Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 28(2): 601 – 608 (2024)
www.ejabf.journals.ekb.eg

A Biotechnological and Illustration Study of *Eichhornia crassipes* Hydrophyte Growing in Sewage

Ahmed M. Sadek*, Alaa A. Elkady, Mohamed A. Mousa
Botany & Microbiology Department, Faculty of Science, Al-Azhar University, Cairo, Egypt

***Corresponding Author : a.sadek@azhar.edu.eg**

ARTICLE INFO

Article History:
Received: Dec. 20, 2023
Accepted: Feb. 7, 2024
Online: April 11, 2024

Keywords:
Plant biotechnology,
rbcL gene,
Palynology,
Eichhornia crassipes,
Sewage

ABSTRACT

The water hyacinth has garnered significant attention due to its harm effect on economics and ecology. Thus, understanding *Echhornia crassipes* at different levels is necessary to figure out the suitable methods either for withstanding or using this species positively. *Eichhornia crassipes* is a synonym of *Pontederia crassipes*, a fast-growing hydrophyte, native to South America, belonging to genus *Pontederia* and family Pontederiaceae, monocots. The anatomy of different organs was addressed to provide a clear image of the internal structure of the species under study. This could explain the survival of this plant in the sewage ecosystem. Aerinchyma tissue was found in all organs, and water was obviously stored in the root to balance plant floating. In addition, pollen grains' characterization was recognized on SEM stubs with their monads, heteropoler, radiosymetry and large size (P-56/E-45µm). Identification of *Echhornia crassipes* was approved via PCR and partial gene sequencing of rbcL gene (Ribulose Bisphosphate Carboxylase), which coincides with the gen bank. The present study encompasses molecular identification, as well as an anatomical and paleontological illustration.

INTRODUCTION

Eichhornia crassipes is the synonym of *Pontederia crassipes* which refers to a fast-growing hydrophyte, native to South America (**Sculthorpe, 1967**) and belonging to genus *Pontederia* and family Pontederiaceae, monocots (**WFO, 2023**). Noticeably, it has garnered significant attention as its remarkable ability to increase and form dense mats on the water's surface, leading to detrimental ecological and economic consequences (**Hossain *et al*., 2008; Nico *et al*., 2009**). These mats can impede water flow, reduce oxygen levels, and disrupt aquatic ecosystems (**Holm *et al*., 1977**). In addition to its invasive nature, *E. crassipes* has been the subject of scientific inquiry owing to its potential applications in wastewater treatment, biofuel production, and phytoremediation (**Zheng *et al*., 2008; El-Shafai *et al*., 2010**).

The molecular and anatomical data of *E. crassipes* are essential for comprehending its unique characteristics and ecological impacts. Though previous studies have explored various facets of this plant, including its genetics, physiology, and ecology, a comprehensive analysis of its molecular and anatomical features is still needed to shed light on its invasive success and potential uses **(Rajasulochana & Preethy, 2010**). This paper pointed to provide an overview of *E. crassipes* at the molecular, anatomicalan and palynoligical levels.

MATERIALS AND METHODS

Mature flowering *E. crassipes* aquatic plant individuals were collected from sewage surface located in Qalubia, Egypt using GPS location (578Q+WH9‹6313701).

Fresh materials of studied species were organized and kept in the Herbarium of the Department of Botany and Microbiology, Faculty of Science (Boys branch), Al-Azhar University, Cairo, Egypt. Identification of studied taxa was assessed by using the plant key of **Tãckholm (1974)** and **Boulos (2002**) in addition to setting a comparison with herbarium specimens from Cairo University Herbarium (CAI).

For anatomical investigation, fresh sample was fixed in F.A.A. (Nassar and El-Sahhar, 1998). Then the leaves, petiole and roots were sectioned by a rotary microtome using the paraffin wax method at 10- 15µm thickness and a double stain (Safranin / Fast green) was applied for microscopic observation (**Dilcher, 1974**). The terminology concerning the mesophyll sorts was given according to data recorded in previous reports (**Fahn, 1974; Metcalfe & Chalk, 1979**; **Bancroft & Gamble, 2008)**.

Pollen grains were isolated using stereomicroscope from hydrated anthers to save them from shrinking and then dehydrated using an absolute alcohol. They were mounted on SEM stubs and Coted with nano-gold that was applied as a conductive material to enhance image quality and reduce charging effects **(Goldstein, *et al*., 2018)**.

Dry leaf fabric was disturbed in person lysing tubes with a globule process. DNA extraction was conducted following the method of **Doyle and Doyle (1990**), who used CTAB buffer with higher 1.4 M NaCl, added an extra chloroform extraction step, and purifyed DNA pellet using cesium chloride density gradient centrifugation. For getting high-quality DNA from previously dried tests, we utilized the QIAamp DNA Stool Scaled down Unit (from QIAGEN) taking after the manufacturer's instructions. Brief parts of particular districts of plastid DNA rbcL (Ribulose-bisphosphate carboxylase chain) arrangements were increased from the dried leaf extricates. Widespread groundworks were utilized coinciding with the study of **Kress *et al*. (2005)**. PCR intensification was conducted employing a standard (non-hot-start) DNA polymerase with roughly 20ng of genomic DNA as a layout in a 20-µL response blend (2µL 109

response buffer Dream TaqTM (Fermentas, Lithuania), 2 pM of each dNTP, 4 pM of each groundwork and 0.2 U Dream TaqTM DNA polymerase). Following the PCR convention, these successive steps were implemented: one cycle for 5min at 94°C; 35 cycles of 30s at 94°C; 30s at 55°C; 60s at 72°C, followed by 10min at 72°C. PCR items were filtered and coordinated sequentially utilizing BigDye Eliminator v3.1 on a 3100 sequencer (Connected Biosystems). Arrangements were adjusted by ClustalW **(Thompson *et al*. 1994)**.

Bioinformatic

Muscle arrangement was utilized to adjust the arrangements utilizing MEGA 11.0 computer program. Arrangement of divergences were calculated by Tamura 3-parameter show. To demonstrate the designs of species uniqueness, Tamura 3-parameter strategy was applied for N.J. trees **(Tamura, 1992)**. Bootstrapping was reached in MEGA 11.0 **(Kumar *et al*., 2004)** with 1000 replications. Visualization improvement was attained utilizing ITOL program **(Letunic and Bork 2021).**

RESULTS AND DISCUSSION

The observation of anatomical structure **(**Plate 1**)** revealed that the aerenchyma tissues were foung in all plant organs to facilitate floating. Aerenchyma tissue seems to be the main and dominant tissue in *Echhornia* organs, whereas in blade epidermis, it has low thickness, uniseriating with thick cuticle and superficial stomata distributed along both adaxial and abaxial surfaces, a finding that matches with that of **Herniwanti *et al*. (2014)**. Additionally, spongy parenchyma is distributed below the epidermis, followed by a parallel small collateral vascular bundles intermediated by a relatively large one resampling the midrib. Then, an aerenchyma tissue was observed forming a large air chamber **(Pereira *et al*., 2011)**. The petioles arc shaped with two long unequal protuberances. The adaxial epidermal cells uniseriating were detected with more thickness than the abaxial ones. This section was followed by 1-5 green thin parenchyma layers and an aerenchyma tissue penetrated by collateral vascular bundles in irregular way.

Similar to the rest of the plant, the rounded peduncle has a thin uniseriating epidermis covered with thick cuticle, while the ground tissue, the aerenchyma, forms large air chambers with irregular distributed collateral vascular bundles.

The adventitious root transection revealed that, the crashed young epidermis was followed by 4-5 layers of thin parenchyma in cortex, and then 6-7 cells of aerenchyma form large air chambers, followed by 6-7 layers of thin parenchyma, which enclosed a medium diagonal vascular bundle, with large xylem vessels. Furthermore, a small pith of thin parenchyma was observed.

Another structure found in the root system was similare to a tube bag used for water storage. The wall of this bag has outer and inner uniseriate epidermis. The outer epidermis is followed by 3-4 layers of thin parenchyma, and then a collateral vascular bundle is arranged in a ring and below them, 10-12 layers of thin parenchyma were detected. In addition, the function of storing water in the air bag of roots system is to maintain the balance for the plant body. In literature, water bags in the root system was assumed to filter water which would help plant survive in a sewage ecosystem (**Ehrenfeld, 2000**).

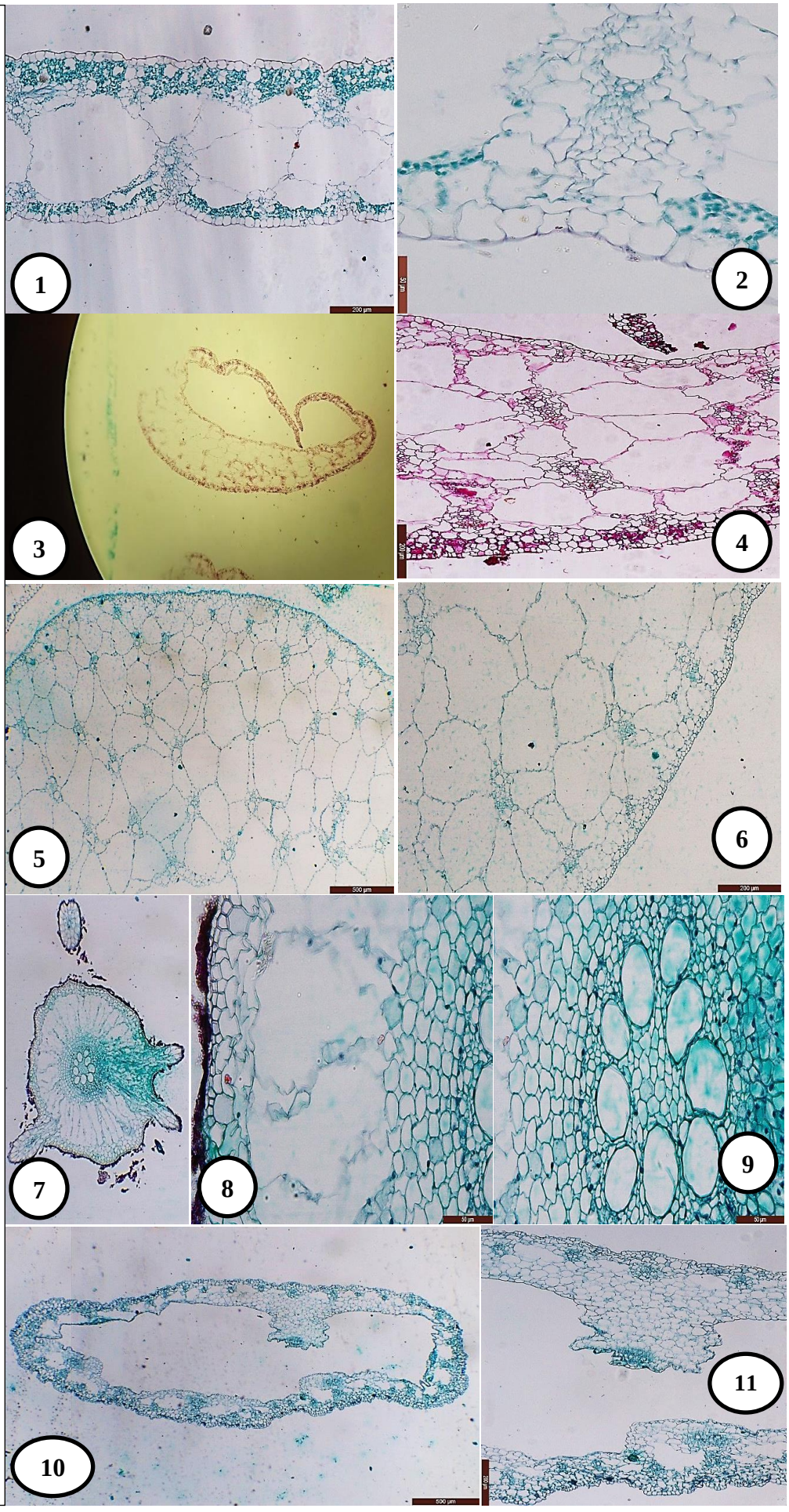
The current study assumed that the spread of aerenchyma tissues (besides flouting) decreases the fungal and bacterial infections as a strategy of resistance and eco-adaptation. In addition, lathery epidermis with the tensioned cuticle was suggested to enhance the penetration into the plant surface.

Pollen grains morphology showed their monads, heteropoler, radiosymetric, and large size (P-56/E-45µm), oblate shape, outlined polar view: elliptic, dry pollen infolding, disulcate and sunken. Ornamentation fossulate, perforate **(**Plate 2**)**.

Neighbor-Joining strategy found the formative history (**Saitou & Nei, 1987**). The ideal tree is apparent **(**Fig. 1**)**. The rate of reproducing trees which the related taxa clustered and organized within the bootstrap test (1000 duplicates) appeared as branches colored and evaluated concurring with the bootstrap esteem (**Felsenstein, 1985**). Tree is graphed to scale with department lengths within the same units as those of the developmental separations utilized to gather the phylogenetic tree **(**Plate 3**)**. The developmental separations were computed utilizing the Tamura 3-parameter strategy (**Tamura, 1992**), and were found to be within the units of the number of base substitutions per location. This investigation included 15 nucleotide arrangements (14 benefactor groupings were assessed from NCBI, and one arrangement acted as an outgroup). Codon positions included were 1st+2nd+3rd+Noncoding. All vague positions were evacuated for each sequence pair (pairwise erasure alternative). There was an add up of 509 positions within the last dataset. Developmental investigations were conducted in MEGA11 (**Tamura *et al.*, 2021**). Then, the visualization improvement was conducted utilizing ITOL program (**Letunic & Bork, 2021**).

Plate 1

Figs. 1-2 Leaf TS and main vascular bundle. Figs. 3-4 exhibite petiole outline with protuperance and vascular bundles distribution. Figs 5-6 rounded peduncle transection and vascular cylinder. Figs. 7-9 . main root structure with airenchyma tissue. Figs. 10-11 resamples a bag for water storage to keep plant balanced.



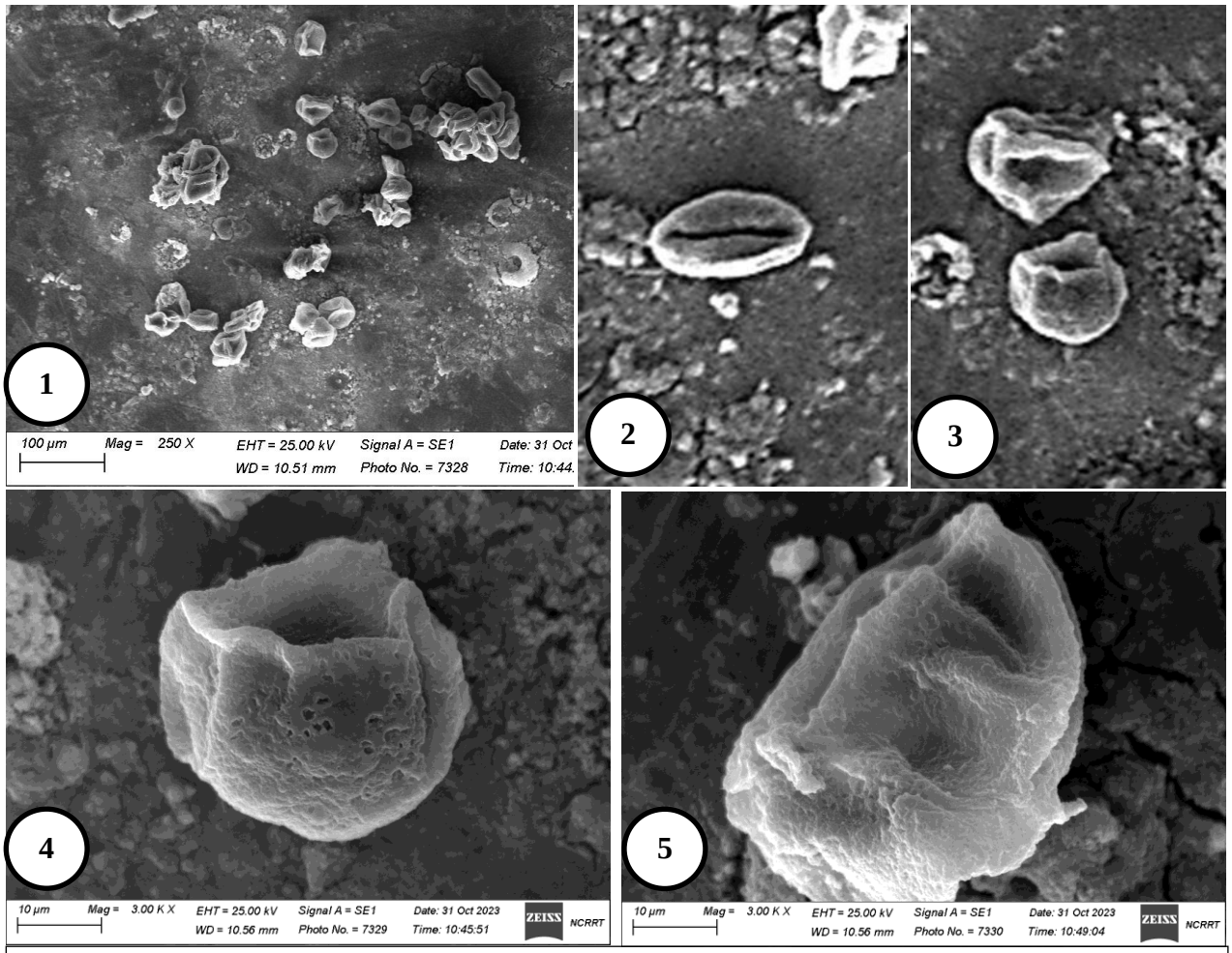


Plate 2: Echhornia crisspies pollen grains on Scanning Electron Microscope.

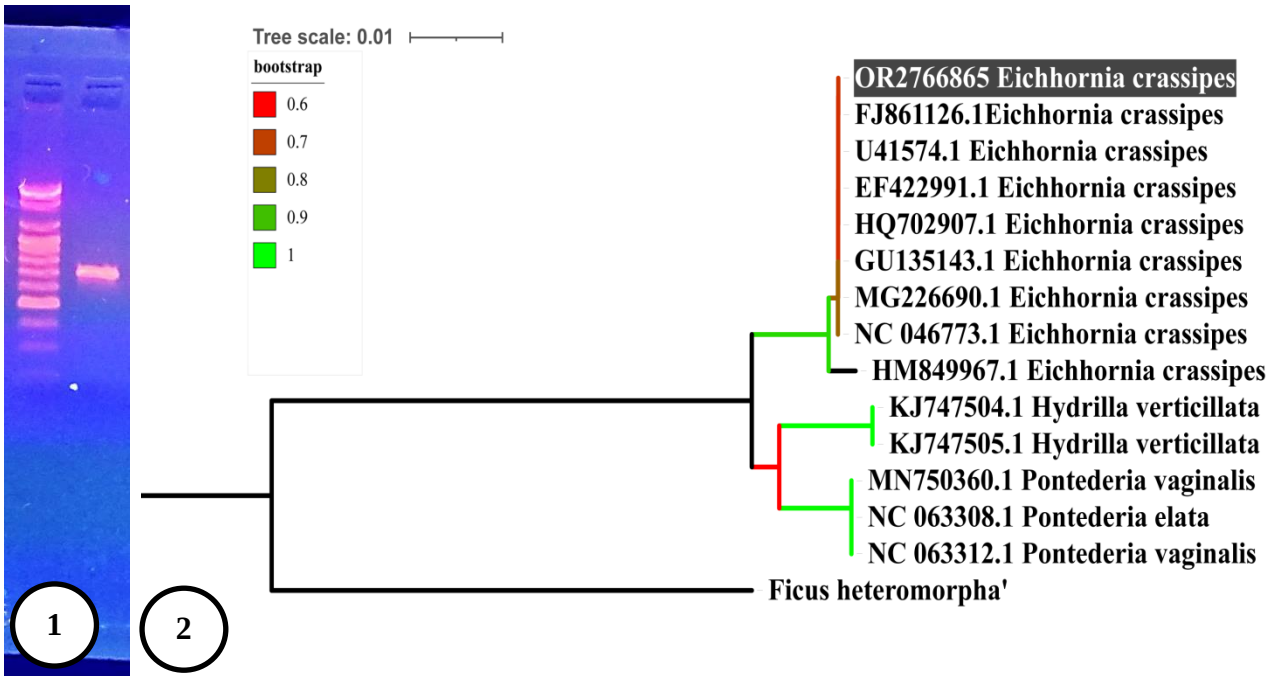


Plate 3: Fig. 1. *Echhornia crisspies* DNA gel documentation under UV. **Fig. 2.** Neighbor-joining phylogenetic evolutionary tree

CONCLUSION

The anatomical observation revealed that the aerinchyma tissue has important role in plant survival preventing the spread of infection in plant organs, in addition to keeping the plant balanced, as well as filtrating and storing water in the root. Pollen grains size, type, ornamentation and symmetry coincide with those recorded in literature, indicating that they were not affected by sewage environment. Additionally, DNA (rbcl) concurring with gen bank ensures plant identification and indicates the non-existance of molecular mutations associated with the sewage ecosystem. Thus, it is highly recommended that, aquatic plants, espicially those found in sewage, require more studies to understand how those plants survive from pollutants, microbial diseases, and infectious diseases of insects.

REFERENCES

Bancroft, J. D.and Gamble, M. (2008). Theory and Practice of Histological Techniques. Elsevier Health Sciences.

Doyle J.J. and Doyle J.L. (1990). Isolation of plant DNA from fresh tissue. Focus, 12**:** 13–15.

Ehrenfeld, J. G. (2000). Evaluating wetland functions. Wetlands, 20(4): 698-712.

El-Shafai, S. A.; El-Gohary, F. A. and Nasr, F. A. (2010). Phytoremediation of euent water from a domestic wastewater treatment plant using the floating macrophyte Eichhornia crassipes. Ecological Engineering, 36(12): 1689-1694.

Felsenstein J. (1985). Confidence limits on phylogenies: An approach using the bootstrap. Evolution 39:783-791.

Goldstein, J.;Newbury, D. E.; Echlin, P.; Joy D. C.; Lyman, C. E.; Lifshin, E. and Fiori, C. (2018). Scanning Electron Microscopy and X-Ray Microanalysis. Springer.

Herniwanti; Yanuwiadi ; ,Priatmadi J.B. and Soemarno (2014). Comparison of Characteristic Aquatic Local Plants for Phytoremediationwith Different Media of Acid Mine Drainage Passive Treatment. J. Appl. Environ. Biol. Sci., 4(3): 167-176.

Holm, L. G.; Plucknett, D. L.; Pancho, J. V. and Herberger, J. P. (1977). The World's Worst Weeds: Distribution and Biology. University Press of Hawaii.

Hossain, M. S.; Rahman, M. A., Rahman; M. M., Uddin, M. J. and Islam, M. S. (2008). Ecological, social and economic aspects of Eichhornia crassipes: a review. Research Journal of Agriculture and Biological Sciences, 4(2): 139-145.

Kress, W. J.; Wurdack, K. J.; Zimmer, E. A.; Weigt, L. A. and Janzen, D. H. (2005). Use of DNA barcodes to identify flowering plants. Proceedings of the National Academy of Sciences of the United States of America, 102(23): 8369-8374.

Nico, L. G.; Williams, J. D. and Jelks, H. L. (2009). Black Carp: Biological Synopsis and Risk Assessment of an Introduced Fish. American Fisheries Society.

Pereira, F. J.; Franca, F. and Ramos, G.(2011). Morpho-anatomical studies of Eichhornia crassipes (Mart.) Solms. Brazilian Journal of Biology. Doi 10.1590/S1519-69842011000100023.

Rajasulochana, P. and Preethy, V. (2010). Comparison on efficiency of various techniques in treatment of tannery effluent. Global Journal of Environmental Research, 4(3): 127-135.

Saitou N. and Nei M. (1987). The neighbor-joining method: A new method for reconstructing phylogenetic trees. Molecular Biology and Evolution 4: 406-425.

Sculthorpe, C. D. (1967). The Biology of Aquatic Vascular Plants. St. Martin's Press.

Tamura K. (1992). Estimation of the number of nucleotide substitutions when there are strong transition-transversion and G + C-content biases. Molecular Biology and Evolution 9: 678-687.

Tamura K.; Stecher G. and Kumar S. (2021). MEGA 11: Molecular Evolutionary Genetics Analysis Version 11. Molecular Biology and Evolution https://doi.org/10.1093/molbev/msab120

WFO, (2023). The World Flora Online. https://www.worldfloraonline.org/taxon/wfo-4000030906.

Zheng, Z.; Cai, T.; Yao, L. and Zeng, G. (2008). Phytoremediation of water polluted with tannery euent by water hyacinth (Eichhornia crassipes). CLEAN - Soil, Air, Water, 36(11): 866-872.