

MORPHOLOGICAL DIFFERENTIATION OF POLLEN OF *LYSIMACHIA VULGARIS* L.
FROM POLAND

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ABSTRACT. The morphology and variability of pollen of *Lysimachia vulgaris* L. were studied on the base of the plant material coming from 21 native localities. 630 pollen grains were examined. The diagnostic features of pollen grains of studied species are: exine sculpture irregularly reticulate with lumina of different diameters, presence of many minute granules of different size inside lumina (a new feature for the examined species), muri with medium thickness, pollen shape most often subprolate, rarely prolate. Obtained results do not fully correspond with the available *L. vulgaris* pollen descriptions. Statistical analysis of nine quantitative grain characteristics showed their variability to be very differentiated. The highest variability was found to occur in trait the Exe/E (the distance between the apices of two ectocolpi). No dependence in pollen morphology differentiation due to the localization of natural populations was observed.

KEY WORDS: *Lysimachia vulgaris*, pollen morphology, statistical analyse, LM, SEM

INTRODUCTION

Genus *Lysimachia* L. consists of ca. 180 species of perennial, biennial or annual herbs with almost all over the world distribution (STÅHL and ANDERBERG 2004). *Lysimachia* is traditionally placed in the tribe *Lysimachieae* of the family Primulaceae (CRONQUIST 1981, TAKHTAJAN 1997) and is one of the largest genera in this family. Results of the recent phylogenetic studies of primuloid families based on molecular and morphological data support the transfer of the genus *Lysimachia* to Myrsinaceae (KÄLLERSJÖ et AL. 2000, APG 2003, MARTINS et AL. 2003, OH et AL. 2008). Genus *Lysimachia* is divided into five-six subgenera and many subgeneric sections (CHEN and HU 1979, BENNEL and HU 1983, ANDERBERG et AL. 2007). *Lysimachia vulgaris* belongs to the subgenus *Lysimachia* and section *Lysimachia* (BENNEL and HU 1983, HAO et AL. 2004).

Lysimachia vulgaris is distributed in Europe, N America, SW Asia and NW Africa (FERGUSON 1972, INTERNET <http://www.eol.org>). In Poland it is a common herb (ATLAS... 2001) occurring in moist habitats, such as fens, wet woods, lake shores, and river banks. It is a medicinal plant and the latest results of chemical studies show its potential cytostatic and anticancer activity (UMEK 1982, ŁUCZAK et AL. 1989, JANIK et AL. 1994, PODOLAK et AL. 1998, PODOLAK and STRZAŁKA 2008).

Morphologically, *Lysimachia* is very heterogeneous with the variation in inflorescence structure, floral morphology, pollination adaptation and growths habit. Studies of pollen morphology of 98 species and varieties

of *Lysimachia* done by BENNEL and HU (1983) resulted in the recognition of ten major pollen-types and four subtypes. *Lysimachia vulgaris* pollen was classified by these authors into Fraseri-pollen type, together with several other species e.g. *L. davurica*, *L. fraseri*, *L. terrestris*, *L. thyrsoiflora*. According to them, the general form of pollen in *Lysimachia* is constant, the grains are isopolar, tricolporate and they vary in size from smallest ($13.0 \times 9.5 \mu\text{m}$ in *L. capillepsis*) to medium (largest $39.0 \times 35.0 \mu\text{m}$ in *L. franchetii*).

We have examined pollen morphology of *Lysimachia vulgaris* from natural habitats distributed all over Poland and from the cultivated population with the aim of (1) the characteristic of *L. vulgaris* pollen morphology on the base of a large sample and (2) the comparative statistical analysis of the examined pollen traits in order to evaluate *L. vulgaris* pollen grains differentiation.

MATERIAL AND METHODS

The examined pollen grains were collected from 20 natural *Lysimachia vulgaris* populations distributed all over Poland and from one cultivated population, which was treated by us as a preliminary attempt to find out if there are differences in pollen morphology in dependence of the plants growing conditions (Table 1). The cultivated population is located in the garden of the Department of Botany of Poznań University of Life Sciences. It was started in 2001 from seeds collected

TABLE 1. Location of studied pollen samples of *Lysimachia vulgaris*

Population number	Localities	Latitude (N) and longitude (E)	Year of collection, herbarium
1	Antoninek	52°24', 17°0'	2005, POZNB
2	Poznań	52°24', 16°55'	2005, POZNB
3	Kamińsko	52°32', 17°3'	2005, POZNB
4	near Trzciel	52°22', 15°52'	2005, POZNB
5	near Świebodzin	52°14', 15°32'	1963, POZNA
6	Oława	50°56', 17°17'	1958, POZNA
7	near Sępólno	53°27', 17°31'	1962, POZNA
8	Mielno	51°32', 14°58'	1957, POZNA
9	near Wejherowo	54°36', 18°14'	1967, POZNA
10	Wilczkowo Lake	53°43', 15°46'	1955, POZNA
11	Puszcza Knyszyńska	53°15', 23°7'	1962, POZNA
12	Puszcza Bukowa near Szczecin	53°25', 14°32'	1961, POZNA
13	Małe Oborowo Lake	54°15', 17° 5'	1954, POZNA
14	near Chojnice	53°41', 17°33'	1969, POZNA
15	Bługowo	53°15', 17°7'	1977, POZNA
16	near Konin	52°13', 18°16'	1977, POZNA
17	Stawek Lake	53°42', 17°33'	1964, POZNA
18	near Zielona Góra	51°56', 15°30'	1977, POZNA
19	Pępowo	51°45', 17°7'	1957, POZNA
20	cultivated population		2006
21	Potasze	52°31', 17°0'	2006, POZNB

PZNB – Herbarium of Department of Botany of Poznań University of Life Sciences, PZNA – Herbarium of Adam Mickiewicz University in Poznań.

from one of the natural populations examined in our present work.

Each pollen sample from herbaria collections contained pollen grains sampled from few flowers of two-three plants, in dependence on the plant material availability. Pollen samples from existing natural populations and from the cultivated one were collected every time from five flowers of 30 plants growing at least two meters one from another, in order to avoid sampling the same individual. Plants in cultivation grew on podosolic soil with the humus level of 0.8% and pH = 6. In the year before the cultivation on the experimental plot lupine was sown in order to plough back. During the vegetation period plants were fertilized twice with NPK in standard doses recommended for ornamental species of *Lysimachia* and watered every second day. Each sample was represented by 30 pollen grains and 630 pollen grains were examined in total. They were analysed for nine quantitative features and the following qualitative ones: shape outline and type of exine sculpture (Table 2). Only grains that were unbroken, symmetrical, and fully expanded in equatorial view were used.

TABLE 2. Pollen grains traits analysed

No	Traits
1	Length of polar axis (P)
2	Length of equatorial axis (E)
3	Thickness of exine along polar axis (Exp)
4	Thickness of exine along equatorial axis (Exe)
5	Length of ectocolpi (Le)
6	P/E ratio
7	Relative thickness of exine (P/Exp ratio)
8	Relative thickness of exine (E/Exe ratio)
9	Distance between the apices of two ectocolpi (d)

All samples were acetolysed according to ERDTMAN'S (1952) method, with insignificant modifications (WROŃSKA-PILAREK 1998). The terminology follows ERDTMAN

(1952), REITSMA (1970), PUNT et AL. (1999) and HALBRITTER et AL. (2007). Relative exine thickness is the ratio of exine thickness measured along the P (Exp) axis to the length of the P axis, and along the E (Exe) axis to the length of the E axis. Pollen grains were mounted in glycerol jelly for LM (Biolar 2308) studies. Surface sculpture of the examined pollen grains was characterised with the use of SEM technique. The microphotographs were taken on a Zeiss EVO 40 electron microscope (Laboratory of Electron Microscopy of Adam Mickiewicz University in Poznań).

The empirical data from grain measurements embraced quantitative features listed in Table 2. Their analysis included descriptive statistics and correlation coefficients, univariate analysis of variance and Tukey's procedure, agglomerative grouping by Ward's method – dendrogram (CALIŃSKI and KACZMAREK 1973, KAROŃSKI and CALIŃSKI 1973).

RESULTS

General characteristic

A description of the pollen grain morphology of the studied species is given below and illustrated with a few SEM photographs (Figs 1-5). The morphological observations are summarised in Table 3.

The pollen of *Lysimachia vulgaris* is three zonocolporate, occasionally four zonocolporate. Pollen size usually medium (75.9%), rarely small (24.1%). Average length of polar axis (P) 25.63 μm (19.8-32,4 μm) and equatorial

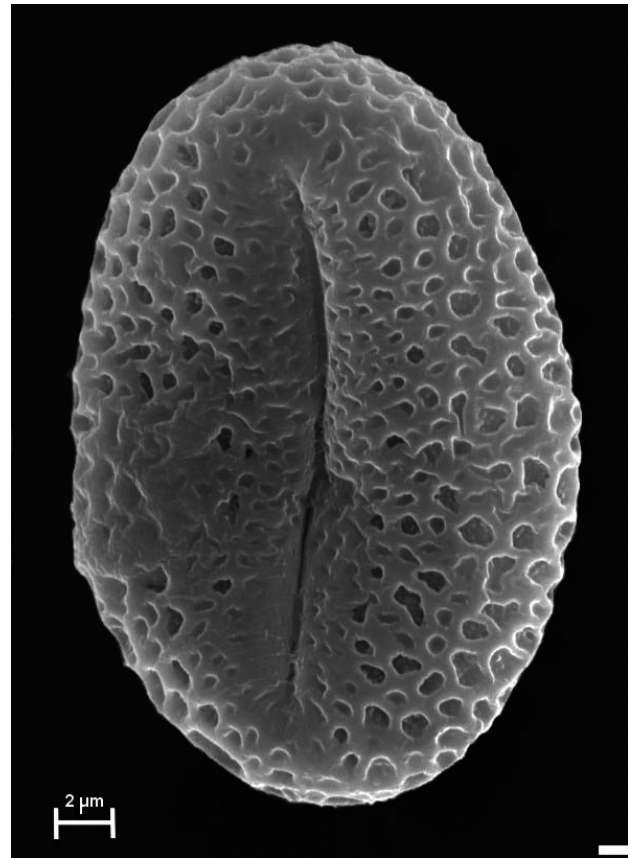


FIG. 2. SEM. Pollen grain in equatorial view; reticulate exine sculpture and ectocolpus visible ($\times 10\ 000$)

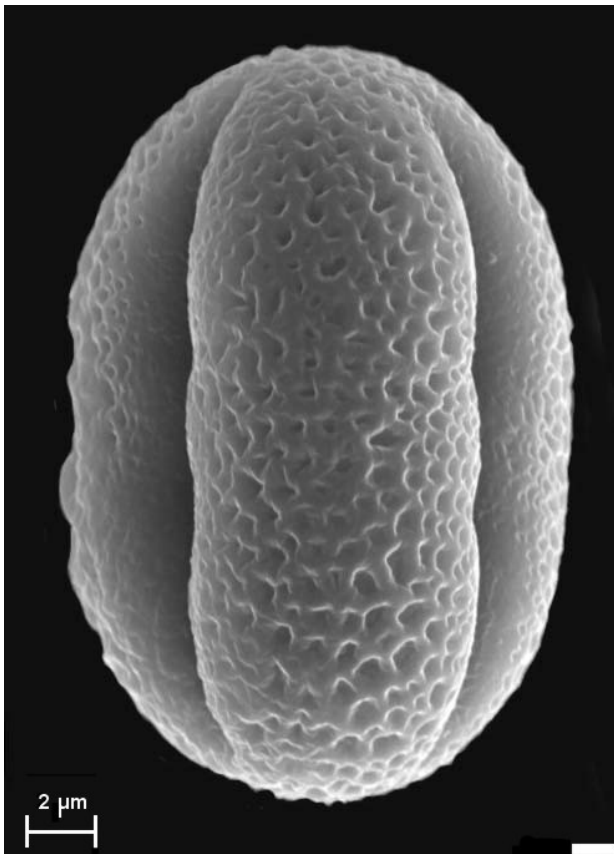


FIG. 1. SEM. Pollen grain in equatorial view; pollen shape, exine sculpture and two ectocolpi visible ($\times 10\ 000$)

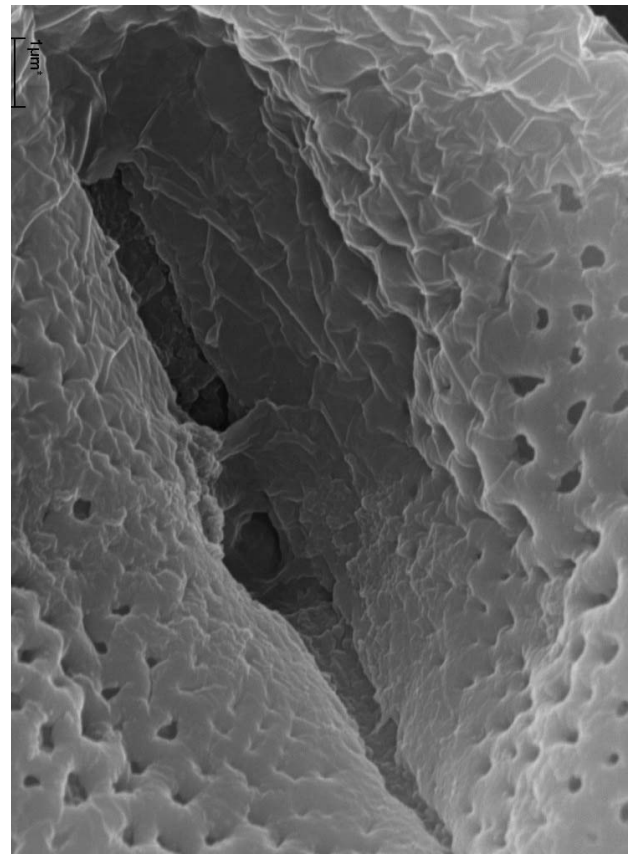


FIG. 3. SEM. Elongated, narrow ectocolpus with colpus margins and opened endopore visible ($\times 30\ 000$)

TABLE 3. Numeral characteristics of traits of studied pollen grains

Localities	Traits																			
	P				E				P/E				Exp				Exe			
	minimum	arithmetic mean	maximum	variance coefficient (%)	minimum	arithmetic mean	maximum	variance coefficient (%)	minimum	arithmetic mean	maximum	variance coefficient (%)	minimum	arithmetic mean	maximum	variance coefficient (%)	minimum	arithmetic mean	maximum	variance coefficient (%)
1	23.4	25.6	27.0	4.7	18.0	20.4	21.6	5.4	1.08	1.26	1.40	6.5	1.44	1.63	1.80	11.2	1.44	1.62	1.80	11.3
2	25.2	29.0	32.4	5.3	19.8	22.1	23.4	4.3	1.08	1.31	1.50	6.8	1.44	1.73	1.80	8.5	1.44	1.69	1.80	9.9
3	23.4	26.9	28.8	6.1	18.0	20.9	23.4	5.4	1.08	1.29	1.50	8.4	1.44	1.48	1.80	7.4	1.44	1.48	1.80	7.4
4	23.4	24.7	27.0	5.0	18.0	19.7	21.6	6.6	1.08	1.26	1.40	6.9	1.44	1.64	1.80	11.0	1.44	1.63	1.80	11.2
5	21.6	25.9	28.8	6.5	18.0	19.3	21.6	5.0	1.17	1.35	1.50	7.5	1.44	1.51	1.80	9.7	1.44	1.51	1.80	9.7
6	19.8	24.7	28.8	8.1	18.0	19.0	21.6	6.4	1.09	1.30	1.45	7.2	1.44	1.54	1.80	10.5	1.44	1.54	1.80	10.5
7	21.6	25.7	28.8	6.4	19.8	21.3	23.4	5.9	1.00	1.21	1.36	8.3	1.44	1.51	1.80	9.7	1.44	1.51	1.80	9.7
8	23.4	25.8	27.0	5.3	18.0	18.9	21.6	5.5	1.18	1.37	1.50	6.9	1.44	1.51	1.80	9.7	1.44	1.51	1.80	9.7
9	21.6	25.7	28.8	6.9	18.0	20.2	21.6	6.9	1.08	1.27	1.45	8.0	1.44	1.48	1.80	7.4	1.44	1.49	1.80	8.4
10	23.4	25.3	27.0	5.3	19.8	21.6	25.2	5.4	1.00	1.17	1.27	6.8	1.44	1.49	1.80	8.4	1.44	1.48	1.80	7.4
11	19.8	22.4	23.4	5.0	16.2	17.2	18.0	5.3	1.20	1.31	1.50	7.2	1.44	1.55	1.80	10.8	1.44	1.52	1.80	10.2
12	23.4	25.6	28.8	4.4	18.0	19.8	21.6	4.1	1.17	1.30	1.50	5.4	1.44	1.44	1.44	0.0	1.44	1.44	1.44	0.0
13	21.6	24.0	27.0	6.0	18.0	19.9	21.6	5.0	1.00	1.21	1.40	7.2	1.44	1.57	1.80	11.2	1.44	1.55	1.80	10.8
14	23.4	25.2	27.0	5.0	18.0	18.6	19.8	4.6	1.27	1.36	1.50	5.2	1.44	1.44	1.44	0.0	1.44	1.44	1.44	0.0
15	23.4	25.8	28.8	5.6	18.0	20.6	23.4	5.5	1.08	1.26	1.40	5.4	1.44	1.55	1.80	10.8	1.44	1.55	1.80	10.8
16	21.6	27.1	30.6	7.6	18.0	20.5	21.6	5.5	1.18	1.32	1.45	6.0	1.44	1.51	1.80	9.7	1.44	1.49	1.80	8.4
17	23.4	25.9	28.8	5.0	18.0	20.3	23.4	5.8	1.08	1.28	1.60	7.6	1.44	1.52	1.80	10.2	1.44	1.52	1.80	10.2
18	21.6	25.0	28.8	6.5	18.0	20.2	21.6	6.5	1.08	1.24	1.60	8.8	1.44	1.68	1.80	10.3	1.44	1.68	1.80	10.3
19	19.8	25.0	28.8	7.9	16.2	18.7	19.8	5.4	1.10	1.34	1.50	6.6	1.44	1.57	1.80	11.2	1.44	1.57	1.80	11.2
20	23.4	27.2	28.8	4.4	18.0	19.9	21.6	6.5	1.25	1.37	1.50	6.8	1.44	1.51	1.80	9.7	1.44	1.51	1.80	9.7
21	23.4	25.8	30.6	7.2	16.2	19.6	21.6	5.8	1.17	1.32	1.55	5.8	1.44	1.50	1.80	9.1	1.44	1.50	1.80	9.1

Localities	Traits															
	Exp/P				Exe/E				Le				d			
	minimum	arithmetic mean	maximum	variance coefficient (%)	minimum	arithmetic mean	maximum	variance coefficient (%)	minimum	arithmetic mean	maximum	variance coefficient (%)	minimum	arithmetic mean	maximum	variance coefficient (%)
1	0.05	0.06	0.08	11.7	0.07	0.08	0.10	14.6	14.4	18.4	21.6	10.2	5.4	8.0	10.8	15.3
2	0.05	0.06	0.07	9.9	0.06	0.08	0.08	10.4	16.2	20.3	23.4	10.4	5.4	8.5	10.8	17.8
3	0.05	0.06	0.07	9.3	0.07	0.07	0.10	9.7	16.2	19.3	23.4	9.2	5.4	7.8	10.8	17.5
4	0.05	0.07	0.08	11.9	0.07	0.08	0.10	11.9	14.4	18.1	21.6	10.3	5.4	6.6	9.0	20.7
5	0.05	0.06	0.08	12.3	0.07	0.08	0.10	11.1	14.4	18.5	21.6	9.6	3.6	6.7	9.0	24.3
6	0.05	0.06	0.08	12.4	0.07	0.08	0.10	10.4	14.4	18.4	21.6	10.2	5.4	7.0	9.0	16.3
7	0.05	0.06	0.08	13.8	0.06	0.07	0.09	12.1	16.2	17.9	21.6	8.9	5.4	7.4	10.8	22.7
8	0.05	0.06	0.08	11.1	0.07	0.08	0.10	11.0	14.4	18.8	21.6	7.4	5.4	7.0	9.0	17.6
9	0.05	0.06	0.08	12.9	0.07	0.07	0.09	10.0	14.4	19.6	23.4	11.3	3.6	7.0	9.0	23.3
10	0.05	0.06	0.08	9.5	0.06	0.07	0.08	8.1	14.4	17.8	19.8	8.9	3.6	7.4	10.8	23.5
11	0.06	0.07	0.09	11.5	0.08	0.09	0.11	12.4	14.4	15.9	18.0	7.3	3.6	6.2	7.2	18.1
12	0.05	0.06	0.06	4.3	0.07	0.07	0.08	4.2	14.4	19.1	23.4	10.4	3.6	7.6	10.8	19.2
13	0.06	0.07	0.08	13.0	0.07	0.08	0.10	12.3	14.4	16.9	19.8	9.1	5.4	7.7	10.8	18.5
14	0.05	0.06	0.06	5.0	0.07	0.08	0.08	4.5	14.4	18.9	21.6	10.2	5.4	6.9	9.0	18.2
15	0.05	0.06	0.08	14.3	0.06	0.08	0.10	13.4	14.4	17.9	21.6	10.0	5.4	7.7	9.0	15.1
16	0.05	0.06	0.08	13.4	0.07	0.07	0.09	8.8	14.4	19.8	23.4	13.3	5.4	7.1	9.0	18.1
17	0.05	0.06	0.07	10.9	0.06	0.08	0.10	12.9	14.4	19.1	21.6	9.1	5.4	7.0	10.8	20.6
18	0.05	0.07	0.08	10.5	0.07	0.08	0.10	12.7	14.4	18.4	21.6	10.8	3.6	7.1	10.8	22.1
19	0.05	0.06	0.08	13.1	0.07	0.08	0.10	11.1	14.4	17.6	21.6	10.2	3.6	6.8	9.0	22.3
20	0.05	0.06	0.07	10.4	0.07	0.10	0.80	131.4	18.0	20.0	23.4	7.2	3.6	7.0	9.0	18.3
21	0.05	0.06	0.08	12.8	0.07	0.08	0.10	11.4	16.2	19.1	23.4	10.1	3.6	6.5	9.0	24.5

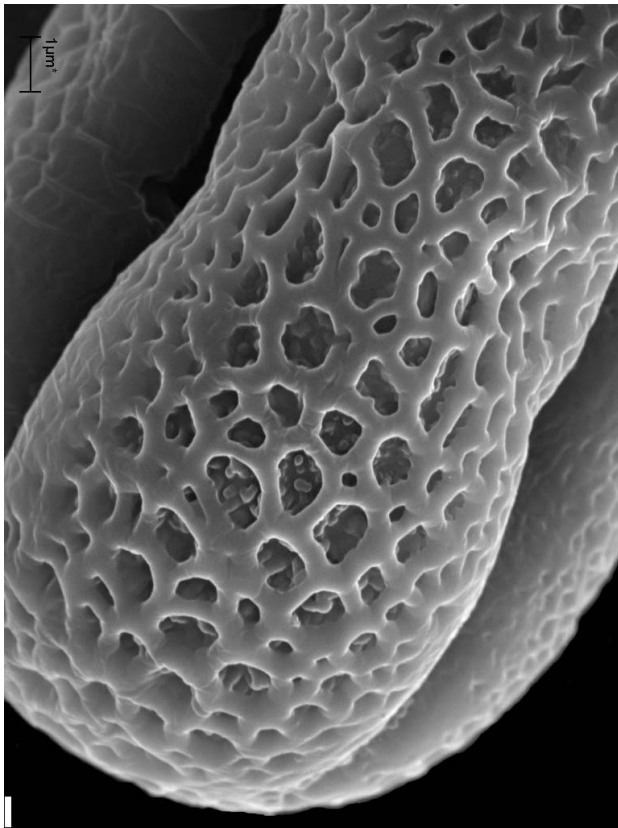


FIG. 4. SEM. Reticulate exine sculpture with lumina with small granules and muri visible ($\times 25\ 000$)

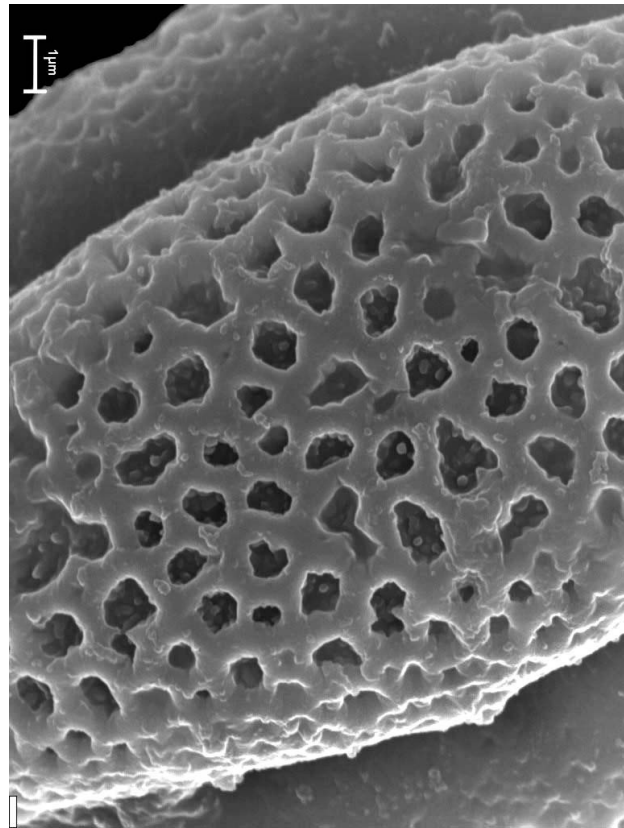


FIG. 5. SEM. Reticulate exine sculpture with lumina with small granules and muri visible ($\times 25\ 000$)

axis (E) $19.94\ \mu\text{m}$ ($16.2\text{--}25.2\ \mu\text{m}$). Average P/E ratio 1.29, with the range from 1.0 to 1.6. Pollen shape spheroidal to prolate, most often subprolate (62.5%) or prolate (31.6%), rarely prolate-spheroidal (5.4%) and spheroidal (0.5%, Table 3). Outline in polar view mostly circular, more rarely elliptic and in equatorial view mostly elliptic, very rarely circular.

Exine 1.54 ($1.44\text{--}1.8$) μm thick, with a partial tectum. Exine is two-layered, with ectexine and endexine of about the same thickness. The relative thickness of the exine measured along P (Exp/P ratio) averaged 0.06 (0.05–0.09), and along E (Exe/E ratio) 0.06 (0.06–0.11). The value and range of the coefficients of the relative thickness of the exine measured along P (Exp) and along E (Exe) similar, what indicates that thickness of exine measured along both axes similar.

Exine sculpture is reticulate. Lumina irregular, with different diameter ($0.5\text{--}1.5\ \mu\text{m}$; average $0.8\text{--}1.0\ \mu\text{m}$) decreasing towards colpi. In the 30–40% of studied pollen grains, inside lumina, minute granules of different size are present. Slightly thickened muri (average thickness $0.5\text{--}0.7\ \mu\text{m}$), usually twice thinner than the lumina diameter.

Apertures 3rd (occasionally 4th), both ectocolpi and endopores. Ectocolpi arranged meridionally, regularly, more or less evenly spaced. Long, narrow, elliptic in outline, sunken, with distinct margins, ends acute with margo and costae. Mean length 18.57 ($14.4\text{--}23.4$) μm ; ectocolpi constitute 72.5% of length of the polar axis (P). Ectocolpus sometimes bridged at the equator. Colpus margins present. Endopores equatorially elongated, forming an almost continuous oral zone. Usually located in the middle of ectocolpi, rarely asymmetrically, usually one. Fastigium present, sometimes indistinct.

Intraspecific variability

Features P, E and P/E ratio showed low variability (P – 7.7%, E – 7.8% and P/E – 8.0%). The features Exp, Exs, Le, d and Exp/P ratio were characterised by moderate level of variability (Exp – 10.5%, Exe – 10.3%, Le – 11.1%, d – 20.8% and Exp/P – 13.1%) and for the Exe/E ratio variability was found to be the highest (38.7%).

On the basis of an analysis of the diagonal elements of the inverse of the R correlation matrix for all the characteristics, those selected for further study were ones with low values of the diagonal element, which is indicative of a weak correlation between the given characteristic and the others. In this way the following arrangement of traits was chosen: P with the diagonal element value of 2.6, E – 1.4, Exp/P – 1.6, Exe/E – 1.1, Le – 1.8 and d – 1.1.

To verify the hypothesis about the effect of the location of 21 analysed populations on pollen grain traits under study, a univariate analysis of variance was carried out. The assumption of distribution normality was verified by Shapiro-Wilk test, and of variance uniformity, by Levene's test. While analysing the empirical value of F statistic and probability p , for each feature under study the differences were found to be significant at the 5% significance level (feature P – $F_{\text{calc}} = 21.1$ and $p = 0.000$, E – $F_{\text{calc}} = 30.8$ and $p = 0.000$, Exp/P ratio – $F_{\text{calc}} = 10.4$ and $p = 0.000$, Exe/E ratio – $F_{\text{calc}} = 1.7$ and $p = 0.030$, Le – $F_{\text{calc}} = 9.5$ and $p = 0.000$ and d – $F_{\text{calc}} = 4.0$ and $p = 0.000$). In analysing the particular localities a lack of significant differences was marked by two points joined with a dashed line (Fig. 6).

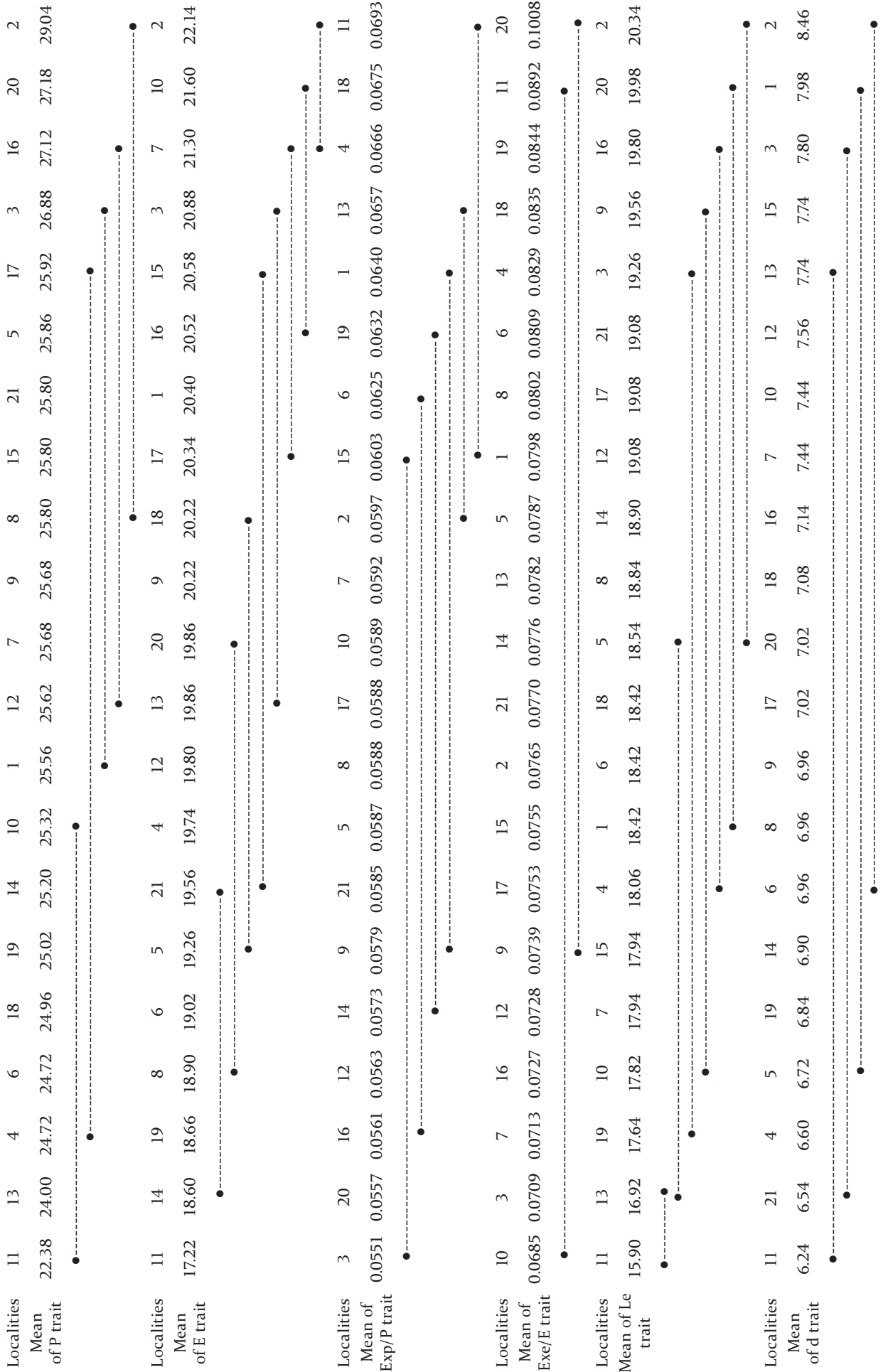


FIG. 6. Multiple comparisons by Tukey's procedure

The highest mean values of P, E, Le and d were noticed for pollen grains from sample no 2, and the lowest for sample no 11. In sample no 11 the value of the Exp/P ratio was highest, and Exe/E ratio was highest in the sample no 20. The lowest values of these features were noticed for samples nos 3 and 10.

In order to distinguish the most homogenous clusters of pollen grains collected in examined populations by the criterion of the features under study (P, E, Exp/P, Exe/E, Le and d), an agglomerative grouping by Ward's method was carried out to obtain a dendrogram (Fig. 7). The grouping revealed that pollen samples from all examined localities formed three groups. The greatest differences occurred between all three of them and the cultivated population (no 20). The first group comprised localities nos 18, 13, 19, 6, 4 and locality no 11 showed similarity to them. The second group included localities nos 21, 8, 14, 12, 5, and the third one localities nos 16, 9, 15, 3, 7, 10, 17, 1. The grouping revealed also similarity to hold among the grains from the latest two groups and the locality no 2.

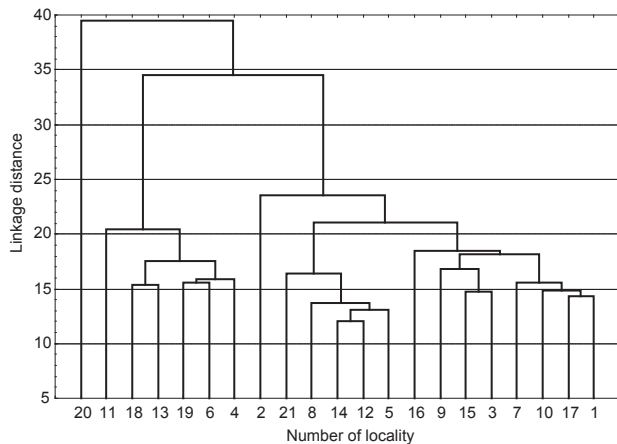


FIG. 7. Dendrogram of 21 localities constructed on the basis of Ward's method

DISCUSSION

According to PUNT et AL. (1976), BENNELL and HU (1983) and FAEGRI and IVERSEN (1993), MOORE et AL. (1991), BEUG (2004) pollen grains from *Lysimachia vulgaris* are tricolporate. BENNELL and HU (1983) described them as small ($22\text{--}24 \times 19\text{--}22 \mu\text{m}$), prolate-spheroidal to subprolate. According to our measurements *L. vulgaris* pollen grains are medium sized and they are most often subprolate or prolate, very rarely spheroidal. PUNT et AL. (1976) includes *L. vulgaris* into *L. vulgaris* – pollen type together with such species as *L. nummularia*, *L. terrestris*, *L. thyrsoiflora* and *L. punctata*. According to BENNELL and HU (1983) *L. vulgaris* belongs to the Fraseri-pollen type together with *L. davurica*, *L. fraseri*, *L. quadrifolia*, *L. serulata*, *L. terrestris* and *L. thyrsoiflora*. They suggested a closer relationship between *L. thyrsoiflora* and *L. terrestris* based on pollen morphology. Results obtained in the presented work are generally in agreement with already published descriptions, but in few cases they are new or not fully congruent with them.

The key to pollen types in genus *Lysimachia* given by BENNELL and HU (1983) classifies *Lysimachia vulgaris* pollen grains into Fraseri-type by, among others things, the reticulate exine sculpture with lumina $<$ muri, the size of lumina $>$ $1.5 \mu\text{m}$ and the small pollen grains size. BEUG (2004) informs, that lumina (brochi) measure $1\text{--}3 \mu\text{m}$ (near colpus only $1 \mu\text{m}$), and muri ca. $0.8 \mu\text{m}$. According to our observations lumina are of irregular shape with the medium size $0.8\text{--}1.0 \mu\text{m}$ and not expanding $1.5 \mu\text{m}$, and muri are slightly thickened up to $0.7 \mu\text{m}$. It is in agreement with the results of HUYNH (1970), who reported that size of lumina for *L. vulgaris* pollen was maximally $1.5 \mu\text{m}$. NOVICKE and SKVARLA (1977) found out that for *L. terrestris* and *L. thyrsoiflora*, the two species belonging to the same pollen type as *L. vulgaris*, the surface of pollen grains was finely reticulate or irregularly perforate and the tectal perforations were more pronounced in the mesocolpial regions. According to our observations *L. vulgaris* pollen grains show similar features. BENNELL and HU (1983) accounted that *L. thyrsoiflora* pollen characterises coarse reticulum. In our investigations it was found out that inside the lumina of *L. vulgaris* pollen grains minute granules of different size are present. Observation of this new feature allows us to suggest that, on the base of pollen morphology, *L. vulgaris* is more alike *L. thyrsoiflora* than *L. terrestris* and it confirms the attachment of these two species to the same pollen type group. It is also congruent with the results of the latest investigations on phylogenetic relations within genus *Lysimachia* based both on the molecular as well as morphological data (ANDERBERG et AL. 2007, OH et AL. 2008).

Experiments on *Lysimachia vulgaris* pollen variability were not carried till now. Examined grains showed different variation level. The lowest variation level was found for the most precisely measured length and thickness of grains (features P, E and P/E ratio). More difficult to exact measurements, and therefore measured roughly, exine thickness, distance between colpis and their lengths showed moderate variability. High level of variation may be correlated with the high value of the one of exine measurements.

The statistical analysis showed that pollen morphological features of one species may be distinctly differentiated. Such a situation was observed concerning pollen samples no 11 and no 2. In the sample no 11 the mean value of the feature P is $22.38 \mu\text{m}$, and in the sample no 2 its value is $29.04 \mu\text{m}$. Furthermore, in sample no 6 the min. and max. values of the feature P differ for $10 \mu\text{m}$. It results from these observations that the number of measured pollen grains is significantly important in obtaining the precise pollen morphology characteristic for the given species.

The performed statistical analysis showed no similarity in the examined pollen features in dependence on the population locality. According to that, grains collected from plants growing near Zielona Góra (lubelskie province), Gostyń (Wielkopolskie province) or Oława (dolnośląskie province) were very similar with each other, while significant differences have been found between pollen morphology of grains collected from very closely located populations. For example, grains from samples no 1 and 2 collected in Poznań and its

surroundings belonged to different groups according to the obtained dendrogram.

CONCLUSIONS

1. The most crucial traits of the pollen grains of the studied species: exine sculpture irregularly reticulate with lumina of different diameters, inside lumina many minute granules of different size (a new feature for the examined species), muri with medium thickness, pollen shape most often subprolate, rarely prolate.

2. Observed features of *Lysimachia vulgaris* pollen do not allow to classify it into given by BENNEL and HU (1983) Fraseri-pollen type.

3. Statistical analysis showed that the variability of the quantitative features of *Lysimachia vulgaris* pollen grains was moderate. The highest level of variability was found for the trait Exe/E, moderate variability level for the features Exp, Exe, Le, d and Exp/P ratio, the rest of the features (P, E and P/E) were characterised by low variability.

4. It is necessary to measure large number of pollen, at least 30 grains in each sample, to obtain precise biometrical data.

5. An agglomerative grouping by Ward's method showed that pollen grains collected from the cultivated population differ from all other pollen samples collected from natural localities. No dependence on population's localization and pollen similarity was observed.

6. To confirm the observed *Lysimachia vulgaris* pollen morphology differentiation between grains coming from natural populations and from the cultivated one it is necessary to examine more pollen samples from higher number of cultivated populations.

7. Obtained results confirm resemblance of *Lysimachia vulgaris* pollen morphological features with *L. thyrsoiflora* and *L. terrestris*, which are, according to recently published phylogenetical relations within genus *Lysimachia*, the closest relatives of the examined species. The new observed feature, the presence of minute granules in lumina, allows to accept *L. vulgaris* as more alike *L. thyrsoiflora* than *L. terrestris*.

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