

MICRO-MACROMORPHOLOGICAL SURVEY OF SOME LATHYRUS SPECIES IN IRAN

F. Sharifnia, R. Soleymani, & F. Salimpour

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Taxonomy of the genus *Lathyrus* with 23 species in Iran has been controversial especially at sectional level. This study is based on the morphological and palynological evidences. 28 morphological and 7 palynological characters belonging to 13 Iranian *Lathyrus* species were examined. Our results showed that *L.* sect. *Nissolia* with simple leaves is monotypic and includes only *L. nissolia*. *Lathyrus vinealis*, *L. sphaericus*, *L. inconspicuus* were transferred from the section *Nissolia* to the section *Linearicarpus* based on the flower and pollen morphology. Morphological and palynological evidences were also used for transferring *L. gorgoni* and *L. rotundifolius* from the section *Gorgoni* to the section *Lathyrus*. In addition, based on the stipule, tendril and pollen morphology, *L. pratensis* was segregated from *L. aphaca* and transferred to *L.* sect. *Pratensis*.

Fariba Sharifnia, R. Soleymani, & Fahimeh Salimpour, Dep. of Biology, Faculty of Sciences, Islamic Azad University, North Tehran Branch, P. O. Box: 19585-936 email.: fa.sharifnia@gmail.com

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بررسی میکروماکرومورفولوژی برخی گونه‌های جنس *Lathyrus* در ایران

فریبا شریف‌نیا، دانشیار گروه زیست‌شناسی، دانشکده علوم پایه، دانشگاه آزاد اسلامی واحد تهران شمال.

روشنک سلیمانی، دانشجوی گروه زیست‌شناسی، دانشکده علوم پایه، دانشگاه آزاد اسلامی واحد تهران شمال.

فهیمة سلیم‌پور، استادیار گروه زیست‌شناسی، دانشکده علوم پایه، دانشگاه آزاد اسلامی واحد تهران شمال.

جنس *Lathyrus* از تیره بقولات (*Fabaceae*) و تبار *Vicieae*، مشتمل بر ۲۳ گونه یکساله و چند ساله در قالب ۱۰ بخشه می‌باشد. مطالعات حاکی از تناقضاتی در بخش‌بندی گونه‌های این جنس می‌باشد. در تحقیق حاضر گونه‌های جنس *Lathyrus* براساس صفات ریخت‌شناسی و گرده‌شناسی مورد بررسی قرار گرفتند و نتایج نشان داد که بخشه *Nissolia* با داشتن برگ‌های ساده، بخشه ای منوتیپیک و مشتمل بر گونه *L. nissolia* می‌باشد. نتایج گرده‌شناسی نیز این مساله را تایید نمود. همچنین گونه‌های *L. sphaericus*، *L. vinealis* و *L. inconspicuus* بر اساس ساختار گل و ریخت‌شناسی گرده این گونه‌ها به بخشه *Linearicarpus* منتقل گردیدند. گونه‌های *L. gorgoni* و *L. rotundifolius* نیز بر اساس شواهد ریخت‌شناسی و داده‌های گرده‌شناسی به بخشه *Lathyrus* انتقال داده شدند. همچنین دو گونه *L. aphaca* و *L. pratensis* به دلیل تفاوت‌های بارزی که از نظر صفات گوشوارک، منشا پیچک و تفاوت در نوع تزئینات سطح آگزین نشان می‌دهند، در دو بخشه مجزای *Aphaca* و *Pratensis* قرار گرفتند.

INTRODUCTION

The genus *Lathyrus* L. (*Fabaceae*, *Vicieae*) comprises ca. 160 annual and perennial species in the world (Allkin et al. 1986). The main centre of biodiversity for *Lathyrus* is located in the Mediterranean region; however some of its species are distributed elsewhere in other regions of Asia, Europe and America (Kupicha

1983, Allkin 1985, Allkin et al. 1986 and Goyder 1986).

Using biochemical markers like anthocyanins, flavonoids and amino acids, Bell et al. (1964) classified the genus into three groups. Based on the vegetative characters like habit, morphology of stipule and tendrils (simple vs. branched) and number of leaflets, Kenicer et al. (2005) classified 53 species of the genus into 11

Table 1. *Lathyrus* species included in the morphological study. Those material which also included in palynological study is marked with asteroid (*).

<i>L. vinealis</i> Boiss. :	Iran, Luristan, Boroujerd, Kioreh village, 1650 m, Soleimani 9000.
<i>L. pratensis</i> L.*:	Iran, Tehran, Chalus road, Pol-Zangouleh, opposite to Siah-Bisheh, 2450 m, Soleimani 9001.
<i>L. aphaca</i> L.*:	Iran, Hamedan, Nahavand, Firozan, Garin mountain, 1875 m, Soleimani 9002.
<i>L. cyaneus</i> (Stev.) C. Koch:	Iran, Azerbaijan, Kaleybar to Hejrandoost, 1700 m, Karafarin 9005.
<i>L. boissieri</i> Sirj.*:	Iran, Lorestan, Sefidasht, 1850 m, Soleymani 9020.
<i>L. roseus</i> Stev.*:	Iran, Tehran, Chapdarre, 2500 m, Salimpour 9006.
<i>L. sativus</i> L.*:	Iran, Gilan, Rostam Abad to Rasht, 1480 m, Salimpour 9007.
<i>L. cicera</i> L.*:	Iran, Tehran, Karaj, 5 Km to Hashtgerd, 1400 m, Salimpour 9010.
<i>L. rotundifolius</i> Willd.*:	Iran, Chalous road, near Siahbisheh, 2350 m, Soleymani 9011.
<i>L. annuus</i> L.*:	Iran, Tehran, Varamin, near terminal, 1050 m, Soleymani 9012.
<i>L. inconspicus</i> L.*:	Iran, Kermanshah, Biston mountain, near Songhor village, 2050 m, Soleymani 9013.
<i>L. pseudocicera</i> Pamp.*:	Iran, Azerbaijan, beach of Uromieh lake(southwestern), 1450 m, Soleymani 9016.
<i>L. chloranthus</i> Boiss.*:	Iran, Kermanshah, 220 m, Salimpour 9017.

sections. Kupicha (1983) defined the monotypic *Lathyrus* sect. *Nissolia* with the simple-leaved species *L. nissolia*, and also transferred the section *Cicerula* into *L. sect. Lathyrus*. In a molecular study performed by Asmussen & Listen (1998), taxonomy of *Lathyrus* has been widely changed.

There are some palynological studies on the genus *Lathyrus*. Using Surface Electron Microscopy (SEM) of eight species belonging to *L. sect. Orobus*, Tosheva et al. (2005) showed that pollen grain in *Lathyrus* is trioclate-porate, and exine ornamentations are very important to circumscribe different species.

Twenty-two annual and perennial species of *Lathyrus* have been reported for Iran. Recently a new species is reported from Iran. (Mozaffarian et al. 2008).

Rechinger (1979) in Flora Iranica grouped those Iranian species into 10 sections, though Nemati (2000) grouped them into two and nine subgenera and sections respectively. Using micro- and macromorphological evidences and electron microscopy, this study aims to clarify the taxonomy of 13 Iranian species of *Lathyrus* especially at sectional level.

MATERIAL AND METHODS

A. Plant material

Herbarium specimens belonging to 13 *Lathyrus* species were collected from different localities in Iran (Tab. 1). Identification of species was performed using Flora Iranica (Rechinger 1979), Flora of Turkey (Davis 1969), Flora of USSR (Fedchenko 1948), Flora Palaestina (Zohary 1972), Flora Europaea (Tutin et al. 1968), Flora of Iraq (Townsend 1974) and the new Flora of Iran (Nemati 2000, in Farsi).

B. Morphological studies

Twenty-eight quantitative and qualitative characters were examined. Qualitative characters include duration, form of stem wing, presence or absence of indumentum on the stem, angle of stems to each other, stem angles, lines on the stem, leaf form, leaf margin, petiole form, tendril origin, indumentum on stipule margin, inflorescence, style form, indumentum on the style. Quantitative characters include plant length, stem in diameter, stipule length and width, stipule length to width ratio, leaflet length and width, leaflet length to width ratio, petiole length, peduncle length, pod length and width and pod length to width ratio, but in this survey the qualitative characters had more impress on separation of taxa.

Characters and character state coding of qualitative characters are presented in Table 2. Hierarchical clustering analysis of 14 qualitative characters was performed using SPSS software version 9 with Ward method (Norusis 1999) and the resulting dendrogram was illustrated. Factor analysis was executed using SPSS software version 9 Principal Component Axes (PCA) were extracted and ordination of taxa was carried out on the first two PCA.

C. Palynological studies

Using a SEM microscope model XL30 (Philips), three individuals from each species were examined. Flowers were opened under a binocular microscope using a needle. Pollen grains were fixed on SEM stubs and then coated with a very thin layer of gold. SEM photographs from the equatorial view and the exine of pollen grains were captured at 2000x, 5000x, 10000 and 15000x magnifications. Character state coding was performed and similar methods for cluster and factor analysis as described above were used (see previous paragraph). Resulting dendrogram and PCA were illustrated.

Table 2. Distribution of morphological qualitative character states among the studied *Lathyrus* species. Character states are as follows: 1. stem wing (short 0/long 1), 2. stem indumentum (glabrous 0/ pubescent 1), 3. indumentum type (absent 0/ one-cellular 1), 4. lines on the stem (absent 0/ present 1), 5. stem with angle to the main axis (no 0/ yes 1), 6. leaf shape (absent 0/ lanecolate 1, elliptic 2, circular 3), 7. leaf margin (smooth 0/ dentate 1), 8. tendrill origin (without tendrill 0/ petiole 1/ leaf axis 2), 9. petiole (absent 0/ present 1), 10. Indumentum of the stipule margin (absent 0/ present 1), 11. inflorescence (single 0/ cluster 1), 12. style morphology (linear 0/ furrowed 1/ winged 2/ spatulate 3), 13. style indumentum (absent 0/ present 1) and 14. duration (annual 0/ perennial 1).

Species (abbreviations)	Characters													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
<i>L. vinealis</i> (vine)	0	0	0	1	1	1	0	1	1	0	0	2	0	0
<i>L. pratensis</i> (prat)	0	1	1	0	1	2	0	1	1	0	1	0	1	1
<i>L. aphaca</i> (apha)	0	0	0	0	1	0	0	2	0	0	0	3	0	0
<i>L. cyaneus</i> (cya)	0	0	0	0	1	1	0	1	1	0	1	0	1	1
<i>L. boissieri</i> (bois)	0	0	0	0	1	1	0	0	1	0	1	0	1	1
<i>L. roseus</i> (rose)	0	0	0	0	0	2	1	0	1	0	1	3	1	1
<i>L. sativus</i> (sati)	1	1	1	1	1	1	0	1	1	1	0	1	1	0
<i>L. cicera</i> (cic)	1	1	1	1	1	1	0	1	1	1	0	1	0	0
<i>L. rotundifolius</i> (rot)	1	0	0	0	1	3	1	1	1	0	1	1	0	1
<i>L. annuus</i> (annu)	1	0	0	0	1	1	0	1	1	0	0	1	0	0
<i>L. inconspicus</i> (inco)	0	1	1	0	1	1	0	0	1	1	0	2	0	0
<i>L. pseudocicera</i> (pseu)	1	0	0	0	1	1	0	1	1	0	0	1	0	0
<i>L. chloranthus</i> (chlo)	1	1	1	0	1	2	0	1	1	1	0	1	0	0

RESULTS & DISCUSSION

A. Morphology

Fig. 2 shows the dendrogram resulting from clustering analysis of morphological data of 13 species. Ordination of studied species based on first two principal components are illustrated in Fig. 4. The variability among species was shown by the first principal components. ca. 31 % of overall variability belongs to indumentum, stem wing, stem angle, leaf shape and margin, stipule indumentum, tendrill origin and inflorescence. ca. 24.5 % of overall variability belongs to stem surface and absence or presence of petiole, and ca. 14 % of overall variation belongs to stigma indumentum and morphology.

As demonstrated in Fig. 4 (PCA), *L. aphaca* is well segregated from other species. Its line is divided from others at the linkage distance 25. *Lathyrus aphaca* has unique combination of characters like leaf-like stipules, annuality, origin of tendrile (leaf axis) and absence of petiole, therefore it is segregated from other species and placed in *L. sect. Aphaca*. Other species divided into two clusters at the linkage distance 13 (Fig. 2). First subcluster of cluster 1 comprises species belonging to *Lathyrus* sect. *Lathyrus* including *L. annuus*, *L. sativus*, *L. chloranthus*, *L. pseudocicera*, *L. rotundifolius* and *L. cicera*. In PCA analysis of first two components, species pair *L. annuus* and *L. pseudocicera*, and species pair *L. sativus* and *L. cicera* from the section *Lathyrus* seem to be at the same

position, and *L. vinealis* and *L. inconspicus* from the section *Linearicarpus* are very close to each other (Fig. 4). A PCA analysis of the first three components will therefore be resulted in a better resolution (not shown).

Six species of subcluster 1 were placed in three different sections by Rechinger (1979) naming *Cicerula*, *Lathyrus* and *Cicera*. These six species are distinguishable from other *Lathyrus* species by having unique furrowed styles and regarding our results should be placed in a separate section. Molecular studies of Asmussen et al. (1998) indicated their close similarity and their placement in a single section.

Second subcluster of cluster 1 includes *L. vinealis* and *L. inconspicus* from the section *Linearicarpus* (Kupicha 1983) which were placed in the section *Nissolia* by Nemati (2000). They are differentiated from other species belonging to *L. sect. Nissolia* by having winged styles, a character common in *L. sect. Leanericarpus*. We here agree with Badr et al. (2002) which suggested their placement in the section *Leanericarpus*.

Lathyrus boissieri and *L. cyaneus* both from *L. sect. Lathyrostylis* characterized by having compound leaves, linear and hairy style are grouped under cluster 2 (Fig. 2).

Table 3. Distribution of palynological character states and measurements among the studied *Lathyrus* species. Characters are as follows: 1. pollen morphology (elliptic 0/ oblong 1), 2. Polar axis length "P" (µm), 3. equatorial axis length „E“ (µm), 4. P/E, 5. distance between grooves (µm), 6. mesoculpium (µm), and 7. Exine surface ornamentation (reticulate 0/ reticulate-porate 1).

Species (abbreviations)	Characters						
	1	2	3	4	5	6	7
<i>L. pratensis</i> (prat)	1	34.32	18.03	1.9	11.95	1.4	1
<i>L. aphaca</i> (apha)	1	31.36	19.84	1.58	9.84	1.75	0
<i>L. boissieri</i> (bois)	0	37.9	27.04	1.4	12.07	1.27	0
<i>L. roseus</i> (rose)	1	33.97	17/13	1.98	7.79	1.09	1
<i>L. inconspicua</i> (incon)	0	24.15	21.44	1.12	7.49	3.01	0
<i>L. sativus</i> (sati)	0	34.09	23.62	1.44	16.91	1.54	1
<i>L. cicera</i> (cic)	0	34.25	24.33	1.4	15.94	3.01	0
<i>L. rotundifolius</i> (rotu)	0	30.39	23.77	1.27	11.42	2	0
<i>L. annuus</i> (ann)	0	27.73	22.13	1.25	12.5	1.88	1
<i>L. chloranthus</i> (chlo)	0	7.11	23.33	1.59	9.43	1.63	0

According to Flora of Iran (Nemati 2000), *Lathyrus* sect. *Orobon* includes *L. roseus* and *L. tuberosus*. However they have different style morphology: *Lathyrus tuberosus* has furrowed style, while *L. roseus* has spatulate style and characteristic aristate leaflets which is more related to morphology of *L. sect. Orobon*. Our results are congruent with those of Dogan et al. (1992) which segregated *L. roseus* from *L. sect. Lathyrus* and transferred it to *L. sect. Orobon*.

Nemati (2000) placed *L. pratensis* in *L. sect. Aphaca*. While *Lathyrus pratensis* is different from the section *Aphaca* in having sagittate stipules, winged petals, and linear and hairy styles. In the dendrogram, *L. pratensis* is however clearly differentiated from the line including *L. aphaca*. In agreement with results obtained from the molecular studies (Asmussen & Liston 1998), our results confirm its placement in the section *Pratensis*.

B. Palynology

The dendrogram resulted from clustering analysis of palynological data of 10 species is illustrated in Fig. 3. Fig. 5 demonstrates the ordination of studied species based on first two principal components. The variability among species was shown by the first 5 principal components. Slightly less than 38 % of overall variability belongs to variability in mesoculpium and exine surface ornamentations, and ca. 36 % belongs to polar axis length, equatorial axis length and the distance between colpi.

Similar to the dendrogram resulted from analysis of morphological data, *L. aphaca* has the minimum similarity with other species and is segregated from them at the linkage distance 25 (Fig. 3). Also PCA

results are highly congruent with those of morphological analysis (Fig. 5). *Lathyrus aphaca* is distinguishable from other *Lathyrus* species with having polar axis length of 31.36 µm, equatorial axis length of 19.84 µm, 9.84 µm distance between grooves, mesoculpium equal to 1.75 µm and perforate exine surface ornamentations (Tab. 3, Fig. 1). These results clearly confirm our morphological conclusions resulted in placing *L. aphaca* in the section *Aphaca*. *Lathyrus inconspicua* from *L. sect. Linearicarpus* is characteristic in having polar axis length of 24.15 µm, equatorial axis length of 21.44 µm, 7.49 µm distance between grooves, mesoculpium equal to 3.01 µm and reticulate exine surface ornamentations (Fig. 1), and was segregated from the rest at the linkage distance 17. The main cluster is divided into two subclusters at the linkage distance 13 (Fig. 3). The subcluster 1 includes *L. pratensis* and *L. roseus* which are very similar in pollen morphology and have low similarity to other species (Fig. 1; Table 3). In the second subcluster, *L. boissieri* from the section *Lathyrus* with its perforate exine ornamentation has less similarity to the rest (Fig. 1; Table 3). Other species including *L. sativus*, *L. cicera*, *L. annuus*, *L. rotundifolius* and *L. chloranthus* from the section *Lathyrus* are grouped in a single subcluster with relatively high similarity between species. *Lathyrus sativus* and *L. annuus* have perforate-reticulate and *L. rotundifolius*, *L. cicera* and *L. chloranthus* have reticulate exine ornamentations (Fig. 1). Results obtained from the PCA analysis of first two components are highly congruent with the dendrogram resulted from the cluster analysis of palynological data (Fig. 3). *Lathyrus pratensis* from the section *Pratensis* and *L. roseus* from the section

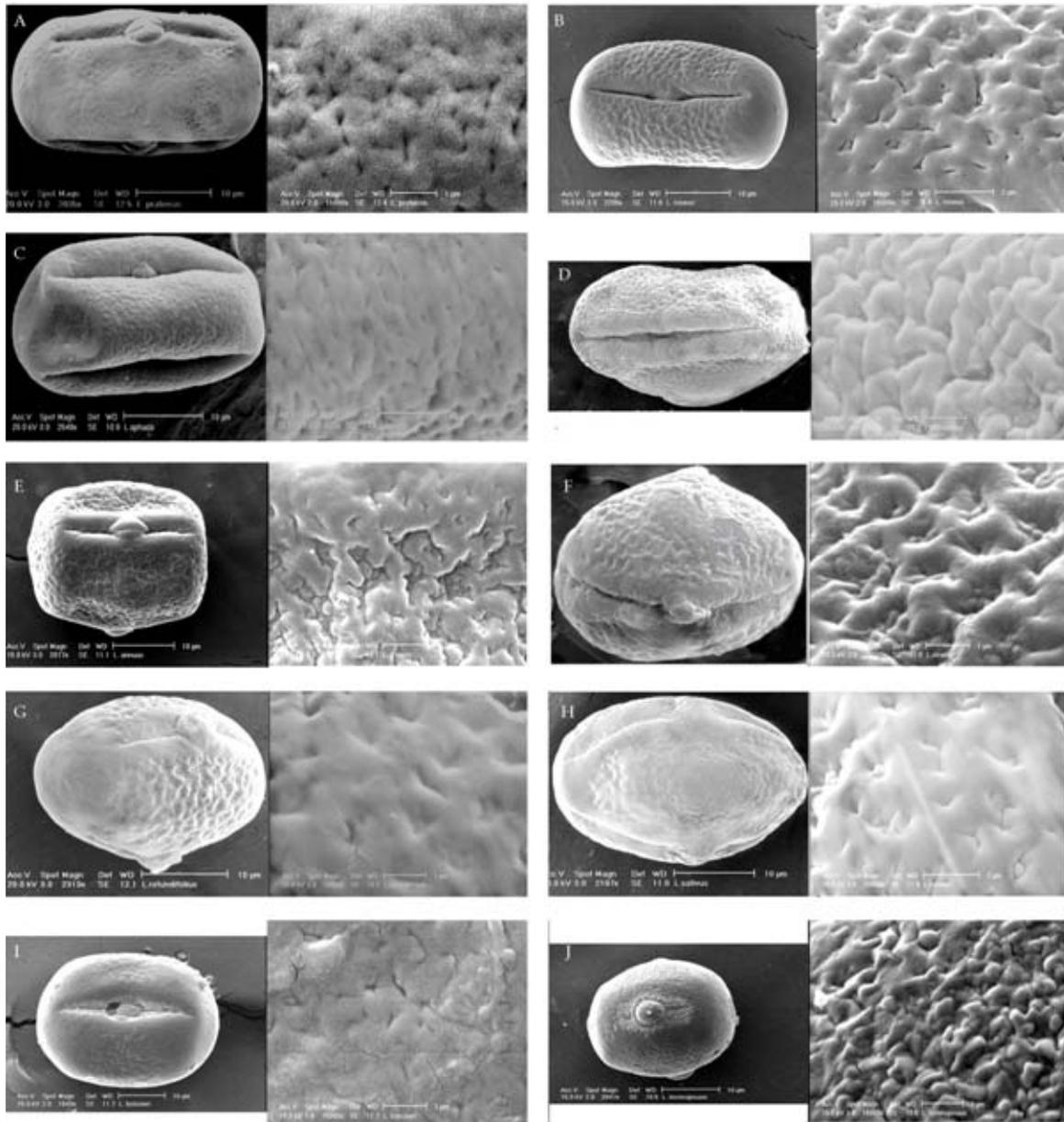


Fig. 1. Pollen morphology and exine surface ornamentation in A: *Lathyrus roseus*, B: *L. pratensis*, C: *L. aphaca*, D: *L. chloranthus*, E: *L. anuus*, F: *L. cicera*, G: *L. rotundifolius*, H: *L. sativus*, I: *L. boissieri* and J: *L. inconspicuus*.

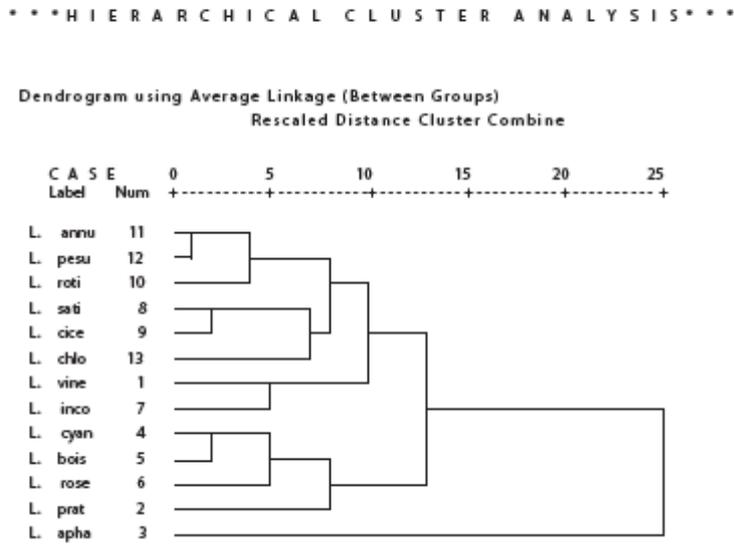


Fig. 2. Cluster analysis of some *Lathyrus* species resulted from analysis of morphological data using Average Linkage with Ward method. Abbreviations are as table 2.

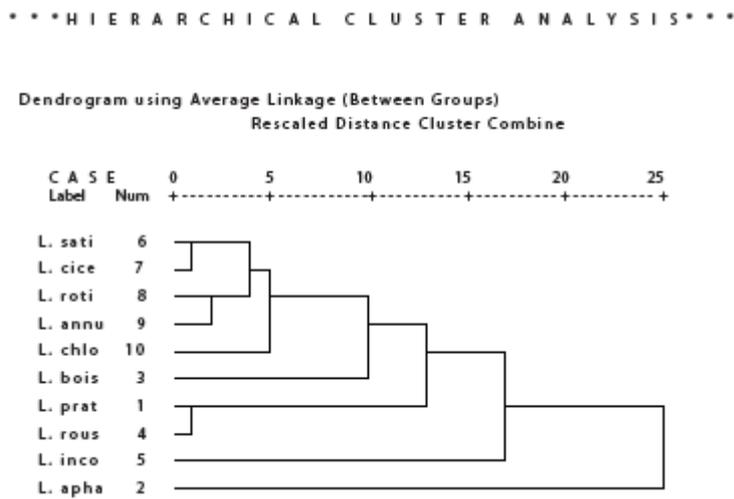


Fig. 3. Cluster analysis of some *Lathyrus* species resulted from analysis of palynological data using Average Linkage with Ward method. Abbreviation. as table of 3.

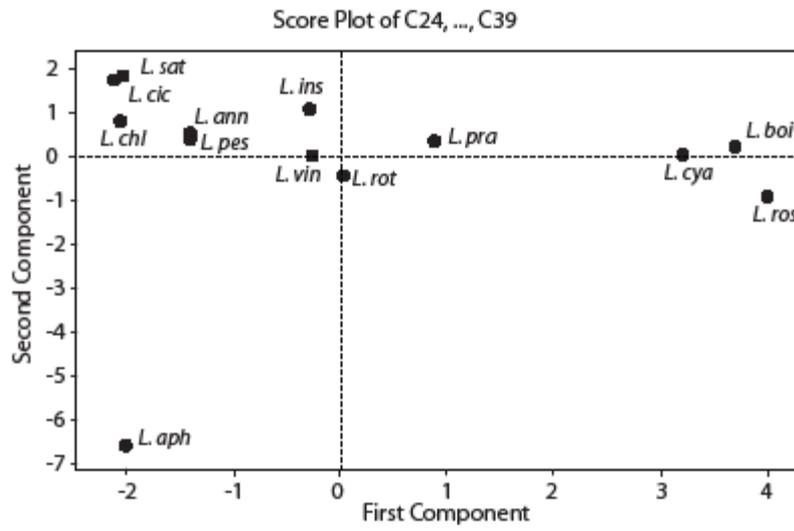


Fig. 4. Ordination of some *Lathyrus* species based on first two principal components using morphological data.

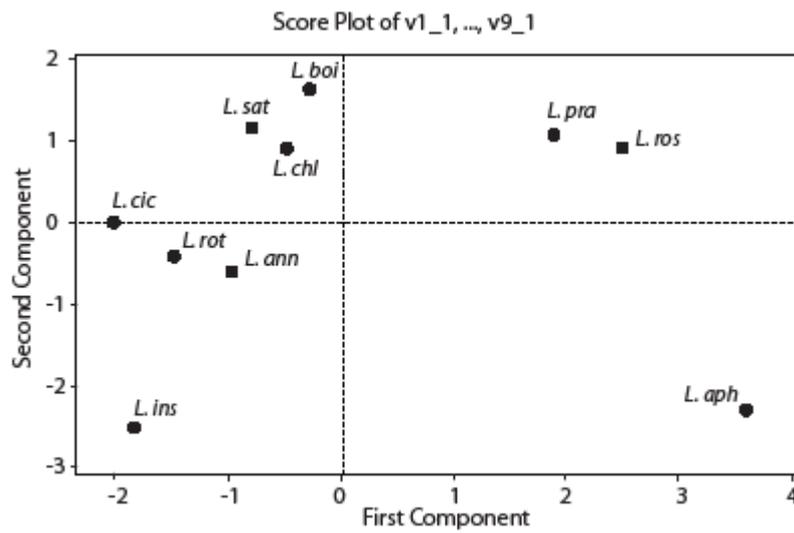


Fig. 5. Ordination of some *Lathyrus* species based on first two principal components using palynological data.

Orobon are close to each other and positioned relatively far to the rest (Fig. 5).

Our results show that the present taxonomy of the genus *Lathyrus* needs to be revised, and that morphology and palynology play an important role in definition of taxa at species and sectional levels.

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