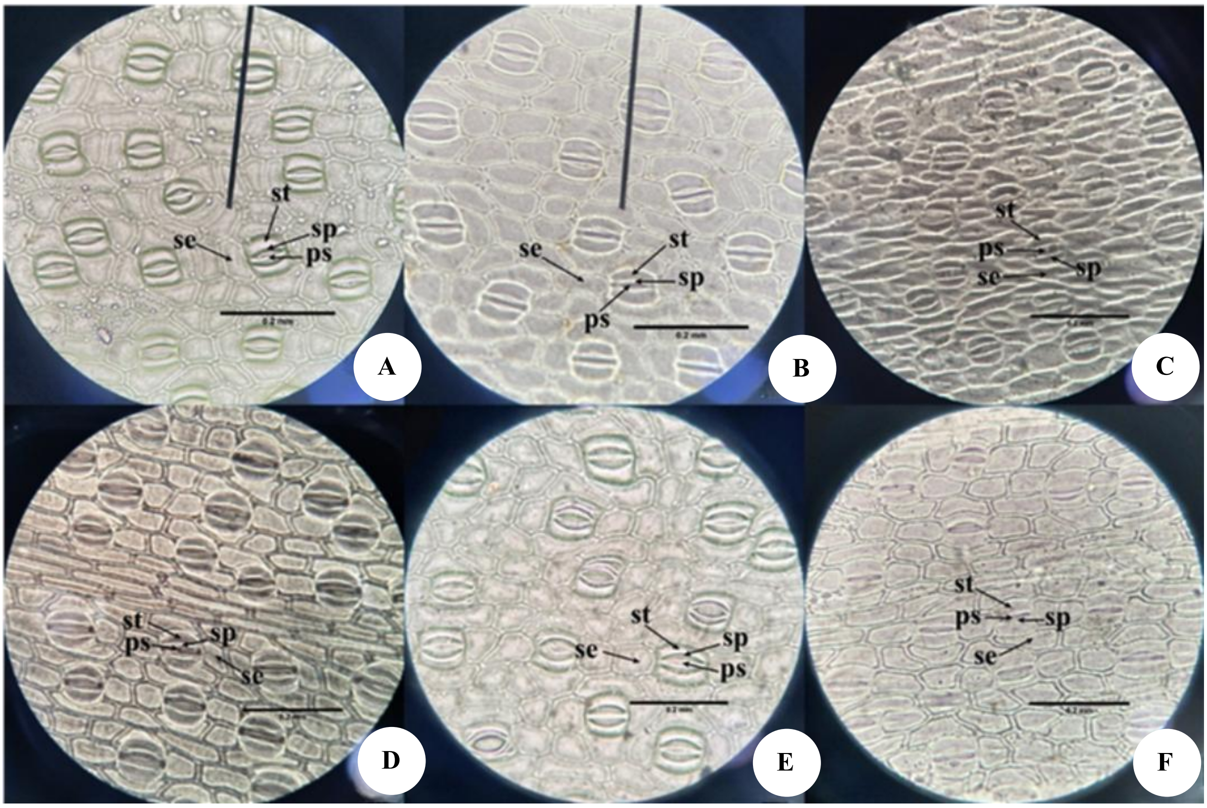

Figure 2. Representative light micrographs of stomata on adaxial and abaxial leaf surfaces of *Pontederia crassipes* from three Bengawan Solo Oxbows, Central Java, Indonesia, at 400× magnification. A-C: Adaxial surfaces of Tangkisan, Sidowarno, and Kadokan; D-F: Corresponding abaxial surfaces; ps: Stomatal pores; sp: Guard cells; st: Subsidiary cells; se: Epidermal cells

Table 4. Mean chlorophyll content (SPAD) and dry weight (± SD) of *Pontederia crassipes* from three Bengawan Solo Oxbow Environments in Central Java, Indonesia. Different superscript letters within each column indicate significant differences according to Tukey's HSD test (α = 0.05)

Oxbow	Chlorophyll content (SPAD)	Dry weight (g)
Tangkisan	45.99^{b} ± 1.7	185.55^{b} ± 2.9
Sidowarno	50.26^{c} ± 1.4	276.11^{c} ± 2.2
Kadokan	37.57^{a} ± 0.8	167.00^{a} ± 1.3
Average	44.61	209.55

Note: Values represent mean ± SD (n = 9 plants per oxbow). Different superscript letters within each column indicate significant differences among oxbow environments based on Tukey's HSD test following one-way ANOVA (α = 0.05)

Dry weight variation

Dry weight of *P. crassipes* differed significantly among the three Bengawan Solo Oxbow Environments (Table 4). Sidowarno exhibited the highest mean dry weight (276.11 ± 2.2 g), followed by Tangkisan (185.55 ± 2.9 g), while Kadokan showed the lowest value (167.00 ± 1.3 g). These differences indicate clear variation in biomass accumulation among oxbows.

Statistical analysis using one-way ANOVA followed by Tukey HSD test confirmed that dry weight differed significantly among all three sites at the 5% significance level. Each oxbow was assigned to a distinct statistical group, indicating that the observed differences in dry weight were consistent across sampled individuals and not attributable to random variation.

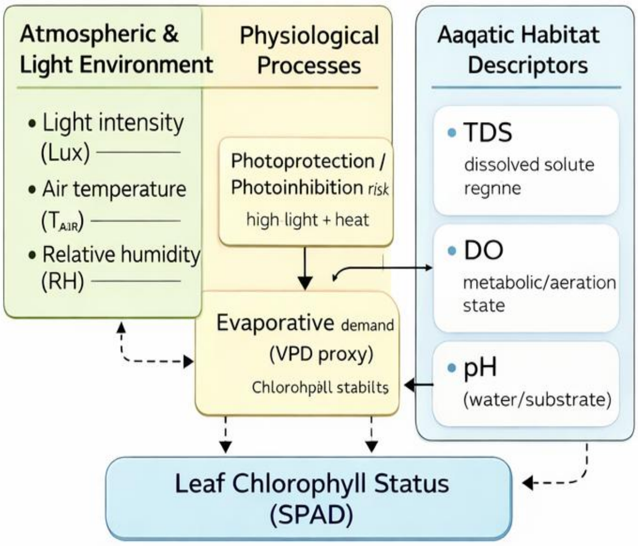

Figure 3. Conceptual model illustrating the relationships between abiotic factors (atmospheric, radiative, and aquatic) and SPAD chlorophyll status of *Pontederia crassipes* across Bengawan Solo Oxbow Environments in Central Java, Indonesia

The ranking pattern of dry weight among oxbows was Sidowarno > Tangkisan > Kadokan. This ordering shows that the oxbow with the highest chlorophyll index did not coincide with the oxbow exhibiting the highest stomatal density, and that biomass accumulation varied independently across sites. Dry weight values thus provide an integrated measure of plant performance that complements, but does not directly mirror, patterns observed in stomatal anatomy and SPAD chlorophyll index.

The dry weight results demonstrate that *P. crassipes* populations in the three Bengawan Solo Oxbows differ markedly in biomass accumulation. These differences establish a clear site-level pattern in whole-plant performance that accompanies the observed anatomical and physiological variation across oxbow environments.

Discussion

Ecological meaning of amphistomatous leaves in floating macrophytes

The amphistomatous leaf condition observed consistently in *P. crassipes* across all Bengawan Solo Oxbows is ecologically coherent with the growth form and functional ecology of this species as a free-floating macrophyte. Amphistomy, defined as the presence of stomata on both adaxial and abaxial leaf surfaces, is relatively uncommon among true floating-leaf hydrophytes but is frequently reported in aquatic or semi-aquatic plants whose leaves are held above the water surface and intermittently exposed to the atmosphere on both sides. In such systems, amphistomatous leaves facilitate gas exchange across both leaf surfaces, enhancing diffusive capacity under variable microclimatic conditions.

In many classic floating-leaf hydrophytes, such as *Nymphaea* and *Nelumbo*, stomata are predominantly or exclusively located on the adaxial surface because the abaxial surface remains persistently submerged, rendering abaxial stomata functionally redundant or maladaptive (Sculthorpe 1967; Rascio 2002). In contrast, *P. crassipes* exhibits inflated petioles that elevate the lamina above the water surface, allowing both leaf surfaces to interact with the aerial environment. This morphological configuration supports amphistomy as an adaptive trait, enabling flexible gas exchange while maintaining high photosynthetic capacity in open, high-light aquatic habitats (Shtein et al. 2017).

Comparative studies across aquatic macrophytes indicate that amphistomatous leaves are often associated with emergent or free-floating species experiencing fluctuating boundary-layer conditions, where both surfaces may alternately experience direct radiation, wind exposure, and high humidity (Maberly and Spence 1983; Mommer et al. 2006). Under such conditions, amphistomy can increase maximum stomatal conductance and carbon assimilation potential, particularly when light availability is high and diffusive resistance at the leaf surface is reduced (Parkhurst 1978; Driesen et al. 2020). The consistent presence of stomata on both surfaces in *P. crassipes* across all oxbows in this study aligns with these broader ecological patterns.

The anomocytic stomatal complex observed in *P. crassipes* further reinforces the interpretation that its stomatal anatomy reflects a generalized angiosperm condition rather than a highly specialized epidermal architecture. Anomocytic stomata, characterized by the absence of morphologically distinct subsidiary cells, are widely reported across diverse angiosperm lineages and are often associated with developmental plasticity in stomatal patterning (Metcalfe and Chalk 1979; Prastika et al. 2023). In aquatic and wetland plants, such plasticity may be advantageous, allowing stomatal traits to adjust during leaf development in response to local environmental cues without being constrained by rigid epidermal patterning.

From a functional perspective, amphistomatous leaves are frequently linked to environments with high light availability and relatively low constraints on water loss, conditions that typify open-water macrophyte stands such as those formed by *P. crassipes* (Parkhurst and Loucks 1972; Mott et al. 1982). Although amphistomy can increase transpirational water loss in terrestrial plants, this cost is mitigated in aquatic macrophytes by continuous water availability and high ambient humidity near the water surface. Moreover, the abaxial surface in free-floating plants often experiences a more humid and thermally buffered microenvironment due to proximity to the water surface, potentially reducing evaporative demand relative to the adaxial surface (Sculthorpe 1967; Badger and Price 1994).

The present findings are consistent with previous anatomical descriptions of water hyacinth, which report large stomata distributed on both leaf surfaces and emphasize the species' capacity for high gas-exchange rates under favorable conditions (Mahmood et al. 2005; Shtein et al. 2017). The persistence of amphistomy across all three oxbows, despite marked differences in microclimate and water chemistry, suggests that the stomatal distribution pattern in *P. crassipes* represents a conserved structural trait rather than a plastic response at the level of presence-absence. Instead, environmental variation among oxbows appears to be expressed more strongly through quantitative traits such as stomatal size and density, which are discussed in subsequent sections.

At a broader scale, the ecological interpretation of amphistomatous leaves in *P. crassipes* aligns with theoretical and empirical work linking stomatal distribution to plant functional strategies along gradients of light, water availability, and boundary-layer conductance (Parkhurst 1978; Buckley et al. 2015). Amphistomy is often associated with acquisitive strategies that prioritize carbon gain under high-resource conditions, a characterization that fits well with the rapid growth and high biomass production typical of water hyacinth populations in nutrient-rich aquatic systems (Villamagna and Murphy 2010). In this context, amphistomatous stomatal anatomy can be viewed as a foundational trait that enables *P. crassipes* to exploit favorable oxbow environments effectively, while quantitative modulation of stomatal traits fine-tunes performance under local environmental constraints.

The consistent amphistomatous and anomocytic stomatal architecture observed in this study supports the interpretation that *P. crassipes* possesses a stomatal design well suited to free-floating, high-light aquatic habitats. This structural baseline provides the anatomical framework upon which site-specific variation in stomatal size and density is superimposed, allowing environmental heterogeneity among oxbows to be reflected in quantitative, rather than qualitative, aspects of stomatal anatomy.

Environmental context of stomatal size and density variation

Variation in stomatal size and density of *P. crassipes* across Bengawan Solo Oxbows can be interpreted within the context of measured microclimatic gradients, particularly differences in air temperature, relative humidity, and light intensity. Because stomatal traits are established during leaf development, they are widely regarded as integrative outcomes of the environmental conditions prevailing during leaf formation rather than direct responses to instantaneous conditions at the time of measurement (Hetherington and Woodward 2003; Driesen et al. 2020). Accordingly, the patterns observed in this study are best discussed as associations with site-level microclimate rather than as evidence of direct causal mechanisms.

Across the three oxbows, Tangkisan exhibited the highest stomatal densities on both adaxial and abaxial leaf surfaces, whereas Kadokan consistently showed the lowest values. This site ranking parallels contrasts in atmospheric conditions, where Kadokan combined the highest air temperature with the lowest relative humidity, while Tangkisan experienced cooler air temperatures and intermediate humidity. Similar associations between drier, hotter air regimes and reduced stomatal density have been reported in a range of terrestrial and wetland plants, where lower stomatal deployment per unit area is interpreted as a conservative anatomical strategy that limits maximum potential conductance under higher evaporative demand (Franks and Beerling 2009; McElwain et al. 2016). In contrast, environments with lower evaporative stress may permit higher stomatal densities without incurring excessive water-loss risk.

Differences in stomatal size also followed site-specific patterns, with Tangkisan generally exhibiting larger stomata than Sidowarno and Kadokan. Across plant functional types, a trade-off between stomatal size and density is often reported, whereby plants may achieve similar maximum conductance through fewer large stomata or many smaller ones (Franks and Farquhar 2007; Dow et al. 2014). In aquatic and semi-aquatic species, however, this trade-off can be relaxed due to continuous water availability, allowing both relatively large and dense stomata to occur under favorable conditions (Maberly and Spence 1983). The combination of larger stomata and higher density observed at Tangkisan is therefore ecologically plausible in a free-floating macrophyte system and should not be interpreted as anomalous.

A light environment provides an additional contextual layer. Tangkisan experienced substantially higher irradiance than the other oxbows, which may favor anatomical configurations that support high diffusive capacity and carbon assimilation under high light availability (Parkhurst 1978; Buckley et al. 2015). Conversely, Kadokan and Sidowarno were exposed to much lower and relatively similar light intensities, suggesting that differences in stomatal traits among these sites may be more closely associated with atmospheric variables than with irradiance alone. Light, temperature, and humidity covary in natural field settings, making it inappropriate to attribute stomatal variation to a single dominant factor without controlled experimentation.

Aquatic parameters such as dissolved oxygen and total dissolved solids provide further habitat context but should be interpreted cautiously in relation to stomatal anatomy. While these variables can influence overall plant vigor and metabolic balance, their effects on stomatal differentiation are indirect and mediated through whole-plant physiological status rather than acting as direct developmental signals (Wetzel 2001; Lambers et al. 2008). Thus, the observed correspondence between higher stomatal density and higher DO or TDS at Tangkisan should be viewed as a site-level association rather than a mechanistic linkage.

The stomatal size and density patterns observed across Bengawan Solo Oxbows are consistent with established ecological theory linking stomatal traits to atmospheric microclimate. However, given the observational nature of the study and the co-variation of environmental factors, these patterns are most appropriately interpreted as environmentally contextualized anatomical variation rather than as evidence of direct cause-and-effect relationships.

Linking stomatal anatomy with SPAD and biomass

Integrating stomatal anatomical traits with SPAD chlorophyll index and dry biomass provides a more comprehensive view of how *P. crassipes* responds to contrasting oxbow environments. In this study, stomatal size and density represent structural attributes at the epidermal level, whereas SPAD and dry weight reflect physiological status and cumulative growth performance, respectively. Considering these components together allows stomatal anatomy to be interpreted within a broader functional context without assigning it a direct mechanistic role in determining physiological or growth outcomes.

The results show that variation in stomatal anatomy does not translate directly into proportional variation in SPAD chlorophyll index or biomass accumulation. For example, Tangkisan exhibited the highest stomatal densities on both leaf surfaces, yet it did not show the highest SPAD values or dry weight. In contrast, Sidowarno achieved the highest SPAD Index and the greatest biomass despite having intermediate stomatal density and a smaller average stomatal size compared with Tangkisan. This decoupling indicates that stomatal anatomical traits alone are insufficient to predict chlorophyll status or biomass and supports the interpretation that SPAD and dry weight function as complementary, rather than derivative, indicators.

From a functional perspective, stomatal anatomy defines the potential capacity for gas exchange, while SPAD reflects the realized status of chlorophyll pigments involved in light harvesting, and dry weight integrates net carbon gain over time (Uddling et al. 2007; Lambers et al.

2008). Numerous studies have shown that high stomatal density or large stomatal size does not necessarily result in higher photosynthetic performance if other environmental constraints limit pigment stability, enzymatic activity, or carbon assimilation efficiency (Murchie and Niyogi 2011; Flexas et al. 2016). The present results are consistent with this view, as the oxbow exhibiting the highest stomatal deployment did not consistently produce the highest physiological or growth metrics.

The SPAD values in this study appear to be more closely aligned with the combined microclimatic context of each oxbow than with stomatal anatomy alone. Sidowarno, which had the highest SPAD Index, experienced relatively high humidity and moderate air temperature compared with Kadokan, conditions that are generally associated with reduced stress on chlorophyll maintenance and photosynthetic machinery (Taiz et al. 2015). Conversely, Kadokan showed the lowest SPAD values alongside the highest air temperature and lowest humidity, a combination that can elevate thermal and oxidative stress and contribute to chlorophyll degradation (Murchie and Niyogi 2011). These patterns reinforce the interpretation that SPAD functions as a physiological indicator integrating multiple environmental influences rather than as a simple reflection of anatomical capacity.

Dry weight further supports this integrative interpretation. Biomass accumulation followed the same ranking as SPAD, with Sidowarno producing the highest dry weight and Kadokan the lowest. This correspondence suggests that chlorophyll status and growth performance are closely linked under the observed field conditions, whereas stomatal anatomy provides structural context rather than direct control. Similar dissociations between stomatal traits and biomass have been reported in other aquatic and wetland plants, where growth outcomes are governed by the balance between carbon gain and stress-related costs rather than by stomatal traits in isolation (Poorter et al. 2012; Buckley et al. 2015).

Positioning SPAD and biomass as supporting indicators avoids overinterpretation of stomatal anatomy as a sole determinant of plant performance. In field-based studies where multiple environmental factors covary, anatomical traits should be interpreted as part of a coordinated response spectrum rather than as linear predictors of physiological output. In this study, stomatal anatomy provides insight into how *P. crassipes* structurally accommodates differing oxbow environments, while SPAD and dry weight contextualize these anatomical patterns within broader physiological and growth outcomes.

The integration of stomatal anatomy, SPAD chlorophyll index, and dry biomass strengthens the ecological interpretation of the results by demonstrating that anatomical variation occurs alongside, but does not strictly dictate, physiological status and growth performance. This framework supports the use of stomatal traits as robust anatomical indicators of environmental context, with SPAD and biomass serving as complementary measures that validate the functional relevance of observed anatomical differences without implying direct causality.

Implications for oxbow habitat assessment

The observed variation in stomatal anatomy of *P. crassipes* across Bengawan Solo Oxbows highlights the potential value of stomatal traits as practical indicators for assessing habitat conditions in oxbow environments. Because stomatal size and density integrate environmental influences experienced during leaf development, these anatomical features can provide insight into site-level differences in microclimate and habitat context without requiring long-term monitoring or complex instrumentation. In oxbow systems where access and resources for repeated sampling may be limited, stomatal anatomy offers a field-relevant indicator that complements conventional physicochemical measurements.

One advantage of using stomatal anatomy in habitat assessment is its relative stability compared with short-term physiological variables. While parameters such as dissolved oxygen, temperature, and humidity can fluctuate diurnally and seasonally, stomatal traits represent cumulative developmental responses that capture environmental conditions over the period of leaf formation (Hetherington and Woodward 2003). As such, stomatal density and size may serve as integrative indicators of prevailing habitat regimes rather than instantaneous snapshots. This characteristic is particularly relevant in oxbow systems, where hydrological isolation and episodic flooding can generate highly variable short-term conditions.

Stomatal anatomical assessment also offers methodological practicality. Leaf impression techniques are low-cost, non-destructive, and can be applied in basic laboratory settings, making them accessible for ecological surveys and monitoring programs in resource-limited contexts (Windarsih et al. 2022). When combined with standardized sampling of leaf developmental stage and surface, as applied in this study, stomatal measurements can yield reproducible data suitable for comparative analysis across sites. This practicality enhances the feasibility of incorporating anatomical indicators into routine assessments of oxbow habitats.

From an applied perspective, integrating stomatal anatomy with supporting indicators such as SPAD chlorophyll index and biomass can improve the interpretation of habitat quality. While stomatal traits alone do not diagnose specific stressors, their variation can signal differences in environmental context that warrant further investigation. For example, consistently low stomatal density in certain oxbows may indicate atmospherically stressful conditions or constrained growth environments, prompting targeted assessment of microclimate or water quality. Conversely, high stomatal deployment may reflect more favorable conditions that support vigorous plant growth.

The use of stomatal anatomy as a habitat indicator should be framed as a screening or comparative tool rather than as a definitive diagnostic measure. Without complementary data on nutrient availability, pollutant concentrations, or long-term environmental trends, stomatal traits cannot be used to attribute observed patterns to specific drivers such as eutrophication or contamination. However, as demonstrated in this study, stomatal anatomy can effectively differentiate oxbow environments within the same river system and provide an anatomical basis for interpreting broader ecological patterns.

The findings support the inclusion of stomatal anatomical traits of *P. crassipes* as part of an integrated assessment framework for oxbow habitats. When combined with basic abiotic measurements and simple physiological indicators, stomatal anatomy offers a cost-effective and biologically meaningful approach for evaluating environmental heterogeneity and plant-habitat interactions in oxbow ecosystems.

Study limitations and future research directions

Several limitations of the present study should be acknowledged when interpreting the results, particularly with respect to temporal resolution and the scope of environmental variables measured. First, sampling and abiotic measurements were conducted during a single field campaign in the rainy season, resulting in a snapshot representation of oxbow conditions. Although stomatal anatomical traits integrate environmental influences over the period of leaf development, the associated abiotic data reflect conditions at the time of sampling and do not capture diel or seasonal variability. Oxbow environments are known to experience pronounced temporal fluctuations in water level, temperature, dissolved oxygen, and light regime, especially between wet and dry seasons. Consequently, the observed associations between stomatal traits and environmental context should be interpreted as site-specific patterns rather than as stable, year-round relationships.

Second, the study relied on bulk physicochemical indicators such as Total Dissolved Solids (TDS), dissolved oxygen, and pH to characterize aquatic habitat conditions. While these parameters provide useful descriptive context, they do not allow differentiation between specific nutrient forms or pollutant loads. In particular, the absence of nutrient speciation (e.g., inorganic nitrogen and phosphorus fractions) limits the ability to distinguish between nutrient enrichment and osmotic or ionic stress as potential contributors to variation in plant physiological status. Similarly, the lack of heavy metal analysis precludes assessment of contaminant-related stress, which has been shown in other studies to influence both physiological performance and anatomical traits of aquatic macrophytes (Ali et al. 2013; Rai 2016).

Future research should therefore prioritize temporal replication and expanded environmental characterization. Repeating sampling across contrasting seasons would allow evaluation of the stability of stomatal anatomical patterns under different hydrological and climatic conditions and help determine whether observed site-level differences persist over time. Inclusion of nutrient speciation, particularly dissolved nitrogen and phosphorus forms, would strengthen the interpretation of plant responses in relation to eutrophication gradients. In oxbows suspected of receiving anthropogenic inputs, targeted analysis of heavy metals and organic pollutants would further clarify the ecological drivers underlying anatomical and physiological variation.

Methodologically, future studies could also incorporate direct measurements of gas exchange, such as stomatal conductance and photosynthetic rate, to better link stomatal anatomy with functional performance. Multivariate analytical approaches integrating anatomical, physiological, and environmental variables could provide a more nuanced understanding of how multiple factors interact to shape plant responses in oxbow ecosystems. Additionally, expanding the number of oxbow sites within the Bengawan Solo basin would enhance spatial generalization and allow broader inference across riverine landscapes. While the present study provides a robust comparative assessment of stomatal anatomical variation across three oxbow environments, addressing these limitations through expanded temporal, chemical, and functional analyses will be essential for advancing understanding of plant-environment interactions in tropical oxbow systems.

In conclusion, this study demonstrates that *P. crassipes* exhibits consistent stomatal structural characteristics across Bengawan Solo Oxbow Environments, with uniformly amphistomatous leaves and anomocytic stomatal complexes. Within this conserved structural framework, quantitative variation in stomatal size and density distinguished oxbows, indicating that localized environmental conditions are reflected at the epidermal anatomical level. Stomatal density was highest in Tangkisan oxbow (101.05 ± 9.3 stomata mm^{-2} on the adaxial surface and 97.62 ± 23.3 stomata mm^{-2} on the abaxial surface), intermediate in Sidowarno (86.06 ± 8.0 and 86.06 ± 8.3 stomata mm^{-2}), and lowest in Kadokan (78.99 ± 10.1 and 64.86 ± 11.9 stomata mm^{-2}). In contrast, physiological and growth indicators did not show a direct correspondence with stomatal anatomical patterns. SPAD chlorophyll index was highest in Sidowarno (50.26 ± 1.4), followed by Tangkisan (45.99 ± 1.7), and lowest in Kadokan (37.57 ± 0.8), while dry biomass exhibited a similar ranking, with the greatest values in Sidowarno (276.11 ± 2.2 g), intermediate values in Tangkisan (185.55 ± 2.9 g), and the lowest values in Kadokan (167.00 ± 1.3 g). These results indicate that stomatal anatomy represents structural potential rather than a direct determinant of chlorophyll status or biomass accumulation, with SPAD and biomass functioning as complementary indicators that contextualize anatomical variation under heterogeneous field conditions. By integrating stomatal anatomy with physiological and growth metrics across multiple oxbows within a single tropical river system, this study provides an anatomically grounded framework for interpreting plant responses to oxbow environments. The results also underscore the practical value of stomatal anatomy as a field-relevant indicator for comparative habitat assessment when combined with basic abiotic measurements and simple physiological indices.

ACKNOWLEDGEMENTS

The authors are grateful to the Faculty of Mathematics and Natural Sciences, Universitas Sebelas Maret (UNS), Surakarta, Central Java, Indonesia, for providing laboratory facilities and technical support during sample processing and microscopic observations. We also acknowledge the assistance of local field guides and community members in Sukoharjo District, Central Java, who facilitated access to the Bengawan Solo oxbow sites during field sampling. Their support was invaluable for the successful completion of this study.

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