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"A comparative study ofthree species of Lemnaceae (*Lemna minor,L. Gibba* and *Spirodela polyrhiza)*"

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ABSTRACT:

As a result certain economically productive traits like vigorous biomass production and high growth pattern, species of lemnaceae are turning out to be appealing for plant science researchers as well as for commercialists. Hence, the correct identification of the species being used for different duckweed research and applications is becoming indispensable. Here, we present an updated comparative study based general characteristics of three species of duckweeds (*Lemna minor*, *Lemna gibba* and *Spirodela polyrhiza*). Lemnoideae is a subfamily of flowering aquatic plants known as duckweeds, water lentils, or water lenses. They are surface or sub surface floating acquatic plants and usually ponder slow-moving water bodies. They usually appear to be thallophyts but astonishingly these belong to Phanerogams. This study proved that the morphological structures are significant in delimiting the three species of Lemnaceae. But the significance of molecular characterization arises where the delimitation becomes hactic as a result of less differences in their morphological characters. Moreover, extensived study is required to fill gaps in our knowledge and to resolve the taxonomic limitations of the species of Lemnaceae family and to develop means and alternatives for processing, management and disposal or reuse of the harvested biomass in order to make the system sustainable and economically feasible for the community.

Keywords: *Lemna minor, Lemna gibba, Spirodela*

INTRODUCTION:

Duckweed are surface or subsurface floating phanerogamous plants having rapid growth and propagation strategies (Skillicorn, Spira, and Journey, 1993). They belong to monocot group and usually ponder the slow moving waters. *Wolffia angusta* represents the smallest flowering plant (Bog et al.,2013). They also show rapid growing pattern (Hicks, 1937). They are called the most mysterious of the flowering plants on the Earth. Because of their small sizes they sometimes remain elusive from casual observation. They are often forming dense, homogeneous clonal populations. Vigorous growth pattern makes them successful colonizers (Bonomo et al 1997, J. Xu, et al., 2011). The plant body of Lemnaceae members are not differentiated into a stem or leaf. It is reduced to a fleshy or thallus like ovoid or flattened structure bearing one-several roots (without root hairs) on the underside, or rootless. Their leaves are fused to form the so called “fronds”. They are easily identified by these fronds. Duckweeds are complex group of vascular plants that had been placed in the family lemnaceae represented by 40 species and 5 genera (Skillicorn,et al., 1993; Lyerly, 2004; Michael et al., 2008). But their structural complexity posed questions regarding their systematic position as a result they have been placed in the sub family lemnoideae of the family araceae based on information provided by chloroplast DNA studies (Duvall, et al., 1993). Wolfia has most advanced traits while, *Spirodela* is the most ancestral. Extensive

comperative study in almost every field of biology (physiological biochemical cytological and molecular study) confirmed their position in the family araceae (Campbell et al., 2002). Duckweeds are natural nutrient removals. Duckweeds could be used to rectify wastewater from municipal and industrial sources. They appear to have economically very fascinating traits in order to get maximum out of them a thorough comperative study becomes necessary. Allozyme studies have been independently by (Crawford and Landolt 1993, 1995; Crawford et al. 1995, 1996, 1997), (Landolt 1986; Jordan et al. 1996), (Les et al. 1997). Keeping these things in view, the study of three different species of the family Lemnaceae which were found in the different lakes of the Srinagar City of Jammu and Kashmir, was done in order to establish the main differences between these species so that there is ease to identify them on the basis of morphological basis.

The aim of this research is to study the characteristic features of the different species of the family Lemnaceae and delimit the same on the basis of morphological and molecular characterization. Main aim is to determine characteristics of the species on the basis of their observed morphological features and distribution pattern

MATERIALS AND METHOD:

1. **Survey and collection**

1. A study on the different species of the family Lemnaceae throughout the different lakes of Srinagar was carried out. A total of 3 species belonging to the same family were collected and identified. Plant species were collected as systematically as possible from the study area which include the banks of Dal lake, Nigeen lake, Gilsar lake and Anchar lake.

2. **DNA Extraction**

The DNA from each species was isolated using CTAB metod.

Table 3.Composition Of Different Constituents for DNA Extraction 1M Tris (pH 8.0)

DNA Extraction	**DNA Extraction**
The DNA from each species was isolated using CTAB metod	

M EDTA (pH 8.0)

E D T A	1 8 6 . 12 g/ l
pH adjusted with 1N NaOH, final volume made to 1 litre and then autoclaved	

1X TBE

Tris base	108 g/l
Boric acid	55 g/l
0.5M EDTA pH(8.0)	40 ml/l
Make final volume with MQ water and then autoclave	

DNA loading dye (10 ml)

Glycerol	50%
Bromophenol blue	0.25%
Xylene cyanol	0.25%
MilliQ water	7 ml

Ethidium bromide

Ethidium bromide	10 mg
MQ water	1 ml

Agarose Gel (0.8%)

Agarose	8mg
1X TBE	50ml
Ethidium bromide	1.25□l
Add EtBr after homogenizing by heating, then cool and pour	

Electrophoresis buffer (500ml)

5X TBE	50ml
MQ water	450 ml

CTAB buffer

CTAB	20 g/l
1M Tris (pH-8)	100 ml/l
5M NaCl	280 ml/l
0.5M EDTA	40 ml/l
β mercaptoethanol	2 ml/1
Polyvinylpyrrolidone	10 ml/l

Add MilliQ to make final volume of solution equal to 1 litre and sterilize by autoclaving (optional). Moreover, β mercaptoethanol is added just before use.

3. Purity Determination

To determine purity of the DNA isolated from the above method. 260/280 ratio for DNA was carried out. 260/280 ratio is the primary measure to assess the purity of DNA/RNA. From this ratio the purity of the DNA isolated above was confirmed. The value was around 1.8.

4. Agarose gel electrophoresis

Agarose Gel Electrophoresis is a technique used very often by scientiststoseparate a mixed population of DNA molecules. The separated DNA may be viewed with stain (EtBr), most commonly under UV light, and the DNA fragments can be extracted from the gel with relative ease. Most agarose gels are made with between 0.7 % (good separation or resolution of large 5–10 kb DNA fragments) and 2 % (good resolution for small 0.2–1 kb fragments) agarose dissolved in electrophoresis buffer (TBE or TAE). Up to 3 % can be used for separating very tiny fragments. 1 % gels are more common for many applications.

Composition Of Different Constituents for PCR

Component	Stock concentration	Working concentration	Volume
Buffer(PH 7-8)	10 X	1 X	2 µl
Magnesium chloride*	50 Mm	1.5 mM	0.5 µl
dNTPs**	2 Mm	0.2 mM	2 µl
Primers (For & Rev)	10 µM	0.5 µM	1 µl
Template	-	-	1 µl
Taq polymerase***	-	1.25 units	0.2 µl
MilliQ	-	-	13.3 µl
Total	-	-	20 µl

*Sometimes $Mgcl_2$ is added in the buffer itself so no need to add separately. Moreover if the concentration of $Mgcl_2$ is 25 mM then double volume has to be taken

**Normal dNTPs comes as 10 mMdNTPs stock, so it has to be converted to 2mM using formula

$M_1V_1=M_2V_2$

***One enzyme unit is that amount of enzyme which fixes 10 nano moles of dNTPs to the DNA in 30 minutes of time at 72(74 to 75 C)

Results:

1. **Results of Morphological Studies:** The three species collected from different lakes of Srinagar were examined morphologically with naked eye and under magnification. The results thus obtained are given below:

a) ***Lemna minor*** **:**

Roots: One root, this rootlet is slender and white with a tip that is usually obtuse. At the base of the rootlet, there is a short cylindrical sheath.

Shape of Plant Body: Flattened, suborbicular to elliptic-obovate in outline, generally symmetrical; dorsal surface smooth (without prominent papules or median ridge), slightly succulent texture and smooth margins; numerous tiny air bubbles that are imbedded within its interior. The upper thallus surface is medium green and slightly convex along a faint longitudinal ridge; the lower thallus surface is light green and flat. Both surfaces are glabrous

Size: 2-4 mm long.

Veins: 3 interior veins are usually visible (a central vein and 2 lateral veins) within the body of the thallus.

b) *Lemnagibba* :

Roots: Single root that is much longer than that of roots of *Lemna minor*.

Shape of Plant Body: It has a simple body that is flattened, orbicular-ovate in outline, often asymmetrical (oblique) at apex; dorsal surface with slight median ridge without distinct papules; ventral side often conspicuously inflated-gibbous which is characteristics of this species ,distinct, large air spaces (often larger than 0.3 mm) and bordered (outlined) in reddish anthocyanin.

Size: 3-5 (6) mm long. **Veins:** 3 (sometimes 5).

c) *Spirodelapolyrhiza* :

Roots: 5 (7)-16 (rarely more) arising from the greatly thickened part of the frond; root cap acute.

Shape of Plant Body: It is oval, broadly obovate, or orbicular in shape and its outer margin is smooth. The texture of the thallus is slightly succulent and it is filled with minute pockets of air, enabling it to float. The upper thallus surface is light to medium green, while the lower thallus surface is usually purplish red (rarely light green); both surfaces are glabrous and nearly flat. Toward one side of the upper surface of each thallus, there is a single node that is often red. . Fronds are solitary or cohering in groups of 2-5, symmetric or asymmetric, 4-10 x 3-8 mm, orbicular-ovate, base obtuse, apex obtuse or rounded; nerves 5-11, conspicuous; upper side flat, smooth, green, lower side flat, red-purple.

Size: : 3-9 mm. long and 2.5-7 mm. across.

Veins: About 5-12 veins originate from this node, curving inward.

Table 5. A comparison of morphological features between *Spirodelapolyrhiza*, *Lemna minor* and *Lemnagibba*.

MORPHOLOGICAL CARACTERS	***Spirodelapolyrhiza***	***Lemna minor***	***Lemnagibba***
Prophyllum at the base of frond	Present	Absent	Absent
No. of roots	7 to 21	2-3	1
No. of veins in frond	7 to 16	3	3 sometimes 5
Root Tracheids	Present	Absent	Absent
Size	10mm	2-4mm	3-5mm
Arrangement of clonal	Solitary or 2-5 connected	Solitary to several(3-5)	Solitary or several
Clusters		Attached	
Budding Pouch Position	2 lateral pouches on either side of basal end	2 lateral pouches on either side of basal end	2 lateral pouches either side of basal End

2. Results of Distributional Studies

These three species of Lemnaceaei.e, *Lemna minor, Lemnagibba and Spirodelapolyrhiza*werecollected from four different lakes of Srinagar. These lakes have different growing conditions in relation with their nutrient constituents. Out of the four lakes (Dal lake, Nigeen lake, Gilsar and Anchar lake), Dal lake has more sewage discharge. *Lemna minor* was found in abundance in the banks of Dal lake, followed by *Spirodelapolyrhiza*and then *Lemnagibba*. Along the banks of Nigeen also sewage discharge from the populations growing in the house boats near this lake is responsible for the addition of the nutrients in the water of this lake. Here, only *Lemna minor* was seen to be growing. But in other Gilsar, *Spirodelapolyrhiza*was seen to grow in large number and other two species were not found densely. These three species have tended to invade the banks of these lakes.*Spirodelapolyrhiza* was not seen to grow in Ancharlake. However in Anchar, *Lemnagibba* was found to grow in large numbers.

Table 6. Dimensions *of Lemna minor, Lemnagibba*and *Spirodelapolyrhiza*found in four lakes of Srinagar City.

1. ***Lemna minor***

Site	**Dimensions of leaf(cm)**	**Dimensions of root(cm)**
Dal Lake	0.1,0.2,0.3	1.5,2.1,2.5
Nigeen Lake	0.1,0.2,0.4	1.2,1.5,2.3
Gilser Lake	0.2,0.4,0.5	2.5,2.6,3
Anchar Lake	0.1,0.3,0.5	2.0,2.5,2.6

3. ***Lemnagibba***

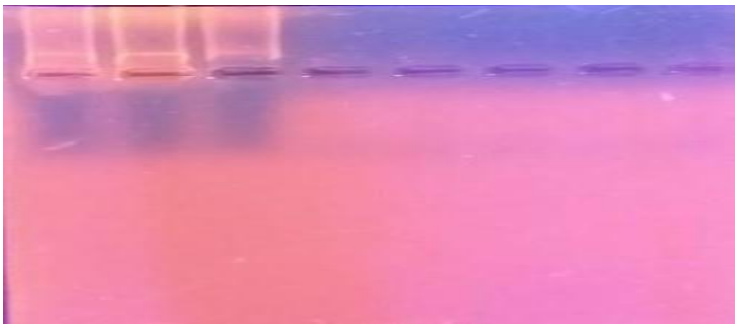
Site	**Dimensions of leaf(cm)**	**Dimensions of root(cm)**
Dal Lake	0.1,0.3,0.4	4.4,5.8,6.7
Nigeen Lake	0.4,0.5,0.7	4.2,4.6,4.7
Gilser Lake	0.1,0.3,0.6	3.3,3.4,5.1
Anchar Lake	0.1,0.2,0.3	2.1,3.4,4.9

4. ***Spirodelapolyrhiza***

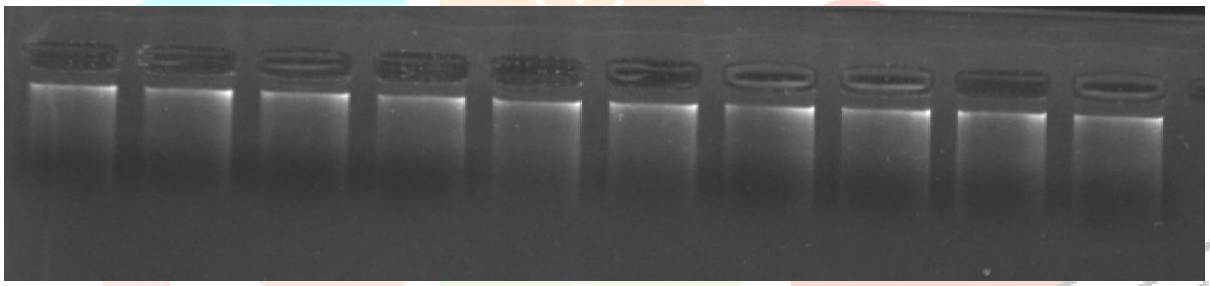
Site	**Dimensions of leaf(cm)**	**Dimensions of root(cm)**
Dal Lake	1.6,1.7,1.8	2.9,4.1,5.6
Nigeen Lake	1.3,1.5 ,1.6	2.8,3.9,4.0
Gilser Lake	1.8,1.9,2.0	2.6,3.2,3.3
Anchar Lake	Not Found	Not Found

2. Results of Molecular studies (Isolation, purification and analysis of genomic DNA)

For molecular characterization of these three species of the family Lemnaceae, samples were collected from each study site and were pooled and used for DNA extraction. The purity and integrity of the community genomic DNA was checked on 0.8% agarose gel. The gel representations are provided below:



Representative gelfor genomic DNA of different species of family Lemnaceae loaded on 0.8% Agarose



Representative gel for PCR products of different species of family Lemnaceae loaded on 1% Agarose gel

CONCLUSION:

Lemnaceae represents the smallest flowering species. They are truly aquatic. The different species present in this family resemble each other to large extents and therefore are very difficuilt to distinguish from each other merely on morphological basis. Fundamental taxonomic problems such as basic species delimitation in a morphologically problematic group such as Lemnaceae, must be reconciled before systematic work can proceed effectively. The species were subjected to several studies, but the taxonomic delimitation of the species of Lemnaceae is still not satisfactorily resolved and there is still much disagreement among botanists.Several different proposals for the classification of these species have been put forward from time to time. First this family was placed together with Aracaceae and Cyclanthaceae in Arales. Many taxonomists supported its helobiae origin. Later, most of the phylogenists agreed that the family represents the offshoot of the Aracaceae with origins probably from Pistia or ancestral stocks of close affinity. Much recently, the works of many scientists represented an effort to supplement available taxonomic criteria by the use of comparative chemical data concerning secondary metabolites and molecular data using molecular markers. But it was never so easy. From time to time taxonomists faced difficulties in delimiting these species. Species identification present obstacles to almost every systematic study. Duckweed identification was extremely

difficult and it was necessary to demonstrate the previous works done on phylogeny before proceeding. In this way we are able to reasonably certain that our results would not be faulted by comparisons of data obtained from specimens that were identified inaccurately. In this study, we report the results of a phylogenetic analysis of the three species of Lemnaceae that is based upon the consideration of characters derived from molecular or non-molecular data. Our results were in accordance with the previous studies on phylogenetic analysis of the three species of Lemnaceae (*Lemna minor*, *Lemnagibba*and*Spirodelapolyrhiza*).

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