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THE DISTINCTION BETWEEN LEMNA GIBBA L. AND LEMNA MINOR L. ON THE BASIS OF VEGETATIVE CHARACTERS

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SUMMARY

Specific identification of flat forms of the *Lemna gibba/L. minor* complex on morphological grounds appears to be possible only when the dimensions are $> 5.0 \times 3.5$ mm or/and the number of veins is > 5 (*L. gibba*), or when the dimensions are $< 3.0 \times 2.0$ mm, the number of veins 3 and/or the width/length ratio < 0.60 (*L. minor*). A consistent diagnostic character is the ability of *L. gibba* of turning gibbous when cultivated on EDDHA containing medium.

1. INTRODUCTION

The segregation of sterile specimens of the flat form of *Lemna gibba* from *L. minor* is still a problem (see, e.g., DE LANGE & SEGAL 1968; DE LANGE & PIETERSE 1973; DE LANGE 1974; LANDOLT 1975; PIETERSE 1975).

DE LANGE (1975) has surveyed various diagnostic characters mentioned in literature and pointed out their inconsistencies. The principal vegetative criteria proposed are: a reticulate structure visible when the fronds are viewed in transmitted light; the size and shape of the fronds; the number of veins; the relative length of the budding pouches and, more recently, the branching of one of the lateral veins (LANDOLT 1975). By taking the capacity of obtaining a gibbous shape as a distinguishing feature, and by assessing it by cultivation in a medium to which the chelating agent EDDHA (ethylenediamine-di-o-hydroxyphenylacetic acid) is added (PIETERSE et al. 1970, DE LANGE & PIETERSE 1973), the consistency of these vegetative morphological characters has been put to the test.

2. MATERIAL AND METHODS

Forty strains of the *Lemna gibba/L. minor* complex, comprising 18 strains studied by DE LANGE & PIETERSE (1973), 16 newly collected strains, and 6 kindly made available by Prof. E. LANDOLT (Zürich), were included in the investigation (see *table 1*). Of all strains clones were reared on M-medium with 1% sucrose and 10 ppm EDDHA (DE LANGE & PIETERSE 1973, under the same conditions of temperature and illumination as stated there). After a few days the tendency towards gibbosity, if present, became noticeable. Then all strains were transferred to Hutner-medium (HUTNER 1953) diluted to 1/3 of the standard concentration. The gibbous strains turned flat again with the exception of the strain G-3. This strain, and the strains 15 and E 9 (which had died off in the diluted

Table 1. Place of origin, reaction on EDDHA and dimensions of the *Lemna*-strains

Strain no.	Place of origin	+	*	Length (mm) mean	max.	min.	Width (mm) mean	max.	min.	Width/Length mean	max.	min.	nr. of nerves
A. Strains determined as L. minor (remaining flat when cultured with EDDHA)													
1	Holland, Naardermeer	D&P	E	2.1	2.2	2.0	1.5	1.6	1.3	0.69	0.75	0.59	3
3	Holland, Hilversum	D&P	E	3.4	3.5	3.2	2.3	2.5	2.0	0.69	0.71	0.63	3
7	Holland, Aerdenhout	D&P	E	3.7	4.5	3.5	2.4	3.0	2.0	0.65	0.80	0.57	3(4)
8	Holland, Aerdenhout	D&P	E	3.6	4.0	3.5	2.6	2.8	2.5	0.71	0.71	0.70	3
11	Holland, Bennebroek	D&P	E	3.2	3.5	3.0	2.3	2.1	2.5	0.71	0.73	0.69	3
12	Holland, Westzijderveld	D&P	E	3.4	3.8	3.0	2.4	2.5	2.0	0.69	0.71	0.66	3(4)
14	Holland, Harmelen	D&P	E	2.9	3.0	2.5	1.9	2.2	1.5	0.67	0.73	0.60	3
F	Laboratory strain	D&P	E	2.9	3.0	2.5	1.9	2.0	1.5	0.64	0.67	0.60	3
Isr.	Israël	D&P	E	3.3	3.5	3.0	2.3	2.5	2.0	0.69	0.71	0.67	3
6580	U.S.A., New Jersy, Harrington	D&P	E	3.3	3.5	3.0	2.1	2.5	2.0	0.65	0.71	0.57	3(4)
6613	Laboratory strain	D&P	E	2.6	3.8	2.2	1.8	2.0	1.5	0.69	0.81	0.54	3
6579	Denmark, Copenhagen	D&P	E	3.2	3.5	3.0	2.0	2.0	1.8	0.62	0.67	0.57	3
6573[1]	U.S.A., Montana, Lincoln	D&P	E	2.9	3.0	2.5	2.0	2.0	1.8	0.68	0.72	0.67	3
7407	Poland, Bialowieza	L	H	3.8	4.0	3.5	2.2	2.5	2.0	0.60	0.63	0.53	3–5
			E	3.0	3.2	3.0	2.0	2.2	2.0	0.67	0.69	0.63	3
6570	U.S.A., Washington, Davenport	L	H	2.9	3.2	2.5	2.0	2.2	2.0	0.71	0.80	0.67	3
			E	3.0	3.2	2.8	1.9	2.0	1.8	0.65	0.70	0.60	3(4)
7283	Libanon, Ammiq	L	E	4.4	4.5	3.8	2.5	3.0	2.0	0.58	0.67	0.53	5
6591	U.S.A., Cal., Escalon	L	E	3.9	4.2	3.5	2.0	2.2	2.0	0.53	0.57	0.48	3(4)
			E	3.0	3.5	2.5	1.8	2.0	1.5	0.60	0.63	0.57	3
8211	U.S.A., Dakota, Split Rock River	L	H	4.4	5.0	4.0	2.9	3.2	2.5	0.67	0.71	0.60	4,5
			E	3.8	4.0	3.6	2.6	2.8	2.5	0.67	0.78	0.63	3,4(5)
7436	U.S.S.R., Lake Onega	L	H	3.5	3.6	3.2	2.1	2.2	2.0	0.62	0.66	0.57	3–5
			E	3.8	4.0	3.5	2.6	2.8	2.4	0.68	0.74	0.63	3–5
E3	Holland, Schermer	W	E	3.5	3.8	3.2	2.6	2.8	2.4	0.74	0.80	0.71	3
E4	Holland, Schermer	W	H	3.0	3.2	2.8	2.0	2.2	2.0	0.68	0.71	0.67	3(4)
			E	3.6	3.8	3.5	2.4	2.5	2.2	0.67	0.71	0.58	4
E5	Holland, Schermer	W	H	3.0	3.0	2.0	2.0	2.2	1.8	0.67	0.73	0.60	3,4
			E	3.8	4.0	3.5	2.6	2.8	2.5	0.69	0.76	0.63	4,5

E6	Holland, Schermer	W	E	2.3	2.5	2.1	1.8	1.9	1.6	0.80	0.86	0.76	3
E7	Holland, Z.O. Beemster	W	E	3.8	4.0	3.5	2.6	2.8	2.2	0.67	0.74	0.58	3
E8	Holland, Ilpendam	W	E	3.8	4.0	3.5	2.7	3.0	2.5	0.72	0.80	0.63	3
E10	Holland, N.W. Overijssel	D	E	3.0	3.1	3.0	2.0	2.0	2.0	0.67	0.67	0.65	3
E11	Holland, Drente	W	E	3.2	3.5	3.0	2.2	2.5	2.0	0.70	0.76	0.67	3
E12	Holland, N.W. Overijssel	D	E	4.2	5.0	3.5	3.0	3.5	2.5	0.72	0.75	0.70	3
E13	Holland, N.W. Overijssel	D	E	3.7	4.0	3.5	2.4	3.0	2.0	0.66	0.79	0.57	3
E16	Germany, München	D	H	3.8	4.0	3.2	2.6	3.0	2.2	0.69	0.79	0.63	3,4
			E	3.7	4.0	3.5	2.2	2.5	2.0	0.60	0.66	0.55	4
B. Strains determined as L. gibba (gibbous when cultured with EDDHA)													
2	Holland, Hilversum	D&P	H	4.2	4.4	4.0	3.0	3.2	2.8	0.74	0.76	0.70	5
			E	4.9	5.2	4.5	3.6	4.0	3.0	0.74	0.87	0.67	5
5	Holland, Spaarndam	D&P	H	4.5	5.0	4.2	3.2	3.5	3.0	0.71	0.78	0.67	5(6,7)
			E	5.6	6.0	5.0	4.3	4.8	4.0	0.76	0.86	0.70	5
6	Holland, Hilversum	D&P	H	3.4	3.8	3.0	2.5	2.8	2.0	0.74	0.93	0.63	5
			E	4.3	5.0	3.5	3.5	4.0	2.5	0.82	1.00	0.71	5
15	Holland, Woerden	D&P	E	4.8	5.0	4.3	3.5	4.0	3.0	0.73	0.80	0.69	5
G3	Laboratory strain	D&P	H	4.0	5.0	3.5	3.2	3.8	2.8	0.79	0.90	0.60	5
			E	4.7	5.0	4.5	3.5	3.5	3.5	0.77	0.88	0.70	5
E1	Holland, Groet	W	H	4.7	5.2	4.5	3.9	4.5	3.5	0.82	0.87	0.78	5(7)
			E	4.6	5.0	3.8	3.6	4.0	2.5	0.78	0.90	0.66	5
E2	Holland, Burgervlotbrug	W	H	4.7	5.0	4.5	3.6	4.0	3.0	0.76	0.84	0.67	5
			E	5.3	5.8	5.0	4.3	4.8	4.0	0.82	0.84	0.80	5
E9	Holland, Monnickendam	W	E	4.4	4.5	4.2	3.7	4.0	3.5	0.83	0.89	0.78	5
E15	Holland, Nijmegen, Dukenburg	W	H	3.9	4.2	3.0	2.9	3.2	2.5	0.75	0.83	0.67	5
			E	3.4	3.8	3.0	2.5	2.8	2.0	0.73	0.83	0.67	5(4)

+ Collection, * Culture medium, D&P = De Lange & Pieterse, L = Landolt, D = De Lange, W = Westinga, E = EDDHA, H = Hutner.

[1] *Lemna turionifera sensu* Landolt (1975).

Hutner-medium) were examined for their morphological characteristics in the gibbous, the other ones in the flat condition. Of each clone 20–50 individual specimens were treated, as a preliminary to an examination of the vasculature, according to Landolt (priv. comm.), as follows: boiling in 70% ethanol for 2 min, bleaching in a technical NaClO solution for about 30 min, rinsing in distilled water twice, and finally staining with Grenacher's carmine alumn overnight followed by a double rinse in distilled water. Of the 30 to 50 largest dyed fronds the number of veins, the mode of vein branching, the relative length of the budding pouches and the shape and size of the fronds (using a sliding gauge, accuracy 0.1 mm) were recorded.

3. RESULTS

The results are tabulated in *table 1* and can be summarised as follows:

a) the potency to become gibbous is in general correlated with the presence of 5 veins, at least in well-developed fronds.
   In exceptional cases 4, 6 or 7 veins occurred;
b) the fronds of strains that remained flat under EDDHA treatment had mostly 3 veins, occasionally 4 or 5;
c) a bifurcation of one of the lateral veins was only noted once (*fig. 1*);
d) *L. minor* was, generally speaking, smaller than *L. gibba* and had a narrower form of the fronds.

Frequence diagrams made of the mean length, width and width/length ratios per clone (*figs. 2–4*) indicate a broad range of overlap regarding these dimensions. This range was for mean values per strain: length 3.4–4.4 mm, width 2.5–3.0 mm and width/length ratio 0.71–0.80. For incidental records these ranges of overlap were, respectively, 3.0–5.0 mm, 2.0–3.5 mm and 0.60–0.86, the total ranges in the material studied being 2.0–6.0 mm, 1.3–4.8 mm and 0.48–1.00, respectively.

The relative length of the budding pouches appeared to vary to such an extent within a clone (from 1/3 to 2/3 of the length of the frond) that it was considered useless to record it. Asymmetry of the fronds proved to be linked with an even number of veins: around the 4th or 6th vein the frond was broadened at that side (*fig. 1*). A separate feature tabulation was, therefore, unnecessary. The mesh-width and discernibility of the reticulate pattern in transmitted light appeared to be variable characters within the clones, and therefore, considered to be unreliable.

4. DISCUSSION

The proposed discriminating criterium for the flat modification of *L. gibba* and *L. minor*, viz., the presence or absence of a capacity to become gibbous under the influence of EDDHA, appears to be satisfactorily reproduceable, the results of the present investigation agreeing fully with the findings of DE LANGE & PIETERSE (1973).

In *table 2* the values for the dimensions of the fronds found in the present

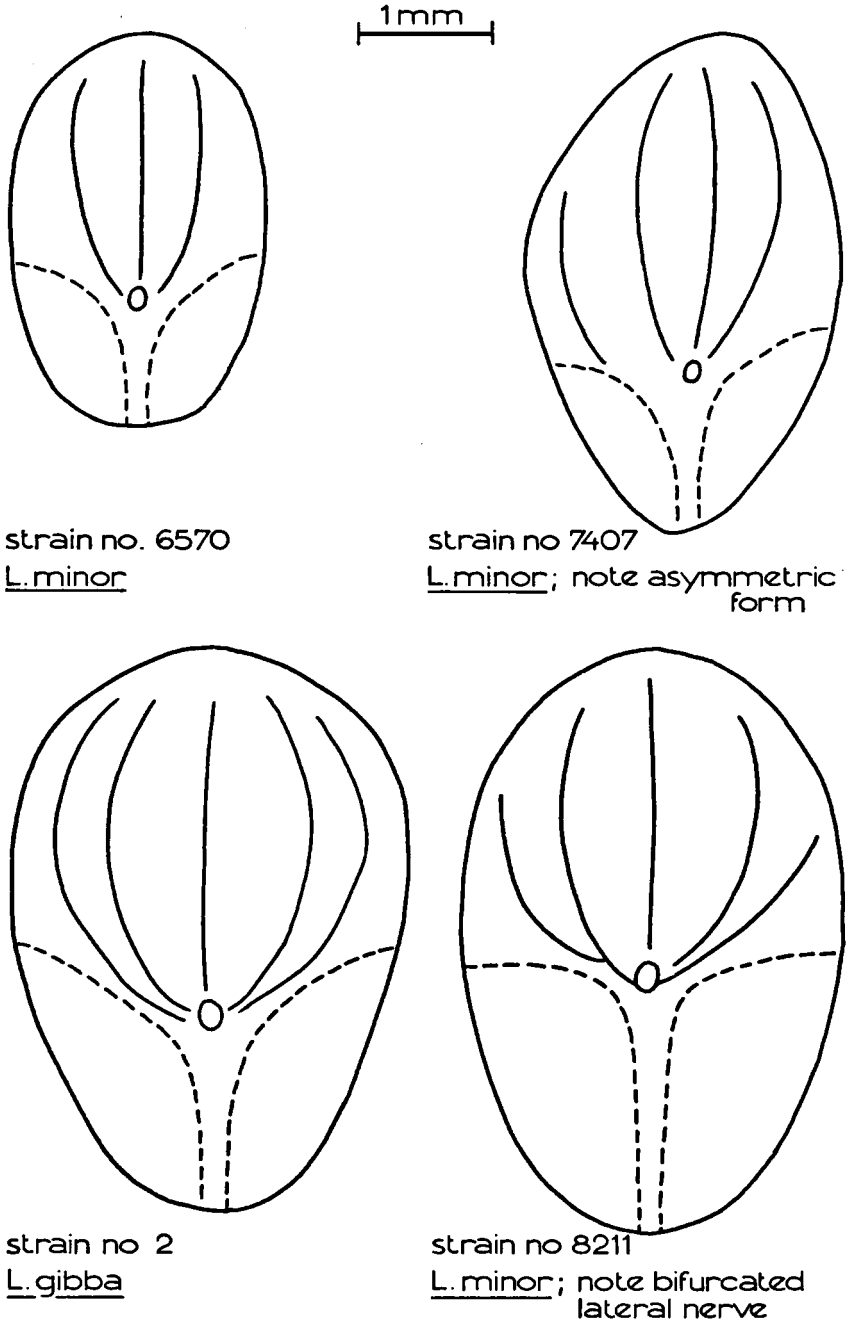

Fig. 1. Different forms of innervation in *Lemna*-strains.

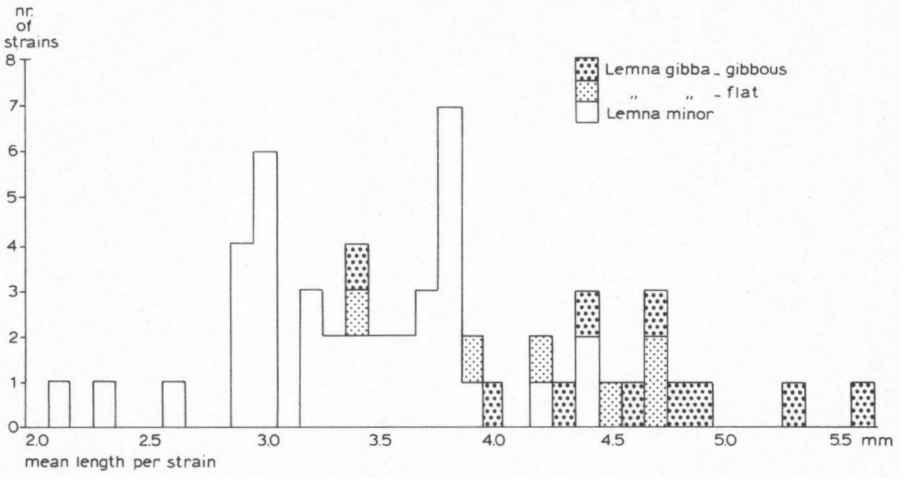

Fig. 2. Distribution of the mean length per strain.

investigation are shown next to previously reported values. The deviations in these data are conceivably attributable to the mistaken identification of flat modifications of *L. gibba* as *L. minor* (in the cases of high records of "L. minor" entries) and to the measuring of juvenile specimens of *L. gibba*, resulting in exceptionally low values.

The visibility of a reticulate pattern as a diagnostic character for *L. gibba* (cf. DE LANGE & SEGAL 1968) appears to be inconsistent, the same holds for the relative length of the budding pouches and the (a)symmetry of the frond. The number of veins may be indicative outside the range of overlap of 4 or 5 veins.

The branching of a lateral vein in *L. minor* (cf. LANDOLT 1975) seems to be inadequate for the recognition of the species.

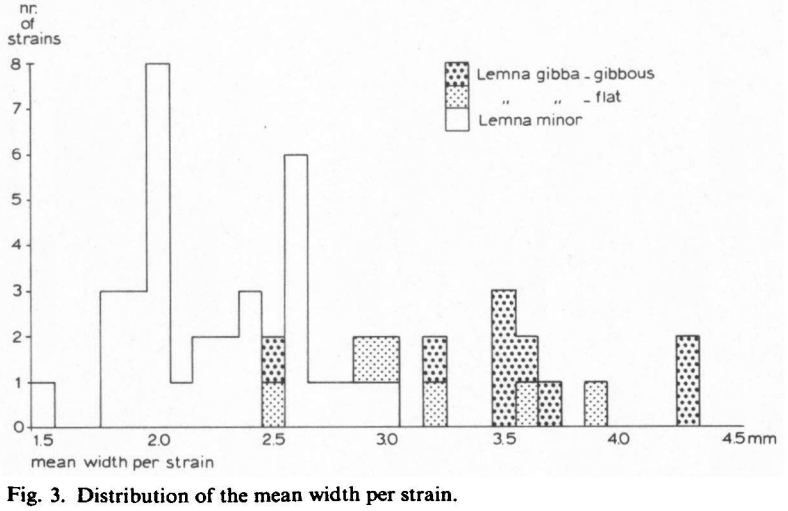

Fig. 3. Distribution of the mean width per strain.

Table 2. Data on dimensions in literature as compared with the results of the present investigation (between () the data for the mean values per clone)

	L. minor		*L. gibba*	
	literature	*pres. invest.*	*literature*	*pres. invest.*
length (mm)	3.0–6.0[a]; max. 5.7[b]; max. 6.0[c]; 2.0–2.5[d]; 2.2–2.8[e]; 2–4[f] 2–3.5(–5)[g]; 2.4–5.0 (2.6–4.6)[h]; 2.5 ± 0.26[i]	2.0–5.0 (2.1–4.4)	3.5–6.0[a]; max. 6.0[b]; 1–5.5[c]; 2.5–4.9[d]; 2.2–4.3[e]; 2–5[f]; 2.5–5(–6)[g] 3.7 ± 0.40	3.0–6.0 (3.4–5.6)
width (mm)	1.5–4.0[a]; max. 4.0[b]; max. 4.5[c]; 1.0–1.7[d]; 1.4–1.9[e]; 1.5–3[f]; 1.7–3.5 (2.0–3.3)[h]; 1.6 ± 0.15[i]	1.3–3.5 (1.5–3.0)	2.5–5.0[a]; max. 4.5[b]; 1–4.0[c]; 2.0–3.9[d]; 1.3–3.7[e]; 2–4[f]; 2.5 ± 0.31[i]	2.0–4.8 (2.5–4.3)
ratio width/length	0.63[e]; 0.48–1.0 (0.63–0.88)[h]	0.48–0.86 (0.53–0.80)	0.74[e]	0.60–1.0 (0.71–0.83)

a: Daubs (1958)
b: Van Horen (1869)
c: Landolt (1957)
d: De Lange & Pieterse (1973)
e: De Lange & Segal (1968)
f: Mason (1957)
g: Van Ooststroom & Reichgelt (1964)
h: Pieterse (1974)
i: De Sloover (1966)

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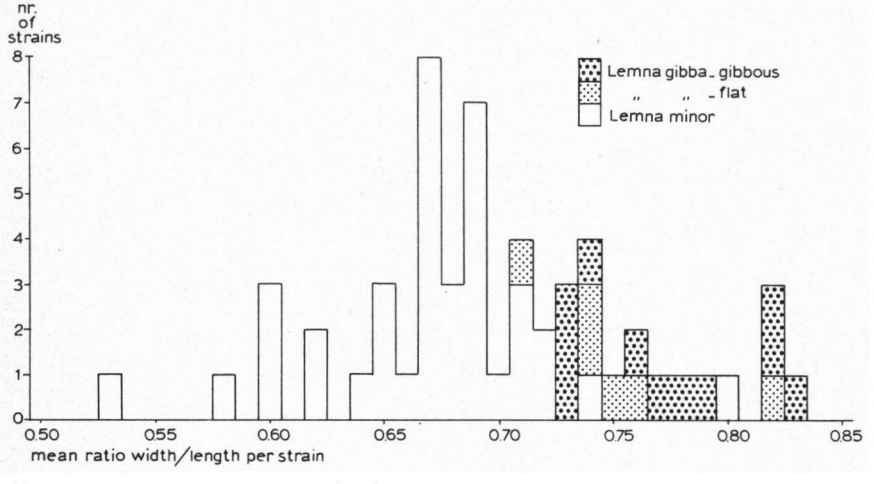

Fig. 4. Distribution of the mean ratio width/length per strain.

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