

CORRELATION BETWEEN THE PHYSICOCHEMICAL
WATER PROPERTIES AND THE MORPHOLOGICAL
CHARACTERS OF *LEMNA GIBBA* L.

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Abstract

This study examined the correlation between physicochemical water properties and morphological characters of *Lemna gibba* L. based on field data from Serbia (South Morava, West Morava, Great Morava, and Danube catchments). Morphology was assessed using macro- (frond and turion number, frond dimensions, and their ratios) and micromorphological characters (epidermal cells and stomatal features). Correlations with water parameters (pH, EC, NO_3^- , NO_2^- , PO_4^{3-} , SO_4^{2-} , Cl^- , Br^- , and F^-) were analyzed using the Pearson correlation coefficient and Canonical Correspondence Analysis (CCA). Pearson correlation analysis showed positive associations of Cl^- and NO_3^- concentrations with frond dimensions (width, length, and area), and negative associations with PO_4^{3-} concentration. The CCA identified Cl^- , F^- , and NO_3^- concentrations as key drivers, linked to reduced frond number, frond length-to-width ratio, and stomatal and guard cell dimensions. In contrast, higher Br^- concentrations were linked to increased frond size and adaxial epidermal cell area, while PO_4^{3-} was associated with greater guard cell width and stomatal density. These results provide insight into the uni- and multivariate influence of certain physicochemical water properties on the morphological variability of *L. gibba*.

Key words: *Lemna gibba*, morphological variability, anions, water physicochemistry

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Introduction. *Lemna gibba* L. is a free-floating aquatic plant belonging to the family Araceae, order Alismatales, subclass Magnoliidae, class Equisetopsida, and phylum Streptophyta [1]. This species consists of one or more oval or oval-oblong fronds [2]. Fronds are 1–8 mm long and 0.8–6 mm wide (1–1.5 times longer than wide), somewhat shiny on the adaxial side, usually with 4–5 nerves, with lateral nerves originating independently from the node. The fronds are convex – with gibbositities (up to 4 mm thick) on the abaxial surface, which is sometimes red, especially near the edges, along the nerves, or on the cells between the air spaces. The adaxial surface occasionally shows pronounced red spots, especially near the apex. The greatest distance between the inner lateral veins occurs at or slightly above the middle of the veins. The largest air spaces are wider than 0.3 mm, and the root cap is generally rounded or sometimes pointed, measuring 0.6–1.8 mm in length. The pistil consists of an ovary with 1–7 ovules and a 0.05–0.1 mm long style. The fruit is 0.6–1.0 mm long and 0.8–1.2 mm wide, with a winged edge measuring 0.1–0.2 mm in width. Each fruit contains 1–5 whitish seeds 0.7–0.9 mm long and 0.4–0.6 mm thick, with 8–16 distinct ribs, which are released from the fruit wall after ripening. The distribution of this species is worldwide, except in Norway, where it is considered extinct. It has been introduced into Japan, Maryland, and Sri Lanka [1].

In phytocoenological terms, *L. gibba* is characteristic of communities of the class *Lemnetea* O. de Bolòs et Masclans 1955. In addition, *L. gibba* can also occur as an accompanying species in communities belonging to other classes, such as *Potamogetonetea* Klika in Klika et Novák 1941 and *Phragmito-Magnocaricetea* Klika in Klika et Novák 1941 [3].

Data on the ecology of *L. gibba* indicate that this species mostly develops in shallow eutrophic, rarely mesotrophic waters, most often under full sun or only slightly shaded conditions. The water pH is usually slightly acidic to slightly alkaline, with a high content of N, sometimes P, Ca^{2+} , and Cl^- (the high concentration of dissolved salts is also reflected in increased water conductivity). *Lemna gibba* often prefers higher water temperatures, typically ranging from 29 to 35 °C [4].

This study aims to determine the influence of water physicochemistry on the variability of morphological characters of *L. gibba*.

Materials and methods. Plant material and water sampling. Plant material was collected from lentic and lotic ecosystems across the territory of the Republic of Serbia (Table 1). From each of the 18 populations, 15 individuals were sampled, washed and preserved in 50% ethanol. Plant material belonging to the genus *Lemna* was identified to species level using Flora SR Srbije VIII: Arales – Lemnaceae: Fam. Lemnaceae [5] and research by CESCHIN et al. [6]. For the evaluation of environmental variables in the study area, water samples were collected concurrently with plant sampling. All samples were transported to the laboratory and analyzed immediately upon arrival or within 24 h of collection.

T a b l e 1

Overview of the study areas, including their coordinates and sampling dates of plant material (1 – plant material collected from ponds; 2 – plant material collected from rivers). Additionally, the table contains the results of measurements of physicochemical water properties

ID	Study area	Latitude	Longitude	Sampling date	pH	EC (µS/cm)	NO ₃ ⁻ (mg/l)	NO ₂ ⁻ (mg/l)	PO ₄ ³⁻ (mg/l)	SO ₄ ²⁻ (mg/l)	Cl ⁻ (mg/l)	Br ⁻ (mg/l)	F ⁻ (mg/l)
1	¹ Batušnac	43.295066	21.803955	05.09.2022	7.87	291	3.760	1.366	0.4016	49.233	18.099	0.006	0.123
2	¹ Ćićina	43.575646	21.638173	08.09.2022	8.37	505	5.682	0.006	0.0055	36.214	20.004	0.006	0.001
3		43.575396	21.635527		7.99	276	2.858	0.006	0.5860	32.483	13.407	0.006	0.095
4	¹ Glogovac	44.039371	21.300100	12.09.2022	7.44	477	0.006	0.006	0.5496	32.277	35.046	0.006	0.544
5		44.038599	21.298386		8.94	294	0.052	0.094	0.3135	132.909	13.754	0.113	0.339
6	¹ Čepure	43.822675	21.348963	14.09.2022	7.67	504	1.099	0.006	0.5409	112.418	30.367	0.006	0.414
7		43.819622	21.347987		7.77	449	0.111	0.092	0.4777	137.793	46.109	0.170	0.012
8		43.819912	21.347625		7.87	291	5.760	1.366	0.5602	51.233	18.099	0.006	0.123
9	¹ Podunavci	43.694732	20.838062	21.09.2022	7.53	328	0.006	0.006	0.9548	9.574	8.031	0.006	0.001
10		43.693990	20.838485		7.49	333	0.006	0.006	0.0002	20.254	11.732	0.006	0.014
11		43.688835	20.838951		7.47	321	0.006	0.006	0.0001	12.198	9.876	0.006	0.056
12	¹ Stopanja	43.594707	21.152327	21.09.2022	7.23	531	0.006	0.006	1.2497	43.115	21.226	0.006	0.332
13		43.595897	21.153455		8.11	341	0.107	0.211	0.7483	15.598	20.328	0.252	0.03
14	¹ Mazarać	42.585436	21.999142	19.10.2022	8.56	392	5.758	0.592	0.3429	64.925	39.476	0.467	0.338
15	² Stopanja – West Morava river	43.596970	21.153323	21.09.2022	7.77	419	2.460	0.532	0.5329	33.001	20.942	0.242	0.025
16	² Radujevac – Danube river	44.279567	22.671818	06.10.2022	7.75	260	0.760	0.603	0.2832	41.425	29.076	0.593	0.379
17	² Kupinince – South Morava river	42.507041	21.888966	19.10.2022	7.75	422	13.755	0.626	0.0003	56.010	67.044	0.006	1.269
18	² Mazarać – South Morava river	42.585566	21.999360	19.10.2022	7.81	423	4.070	0.076	0.0019	63.206	41.005	0.006	0.179

Measurement of morphological characters. Macromorphological frond characters were systematically observed and assessed using a Leica MZ 16 A stereomicroscope. For micromorphological measurements, unstained wet mounts of fronds were prepared for each specimen and examined using a Leica DM 2500 light microscope. Each specimen was photographed and the resulting images were used to assess morphological characters (Table 2) employing the ImageJ 1.47v software package [7].

Chemical analyses. Water pH and electroconductivity (EC) were measured using a sensION+ MM374 multimeter (HACH, Germany). Anions were determined by ion chromatography (Dionex Aquion, Thermo Scientific, Germany) after filtration (0.45 μm microfilter), using Dionex IonPac AS22 column with AG22 guard column, carbonate/bicarbonate eluent (1.2 mL/min), and conductivity detection. Anion concentrations were calculated using Chromeleon 7 software.

Statistical data processing. Data on all characters were statistically processed using the IBM SPSS Statistics 20 software. The analysis included the calculation of descriptive statistics, Pearson correlation coefficient, and canonical correspondence analysis (CCA). The CCA (based on Chi-square distance) was performed at the character-level, to clarify the relationships between the environmental variables and the individual morphological characters.

Results. Basic statistical properties of the examined morphological characters and character-environment associations. The Pearson correlation coefficient was used to examine the relationships between the 9 measured environmental variables (Table 1) and the 17 morphological characters (Table 2). The strongest positive correlations were discovered between Cl^- and NO_3^- concentrations and AFW, FWM, FWA, LFA, FWB, and FL. The strongest negative correlations were found between these characters and PO_4^{3-} concentration (Fig. 1).

The CCA-based assessment of environmental drivers of morphological variability. The multivariate influence of physicochemical water properties on the variability of morphological characters of the studied *L. gibba* populations was evaluated using CCA.

This analysis indicated that Cl^- , F^- , and NO_3^- had the greatest effect on the morphological characters among the populations studied. The EC also loaded negatively on Axis 1, suggesting that it covaries strongly with ion concentrations, whereas pH exhibited a moderate contribution to the overall chemical gradient (Fig. 2).

According to this analysis, FWB, FWM, FWA, AFW, ECAad, FL, and LFA showed a positive correlation with Br^- concentration, while ECAab expressed a positive correlation with NO_2^- concentration, and SD and GCW expressed a positive correlation with PO_4^{3-} concentration (Fig. 2). Negative correlations were observed between BF-R and NO_2^- concentration, as well as between NF, FL/FW, GCL, GCW, SL, and SW and Cl^- , F^- , NO_3^- , and SO_4^{2-} concentrations, as well as EC and pH. These characters correspond to areas with comparatively lower ion

T a b l e 2

Descriptive statistics for all 17 examined quantitative characters: $\bar{x}$ – mean value; σ – standard deviation

Character description	Character acronym	Min	Max	$\bar{x}$	σ
Number of fronds	NF	1.00	4.00	1.69	0.64
Distance from the base of the frond to the point of origin of the roots (mm)	BF-R	0.13	2.34	1.16	0.27
Largest frond area (mm ²)	LFA	1.41	17.95	5.43	2.93
Frond length (mm)	FL	1.72	5.81	3.09	0.72
Frond width at the base – at 5% of length (mm)	FWB	0.44	2.27	0.98	0.31
Frond width at the middle – at 50% of length (mm)	FWM	1.13	4.46	2.20	0.67
Frond width at the apex – at 95% of length (mm)	FWA	0.54	2.62	1.23	0.40
Average frond width (mm)	AFW	0.83	2.87	1.47	0.43
Frond length-to-width ratio	FL/FW	0.99	1.88	1.44	0.18
Number of turions	NT	0.00	3.00	0.18	0.52
Epidermal cell area on the adaxial side of the frond (μm ²)	ECAad	129.63	714.46	308.28	107.14
Epidermal cell area on the abaxial side of the frond (μm ²)	ECAab	233.31	1898.58	799.02	299.46
Guard cell length (μm)	GCL	13.93	33.60	21.17	3.36
Guard cell width (μm)	GCW	2.90	20.01	4.94	1.32
Stomatal length (μm)	SL	7.99	24.27	14.97	3.05
Stomatal width (μm)	SW	2.75	10.38	6.21	1.44
Stomatal density per mm ²	SD	24.81	297.77	151.92	54.23

	pH	EC	NO ₃ ⁻	NO ₂ ⁻	PO ₄ ³⁻	SO ₄ ²⁻	Cl ⁻	Br ⁻	F ⁻		
NF	-0.004	-0.080	-0.118	-0.031	-0.034	0.293	0.012	-0.016	0.070		
BF-R	0.127	0.090	0.233	-0.055	-0.286	0.140	0.392	0.113	0.145		
LFA	0.224	0.140	0.512	0.079	-0.459	0.100	0.487	0.052	0.190		r
FL	0.217	0.117	0.468	0.086	-0.448	0.118	0.478	0.090	0.151		1.00
FWB	0.111	0.165	0.492	-0.009	-0.333	0.057	0.534	0.157	0.322		0.75
FWM	0.227	0.117	0.534	0.126	-0.480	0.108	0.506	0.080	0.225		0.50
FWA	0.132	0.100	0.518	0.046	-0.407	0.020	0.541	0.181	0.258		0.25
AFW	0.187	0.132	0.558	0.078	-0.457	0.076	0.561	0.136	0.275		0.00
FL/FW	-0.142	-0.032	-0.394	-0.153	0.320	-0.049	-0.316	-0.058	-0.259		-0.25
NT	-0.040	-0.212	0.277	0.347	-0.090	-0.045	0.174	0.185	0.228		-0.50
ECAad	0.168	0.241	0.250	0.093	-0.086	0.206	0.473	0.270	0.234		-0.75
ECAab	0.172	0.201	0.317	0.147	-0.133	0.129	0.364	0.163	0.270		-1.00
GCL	0.182	-0.026	0.270	0.221	-0.137	0.034	0.319	0.313	0.194		
GCW	0.089	-0.003	0.145	0.078	-0.098	0.028	0.147	0.109	0.143		
SL	0.193	0.033	0.185	0.224	-0.098	0.113	0.263	0.300	0.106		
SW	0.155	-0.023	0.029	-0.069	-0.112	0.006	0.037	0.052	0.020		
SD	-0.017	-0.103	0.004	0.011	-0.080	-0.011	0.020	0.050	0.023		

Fig. 1. Pearson correlation matrix (heatmap) showing correlations between each pair of environmental variables and morphological characters. The list of character acronyms with their meanings is given in Table 2

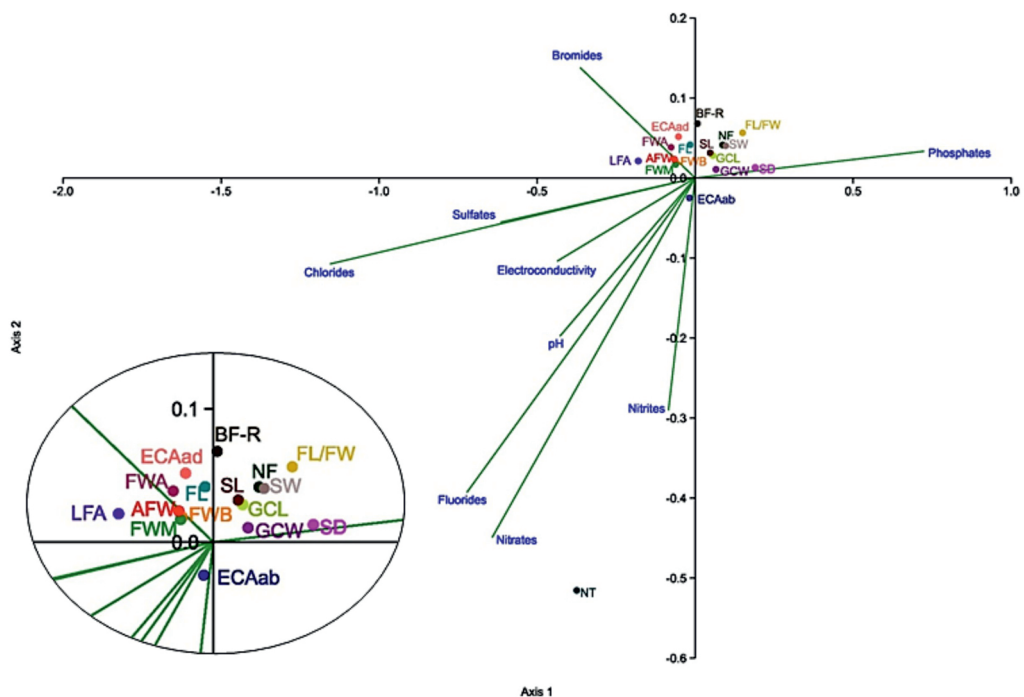


Fig. 2. Graphical representation of the results of Canonical Correspondence Analysis (CCA), showing the correspondence between 9 environmental variables and 17 morphological characters of *L. gibba*. The list of character acronyms with their meanings is given in Table 2

contents. The NT appears positioned further on the negative side of Axis 2, indicating an unique chemical signature, most likely associated with elevated NO_3^- and NO_2^- levels (Fig. 2).

Discussion. In situ data on the influence of environmental variables on the variability of morphological characters of *L. gibba* are poorly represented in the literature. The characters that can be strongly modified by environmental variables include frond size and shape, gibbosity, root length, size of air spaces, and frond colour and pigmentation [8]. Most research has focused on the influence of environmental variables on biomass production and relative growth rate in this species [9–14]. Therefore, our present study aimed to assess the correlation between physicochemical water properties and morphological characters of *L. gibba*.

The effect of changes in pH on the relative growth rate of *L. gibba* has previously been reported [9]. Up to pH 7.8, significant dry matter production was observed, whereas at pH values close to or above 9.8 growth became almost impossible. Similarly, our results associate higher pH values with smaller NF, as well as with smaller FL/FW, GCL, GCW, SL, and SW (Fig. 2). *Lemna gibba* can grow successfully and even tolerate stress from other environmental variables until pH exceeds a certain threshold, above which it becomes more sensitive. Although HILT et al. [9] did not directly measure morphological characters of this species, the effect on relative growth rate implies that biomass, area, and frond number decrease as pH increases beyond optimal range [9]. This may explain the smaller frond length in the populations analyzed in our study, as the pH at our sites ranged from 7.23 to 8.94 (Table 1), while the optimal pH range for this species, according to BAEK et al. [15], is usually ~ 5.5 – 7.5 , and according to KHELLAF and ZERDAOUI [10] the best growth rate of this species occurs at pH of 5–6. The maximum frond length recorded in our study was 5.81 mm, while under optimal conditions it may reach 8 mm [1]. Low pH values (3–4) were also found to decrease growth rate in this species [10].

Information on the effects of EC on the morphology of *L. gibba* in the literature is scarce. LANDOLT [8] highlighted the influence of EC on gibbosity, showing that higher EC promotes the appearance of gibbous forms in this species.

Nutrient availability strongly influences morphology in this species. Higher N and P levels promote larger and healthier fronds, as well as increased frond number, whereas nutrient limitation results in smaller, chlorotic fronds [16]. Nutritional status also affects stress responses, with nutrient-poor plants showing reduced plasticity and slower recovery [16]. Nitrates and nitrites play a central role in N metabolism, influencing growth, biomass allocation, and indirectly morphology in duckweeds [11–13]. Consistent with this, NO_2^- was positively associated with ECAab and negatively with BF-R, while NO_3^- showed negative relationships with NF, FL/FW, GCL, GCW, SL, and SW (Fig. 2).

Phosphates availability similarly affects growth strategies. Higher PO_4^{3-} levels promote faster growth and smaller fronds, whereas limitation induces smaller

size, higher dry mass allocation, and slower reproduction [17]. In our study, PO_4^{3-} was positively associated with GCW and SD (Fig. 1). According to Landolt [8], $\sim 0.1 \text{ mg/l } \text{PO}_4^{3-}$ corresponds to flat forms of *L. gibba*, whereas concentrations $> 0.3 \text{ mg/l } \text{PO}_4^{3-}$ are associated with gibbous forms. However, all specimens in our study were gibbous despite wide variation in PO_4^{3-} concentrations at the moment of measurement (0.0001–1.2497 mg/l) (Table 1).

The influence of SO_4^{2-} and Cl^- concentrations has mainly been examined in relation to protein content in dry matter and relative growth rate in duckweeds [14]. However, data on their impact on *L. gibba* morphological characters remain limited, making the present study among the first to report the effects of these anions on the morphology of this species. Studies on related species of the genus *Lemna* and other macrophytes show that elevated concentrations of these anions can lead to reduced frond area, decreased frond and root number, chlorosis, and damage to chloroplasts. These changes are caused by osmotic and ionic stress, which disrupts cellular water and ion balance, induces oxidative damage, and leads to degradation of chloroplasts. Morphologically, this is manifested through yellowing of fronds, reduced growth, and lower biomass production [18]. In addition, increased concentrations of chloride ions can reduce relative growth rate and total biomass and, at higher levels, may lead to reduction of roots and fragility of root structures [19]. Elevated concentrations of SO_4^{2-} in water may also result in growth inhibition and decreased biomass production [20].

Although direct evidence linking F^- exposure to morphological changes in *L. gibba* remains limited, it has been shown to have inhibitory effects on frond production, indicating that F^- may influence growth-related morphological traits [21].

Direct experimental evidence on the effects of Br^- on the morphology of *L. gibba* is also currently lacking. However, studies on duckweeds indicate that it may affect chloroplast morphology (e.g., increased size and irregular shape) in closely related species (*L. minor* L.), suggesting its potential role in shaping morphological variability [22].

Conclusion. By identifying specific physicochemical water characteristics significantly associated with morphological variability in *L. gibba*, this work provides the first integrative evidence that water anion composition, pH, and EC play a significant role in shaping phenotypic expression in this species under natural conditions. Our results go beyond descriptive morphology by quantitatively linking environmental gradients with plant morphological responses, thereby contributing to a deeper understanding of plant-environment interactions in aquatic ecosystems.

Furthermore, the results indicate that morphological plasticity in *L. gibba* is not driven by single environmental variables alone, but rather arises from the combined influence of multiple physicochemical variables, with anion-related effects showing particular ecological significance.

Future research should integrate experimental and field-based approaches, incorporate additional environmental variables, and evaluate genotype-specific re-

sponses to environmental gradients. Expanding analyses to include biochemical and physiological traits could further clarify the mechanisms underlying morphological plasticity. Such efforts would improve our understanding of how this species responds to changes in water chemistry.

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