

## Pollen morphology of the genus *Lycopus* (Lamiaceae)

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The pollen morphology of 15 species (34 specimens) of the genus *Lycopus* (Lamiaceae, Mentheae) was studied and documented in detail using light microscopy (LM), scanning electron (SEM), and transmission electron microscopy (TEM). The pollen is mostly medium or sometimes small in size, with a circular amb, oblate to prolate in shape, hexacolpate with granular membranes; the exine is bi-reticulate, with unbranched columellae and a continuous, lamellated endexine. The results indicate that *Lycopus* is stenopalynous; thus the value of pollen characters for taxonomic applications is limited. Some phylogenetic relationships with other related taxa within the tribe Mentheae are also briefly discussed.

Key words: Lamiaceae, *Lycopus*, Mentheae, pollen morphology, SEM, systematics, TEM

### Introduction

The genus *Lycopus*, comprising ca. 15 species of herbaceous perennial plants, occurs mostly in low wetland areas and is distributed primarily in Europe, Asia and North America (Henderson 1962). The genus is characterized by a combination of the following characters: dentate or pinnatifid, opposite leaves, an inflorescence compacted with 12–27 flowers (i.e., verticillaster), a 4–5 lobed tubulate/campanulate calyx, a dry, tetrahedral, one-seeded nutlet. However, the vegetative characters of *Lycopus* show a high degree of variability which has led to much difficulty in characterizing the taxa (Henderson 1962, Li & Hedge 1994).

Briquet (1896) assigned *Lycopus* to the subfamily Stachyoideae, tribe Satureieae and subtribe Menthinae. He considered *Lycopus* to

be most closely related to *Mentha*, from which it differs in having two fertile stamens instead of four, and bearing nutlets with corky crests, the nutlets of *Mentha* being smooth or roughened but without a crest. Recently, Cantino *et al.* (1992) revised the classification of all genera in Lamiaceae, and placed *Lycopus* within the subfamily Nepetoideae, tribe Mentheae, following Erdtman's (1945) circumscription. On the basis of vegetative characters, Briquet (1896) divided the genus into two sections: sect. *Stoloniferi*, in which the plants have long runners or stolons from the lower nodes of the stem, and sect. *Astolonosi*, in which the plants had rootstocks, but lacked runners. He suggested the presence of only a single taxon, *L. sinuatus*, placed within sect. *Stoloniferi*. His infrageneric delimitation of the genus, however, is in our opinion very vague and should be treated with caution.

**Table 1.** Summary of pollen morphological data for *Lycopus*. *P* = polar axis ( $\mu\text{m}$ ), *E* = equatorial diameter ( $\mu\text{m}$ ), *CL* = colpus length ( $\mu\text{m}$ ), *ET* = exine thickness ( $\mu\text{m}$ ), *P/E* = ratio of polar axis and equatorial diameter. Taxa are listed in alphabetical order.

	<i>P</i>		<i>E</i>		<i>CL</i>		<i>ET</i>		<i>P/E</i>	
	min.	max.	min.	max.	min.	max.	min.	max.	min.	max.
1. <i>L. americanus</i>	27.59	(29.75)31.25	20.00	(23.50)26.25	17.50	(18.88)21.25	2.00	(2.10)2.50	1.16	(1.27)1.39
2. <i>L. amplexans</i>	26.25	(28.94)32.50	22.50	(25.87)28.75	18.75	(22.19)25.00	2.00	(2.00)2.25	1.00	(1.12)1.24
3. <i>L. asper</i>	31.25	(35.56)38.75	27.50	(30.69)33.75	20.00	(22.22)25.00	1.75	(1.92)2.00	1.00	(1.16)1.36
4. <i>L. australis</i>	30.33	(32.13)35.00	23.75	(26.13)27.50	17.50	(19.50)22.50	2.00	(2.08)2.50	1.09	(1.23)1.27
5. <i>L. communis</i>	30.00	(32.50)35.00	27.50	(29.88)35.00	20.00	(22.63)25.00	1.75	(2.03)2.25	1.00	(1.09)1.18
6. <i>L. europaeus</i>	27.50	(30.69)32.50	22.50	(26.06)31.25	21.25	(23.38)25.00	2.00	(2.18)2.50	1.00	(1.18)1.44
7. <i>L. exaltatus</i>	22.50	(25.38)28.75	20.00	(22.38)27.50	15.00	(19.69)22.50	1.75	(2.03)2.50	0.90	(1.13)1.35
8. <i>L. laurentianus</i>	22.50	(25.25)30.00	27.50	(29.88)33.75	15.00	(18.62)20.00	1.50	(1.90)2.00	0.75	(0.85)0.92
9. <i>L. lucidus</i>	22.50	(26.13)28.75	20.00	(22.44)27.50	15.00	(16.81)18.75	2.00	(2.41)2.50	0.95	(1.16)1.35
10. <i>L. maackianus</i>	23.75	(26.03)31.25	22.50	(24.93)28.75	15.00	(16.91)18.75	1.75	(2.13)2.50	0.91	(1.04)1.25
11. <i>L. membranaceus</i>	25.00	(25.75)28.75	23.75	(26.00)28.75	16.25	(17.63)20.00	1.75	(2.00)2.25	0.87	(0.99)1.21
12. <i>L. ramosissimus</i>	23.75	(26.13)30.00	22.50	(25.13)27.50	15.00	(16.13)17.50	1.75	(1.93)2.25	0.95	(1.04)1.14
13. <i>L. rubellus</i>	23.75	(25.81)30.00	23.75	(26.44)30.00	17.50	(19.25)22.50	2.00	(2.13)2.50	0.83	(0.98)1.16
14. <i>L. sinuatus</i>	22.50	(25.78)28.75	20.00	(23.17)27.50	15.00	(17.93)22.50	1.50	(1.90)2.50	0.97	(1.11)1.38
15. <i>L. uniflorus</i>	25.00	(26.94)30.00	25.00	(27.63)31.25	15.00	(18.75)21.25	1.75	(2.00)2.25	0.88	(0.98)1.04

Investigations of pollen morphology in the Lamiaceae have been essential as an aid to classification within this family (Erdtman 1945, Harley *et al.* 1992, Abu-Asab & Cantino 1994). However, the pollen morphology of the genus *Lycopus* is poorly known, and only a few previous studies have been conducted on it. Only brief notes without descriptions of one or a few taxa in *Lycopus* have been given by Erdtman *et al.* (1963), Moore *et al.* (1991), Wang *et al.* (1991), Pozhidaev (1992), and Trudel and Morton (1992) so far. Recently, Wagstaff (1992) studied the pollen morphology of the tribe Menthaeae *sensu* Bentham (1876), but he did not treat any taxa of *Lycopus*.

The objectives of this paper are to provide a detailed account of the pollen morphology of *Lycopus* as a whole by light microscopy (LM), scanning electron and transmission electron microscopy (SEM & TEM), and to determine the extent to which these palynological data can be used as a taxonomic character in the genus.

## Material and methods

Pollen grains of the 15 recognized species (34 specimens) of *Lycopus* were mostly taken from herbarium material housed at the following herbaria: GH, KHUS, S, UPS and US (acronyms follow Holmgren *et al.* 1990), and also in part from plants recently collected in Korea by the authors (vouchers listed in Appendix).

Fully matured anthers were removed from the specimens and were prepared by the standard acetolysis method (Erdtman 1960), after which they were mounted in glycerin jelly and sealed with paraffin wax for light microscopy (LM). Measurements and morphological observations were made with an Olympus BX-41 microscope. Measurements of polar axis (*P*), equatorial diameter (*E*), colpus length (*CL*), and exine thickness (*ET*) were taken of 20 pollen grains per species under the LM ( $\times 400$ ). The quotient *P/E* is given in Table 1, and histograms are also provided for comparing the frequency of pollen shapes among the taxa.

For scanning electron microscopy (SEM), acetolysed pollen grains in a 70% ethanol solution were pipetted directly onto aluminium

stubs with double sided cellophane tape, and air-dried at room temperature under an inverted flask. Specimens were coated with gold using a JFC-1100E ion sputter, then examined in a JEOL JSM-5200 at 20 kV and photographed.

For transmission electron microscopy (TEM), fresh pollen grains of two taxa (*Lycopus sinuatus* and *L. lucidus*) were maintained for ca. 50 hours in TAG-solution (1% tannic acid + 1 glutaraldehyde in 0.1 M phosphate buffer, pH = 7.4). Following dehydration in alcohol solutions (Ruzin 1999), grains were embedded in araldite resin using a rapid embedding technique described by Skvarla (1966). Sections were cut with a Sovall MT 6000 ultramicrotome, stained with 1% aqueous uranyl acetate for 20 min at room temperature and with lead citrate for 5 min. The sections, on copper grids, were examined in an Hitachi H7100.

The pollen terminology in general follows Harley (1992), Harley *et al.* (1992) and Punt *et al.* (1994).

## Results and discussion

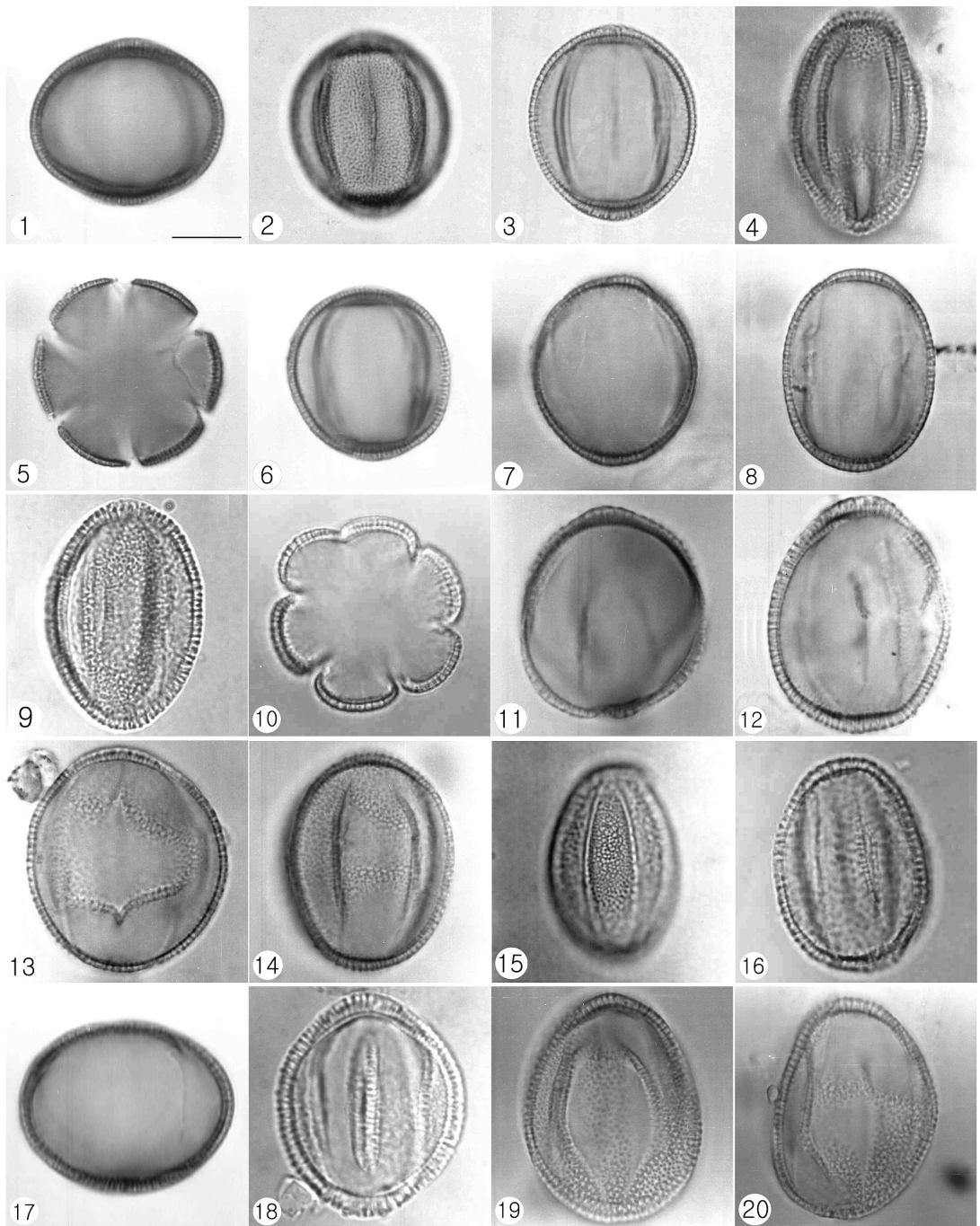
The pollen size and shape measurements are provided in Table 1. Representative pollen grains are illustrated in Figs. 1–34, and the shape frequency of pollen for each taxon in equatorial view is presented graphically in Fig. 35.

The pollen grains are monad and mostly medium or sometimes small: size *P* (polar axis) = 22.5–38.7  $\mu\text{m}$ ; *E* (equatorial diameter) = 20.0–35.0  $\mu\text{m}$ . Among the species investigated, the largest pollen grains on average were observed in *Lycopus asper*. Trudel and Morton (1992) provided brief pollen data without descriptions of the six North American taxa. Most of the taxa they investigated had more or less similar pollen morphologies to these examined by us, except for the differences in size and in the ratio of the polar axis and equatorial diameter. In two taxa, there are striking size differences when compared with our data, the pollen being notably larger: the polar axis in *L. asper* and *L. rubellus* is 14.4–(17.3)–24.0  $\mu\text{m}$  and 12.0–(13.7)–16.8  $\mu\text{m}$ , while our results for the same taxa are 31.25–(35.56)–38.75  $\mu\text{m}$  and 23.75–(25.81)–30.00  $\mu\text{m}$ , respectively. However,

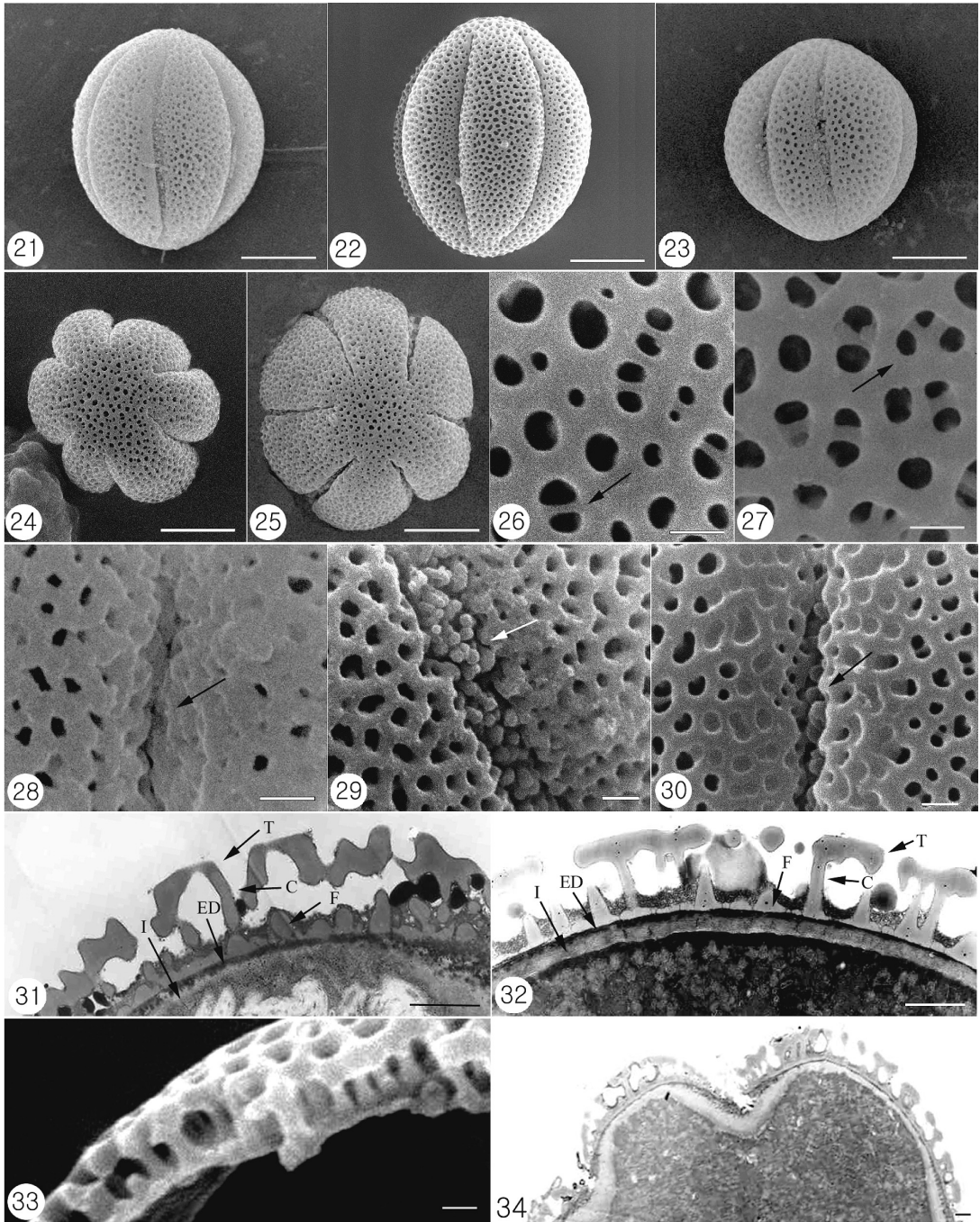
it is difficult to ascertain whether or not these size differences relate either to the geographical variation or chromosome number (especially polyploidy). So far, chromosome numbers for only a few taxa (e.g., *L. americanus*, *L. asper*, *L. europaeus*, *L. rubellus*, *L. uniflorus*) in the genus have been reported (Gill 1980, Webber & Ball 1980). Nevertheless, the reported taxa of *Lycopus* are all diploid and have the same chromosome numbers,  $2n = 22$  ( $n = 11$ ). Thus, at the moment, no association seemingly exists between pollen grain size and chromosome number.

It is interesting to note that the breeding system of many *Lycopus* taxa is known to be gynodioecy, i.e. plants with hermaphrodite flowers and plants whose flowers are functionally female, the male organs being reduced in size and sterile (cf. Owens & Uberta-Jiménez 1992). Trudel and Morton (1992) reported two malformed pollen grains in *L. amplectans* and *L. rubellus*, however, they did not consider these aberrant pollen types in connection with the possibly different sexual condition (in particular female) of studied taxa. Thus, this observation should be more carefully reexamined, particularly in respect to the sexual system of the genus *Lycopus*.

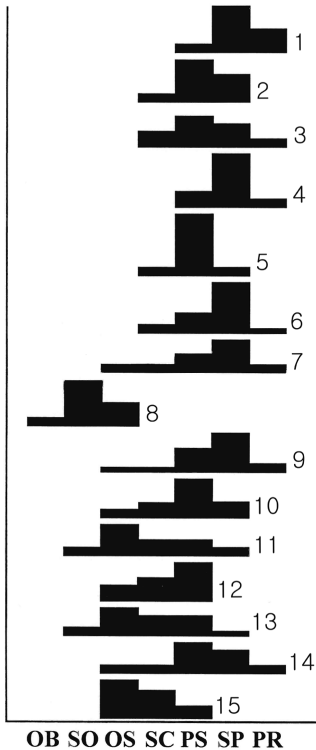
The polar outlines are more or less circular (Figs. 5, 10, 24–25), oblate to prolate ( $P/E = 0.85$ – $1.27$ ). However, the shapes of pollen in equatorial view are variable among the taxa, even within the same taxon (Figs. 1–8). They are most frequently oblate-spheroidal or prolate-spheroidal in the majority of studied taxa, except in *Lycopus laurentianus*, in which they are oblate to oblate-spheroidal (Fig. 35). León-Arencibia and La-Serna Ramos (1992) reported similar intraspecific heterogeneity for pollen shape in the genus *Lavandula*. It is, however, noteworthy that the shape of Lamiaceae pollen is often affected by the state of hydration and/or fixation (Sebsebe & Harley 1992). The pollen grains in this group frequently undergo a dramatic shape change as a result of colpal membrane loss during acetolysis. As a result of hot acid treatment a naturally hydrated, oblate or suboblate grain frequently becomes subprolate or prolate, because in the absence of the colpal membranes the inter-colpal areas of tectum tend to close in (Harley 1992). Therefore, it may be considered that differences



**Figs. 1–20.** LM micrographs of pollen grains of *Lycopodium*. — 1–5: *L. lucidus*. — 6–8: *L. rubellus*. — 9–10: *L. amplexens*. — 11: *L. asper*. — 12: *L. australis*. — 13: *L. ramosissimus*. — 14: *L. americanus*. — 15: *L. europaeus*. — 16: *L. exaltatus*. — 17: *L. laurentianus*. — 18: *L. maackianus*. — 19: *L. uniflorus*. — 20: *L. sinuatus*. 5, 10 (polar view), the remainder (equatorial view); 1, 3, 5–8, 10–13, 16, 17, 18 (low focus); 2, 4, 9, 15 (high focus); 14, 19–20 (high-mid focus). 1–20 to same scale; scale bar on 1 = 10  $\mu\text{m}$ .



**Figs. 21–34.** SEM and TEM micrographs of pollen grains of *Lycopus*. — 21: *L. europaeus* (equatorial view: EV). — 22: *L. lucidus* (EV). — 23: *L. exaltatus* (EV). — 24: *L. lucidus* (polar view: PV). — 25: *L. laurentianus* (PV). — 26: *L. lucidus*. — 27: *L. exaltatus* (26–27: surface detail towards pole). — 28–29: *L. maackianus*. — 30: *L. lucidus* (28–30: surface detail, mesocolpium and colpus margin). — 31: *L. sinuatus*. — 32–34: *L. lucidus* (31–32: transverse section, mid mesocolpium). 21–30, 33 (SEM); 31–32, 34 (TEM); T (tectum), C (columella), F (footlayer), ED (endexine), I (intin). Scale bars on 21–25 = 10  $\mu\text{m}$ ; 26–34 = 1  $\mu\text{m}$ , respectively.



**Fig. 35.** Histograms of the frequency of pollen grains in the studied *Lycopus* taxa, according to their shapes in equatorial view. The number of each histogram is correlated with taxon in Table 1. OB = oblate, SO = sub-oblate, OS = oblate-spheroidal, SC = spherical, PS = prolate-spheroidal, SP = subprolate, PR = prolate.

in shape of *Lycopus* pollen grains are neither particularly significant nor useful in taxonomic applications. In order to keep the natural form of the pollen grains, more sensitive treatments (e.g., critical pointing drying process for the fresh material) will be needed.

All species examined in the present study are 6-zonocolpate. The ectocolpi are distributed symmetrically, (Figs. 5, 10, 24–25), elongated, usually shallow, narrowing at the poles with weakly developed granular membranes, mostly visible in SEM (Figs. 28–30). Some colpi are formed the rounded amb into three big and three slightly smaller mesocolpia like many hexacolpate Lamiaceae pollen grains as discussed by Pozhidaev (1992). The range of colpi length of all studied taxa is 15.00–25.00  $\mu\text{m}$  (15.00–17.50  $\mu\text{m}$  in *Lycopus ramosissimus* and 21.25–25.00  $\mu\text{m}$  in *L. europaeus*; cf. Table 1). The length

of colpi is not correlated with the whole pollen size;

The exine is slightly thicker at the poles than at the equator (1.5–2.5  $\mu\text{m}$ ; Figs. 3, 8–9, 12, 18), bi-reticulate (terminology as discussed in Harley *et al.* 1992, Harley 1992). Lumina of the primary reticulum are shallow, usually rounded (Figs. 26–27) or occasionally more or less polygonal, especially near the apertural area (Figs. 28–30); the average diameter of lumina is relatively small (usually less than 1  $\mu\text{m}$  long, at longest axis).

TEM details of *Lycopus sinuatus* and *L. lucidus* (Figs. 31–32, 34) are as follows. The tectum is slightly thicker than the foot layer. Columellae are more or less closely spaced (Figs. 31–33), unbranched, comparatively long, nearly twice the thickness of the tectum. Interbacular and infratectal areas are filled with a dark-stained, compact, probably lipidic material (Fig. 34). The foot layer is distinctly continuous, thin or slightly thick in places where it anastomoses with the base of bacula, thicker and cone-shaped. Endexine is continuous, lamellated, slightly thinner than the foot layer or tectum. Such lamellations appear to be characteristic of pollen wall stratification of many species in the Lamiaceae (Nabli 1976, Harley 1992). Intine is nearly as thick as the foot layer/tecutum, and there is an underlying light-stained, thin layer of granular composition, sparse in the mesocolpium, denser in apertural region (Figs. 31–32, 34).

Briquet (1896) divided the genus *Lycopus* into two sections: sect. *Stoloniferi* and sect. *Astolonosi* by root systems. According to his classification, only *L. sinuatus* is included in sect. *Stoloniferi*, and the remaining species are included in sect. *Astolonosi*. However, on the basis of the present data, there are no significant differences in pollen morphology between *L. sinuatus* and the other taxa in the genus. Thus, pollen morphology does not appear to support Briquet's (1896) proposal that *L. sinuatus* be assigned to sect. *Stoloniferi* and segregation of two sections in *Lycopus*.

Abu-Asab and Cantino (1992) presumed that the pollen with unbranched columellae is the plesiomorphic condition on the basis of outgroup comparison in the Lamiales. If so, it can be speculated that phylogenetically, the pollen types

of *Lycopus*, which also has unbranched columellae (Figs. 31–34), may occupy a basal position within tribe Mentheae. In addition, Wagstaff (1992) suggested that some taxa (e.g., *Elsholtzia*, *Perilla*, *Perillula*, etc.), which have the plesiomorphic pollen type with ten calyx nerves, are primitive groups, while the other taxa, which have 13 or 15 calyx nerves, are considered to be advanced within the tribe Mentheae. Since the number of calyx nerves of *Lycopus* is 13 (H.-K. Moon & S.-P. Hong pers. obs.), if we followed Wagstaff's (1992) speculation, the phylogenetic position of *Lycopus* certainly is in the latter group. Nevertheless, the phylogenetic condition of pollen data combined with other characters from other fields of botany within the tribe Mentheae should be considered with great care.

The genus *Mentha* has been considered to be a most closely related taxon to *Lycopus* in the tribe Mentheae (Briquet 1896, Henderson 1962). As already concluded in earlier studies (Jančić & Polić 1989, Trudel & Morton 1992, Wagstaff 1992), the pollen morphology in *Lycopus* and *Mentha* is very homogenous, the grains having no clearly defined suprategular reticulum, and does not provide much useful information for the intergeneric delimitation within the tribe Mentheae. Our present results are also in agreement with the earlier studies. However, further detailed study of pollen morphology in *Lycopus* including extended TEM treatment in this genus as well as its relatives in Mentheae, may be helpful in elucidating intergeneric relationships between them.

In conclusion, *Lycopus* is a stenopalynous genus with only slight variation (e.g., size differences) in pollen features. The similarity in exine structure and ornamentation, as well as the variability of the various parameters analyzed at interspecific level makes it hard to establish taxonomical boundaries and clearly shows the affinity of species as far as pollen morphological characteristics are concerned.

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## Appendix

SPECIMENS EXAMINED. — *Lycopus americanus*: **USA**. New York, Tompkins Co. Townley Swamp, 1917 *Gershoy* 8714 (S). — *L. amplexans*: **USA**. New Jersey, Camden, 1974 *Parker s.n.* (UPS); Massachusetts, 1901 *Kennedy & Williams & Fernald* 19 (S); Indiana, Jasper Co., 1949 *Friesner* 22967 (S). — *L. asper*: **Germany**. Otterburne, 1954 *Anonym.* 54/532 (S). — *L. australis*: **Australia?** Tarranatta, 1801 *Caley s.n.* (UPS). — *L. communis*: **USA**. St. George Bay, 1901 *Howe & Lang* 999 (UPS); Columbia, 1898 *Steele s.n.* (S). — *L. europaeus*: **Estonia**. Tallinn, 1860–1870 *Sengbusch* 193 (S). **Poland**. Troki Distr., Nowy Sącz, 1952 *Staszkiwicz* 180 (UPS). **Sweden**. Skåne, Borby, 1950 *Johansson s.n.* (S); Småland, Westervik, 1872 ?*Axelund s.n.* (S). — *L. exaltatus*: **Hungary**. Without precise locality, 1871 ?*Jauscher s.n.* (UPS). **Sweden**. Standort, 1904 *Vetter s.n.* (S). — *L. laurentianus*: **Canada**. Quebec, 1945 *Raymond & Kucyniak*

1459 (UPS); Quebec, 1943 *Victorin & Rolland* 56804 (S). — *L. lucidus*: **South Korea**. Kyungi-Do, Gaewee Mt., 1999 *Hong & Moon* 99817 (KHUS). **China**. Yang-hin-paing, 1955 *Anon.* 353 (UPS). **Russia**. Chabarovsk Prov., 1910 *Anon. s.n.* (S); *ibid.*, 1910 *Desoulavy* 3647a (S). — *L. maackianus*: **South Korea**. Kyungi-Do, In-Cheon, 2001 *Hong & Moon* 01913 (KHUS). **Japan**. Hondo, Musashi, 1951 *Togashi* 381 (US); Hondo, Ugo Prov., Minamiakita-gun, 1953 *Kato s.n.* (S). — *L. membranaceus*: **USA**. Michigan, Emmett Co., 1933 *Gleason s.n.* (S). — *L. ramosissimus*: **Japan**. Kai Prov., Kawaguchi Lake, 1958 *Furuse s.n.* (S); Yokohama, 1862 *Anon. s.n.* (GH). — *L. rubellus*: **USA**. North Carolina, Biltmore, 1903 *Anon.* 2766d (S); Missouri, Campbell Mt., 1895 *Bush* 469 (S); South Carolina, Berkeley Co., 1966 *Bradley & Sears* 3525 (S); North Carolina, Columbus Co., 1968 *Leonard* 2198 (S); Arkansas, Ashley Co., 1938 *Demaree* 18586 (S). — *L. sinuatus*: **USA**. Wyoming, 1894 *Nelson* 542 (S); Iowa, Armstrong, 1883 *Cratty s.n.* (UPS). — *L. uniflorus*: **Canada**. Vancouver, Forest Distr., 1959 *Haney* 59 (UPS).