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# A numerical taxonomic study of *Euphrasia paucifolia* complex (Scrophulariaceae) from Pakistan and adjoining areas

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&  
S. SHAHID SHAUKAT

## ABSTRACT

SIDDIQUI, T., M. QAISER & S. SHAHID SHAUKAT (1989). A numerical taxonomic study of *Euphrasia paucifolia* complex (Scrophulariaceae) from Pakistan and adjoining areas. *Candollea* 44: 521-530. In English, English and French abstracts.

A morphological analysis of *Euphrasia paucifolia* complex from Pakistan and adjoining areas was carried out. Principal component analysis of 53 OTUs was employed, which resulted in the recognition of *E. paucifolia* Wettst. and a new species *E. pseudopaucifolia* T. Siddiqui & Qaiser. The scanning electron microscopic studies of leaf, bract and calyx indumentum, pollen and seed surface showed the differences in both the species.

## RÉSUMÉ

SIDDIQUI, T., M. QAISER & S. SHAHID SHAUKAT (1989). Etude taxonomique du complexe *Euphrasia paucifolia* (Scrophulariaceae) du Pakistan et des régions voisines. *Candollea* 44: 521-530. En anglais, résumés anglais et français.

Une analyse morphologique du complexe *Euphrasia paucifolia* du Pakistan et des régions voisines a été faite. L'analyse par covariance de 53 éléments a été employée, avec pour résultat la délimitation de *E. paucifolia* et l'établissement de l'espèce nouvelle *E. pseudopaucifolia* T. Siddiqui & Qaiser. L'examen au microscope électronique à balayage de la pilosité des feuilles, des bractées et du calice, du pollen et de la surface des graines montre bien les différences entre les deux espèces.

## Introduction

*Euphrasia paucifolia* Wettst. is distributed in Himalayas and Karakorum from Kashmir and Astor to Spiti at an elevation of 3000-4000 m (Fig. 5).

During the preparation of an account of the genus *Euphrasia* L. for the Flora of Pakistan the senior authors came across several specimens identified as *E. paucifolia* Wettst. which were significantly different from each other. PENNELL (1943) has also observed the variability in the indumentum and the leaf size of *E. paucifolia* Wettst. However, it appears that he had not enough material at hand to reach any definite conclusion. The present authors had an opportunity to examine large number of specimens from Pakistan, Kashmir and India present in A, BM, GH, K, KUH, MICH, NA, NY, PH, RAW and US.

In order to assess and evaluate the variability found in this complex the present study was undertaken.

Quantitative			Qualitative		
1.	BL	Bract length	11.	BIT	Bract indumentum type: non-glandular hairy/glandular and non glandular
2.	BB	Bract breadth	12.	BS	Bract shape: ovate to broadly ovate/elliptic
3.	TKL	Total calyx length	13.	BA	Bract apices: acute-acuminate/obtuse
4.	KTL	Calyx tube length	14.	BLC	Bract lobe curvature: incurved/not incurved
5.	KLL	Calyx lobe length	15.	DBI	Density of bract indumentum: density hirsute with long hairs/sparsely hirsute with short hairs
6.	KLB	Calyx lobe breadth	16.	EKL	Equality of calyx lobes: lobes equal/lobes unequal
7.	TCL	Total corolla length	17.	NLL	Notch of lower lip of corolla: erose-slightly notched/widely notched
8.	CTL	Corolla tube length	18.	ECL	Equality of lower lip of corolla: left and right lobe of lower lip equal/left and right lobe unequal.
9.	ULL	Corolla upper lip length	19.	DKI	Density of calyx indumentum: densely hirsute/sparsely hirsute
10.	LLL	Corolla lower lip length	20.	KIT	Calyx indumentum type: non-glandular hair/glandular and non-glandular hairy

Table 1. — Variables used in the analysis of *Euphrasia paucifolia* complex.

## Materials and methods

### *Numerical methods*

A total number of over 150 specimens were studied and variation in 20 characters was observed (Table 1). The data thus obtained was subjected to numerical analysis. However, for numerical analysis complete specimens with flower and fruits were considered. Therefore 53 OTUs were used. To unravel the underlying structure in the OTUs by character matrix a trend seeking and data summarizing technique namely principal component analysis (PCA) was employed. R-technique of component analysis was performed using covariance as the resemblance function (ORLOCI & KENKEL, 1985). A stereogram of the fiftythree OTUs on the first three principal components was plotted on an Apple IIe microcomputer using the method of FEWSTER & ORLOCI (1978).

### *Scanning electron microscopy*

In order to study the indumentum of leaf, bract and calyx lobes, scanning electron microscopic studies were carried out using Jeol JSM-T200. The material was coated with gold by the conventional method. Seed surface and the exine stratification of the pollen were also studied with the help of SEM.

## Results and discussion

Figure 1 shows the biplot of first two principal components while Figure 2 shows the stereogram based on first three principal components. The first two components together account for 38.32% while the first three components together account for 47.95% of the total variability of the data matrix. The first principal component which explains 25.44% of the total variability is primarily a function of the following characters viz., bracts lobe curvature, density of the indumentum of bract, density of the indumentum of the calyx, length of the upper lip of the corolla and the breadth of the bract as indicated by the eigenvector coefficients (Table 2). The second component that accounts for 12.87% of the total variation is chiefly controlled by the total calyx length, length of the calyx lobes density of the indumentum of the calyx, length of the bract and breadth of the bract. The third component accounts for 9.63% of the total variability and is chiefly a function of the indumentum type of the bracts, total length of calyx, equality of lower lip of corolla, breadth of the bract and length of the corolla tube. The scatter of points in Figure 1 clearly indicate the

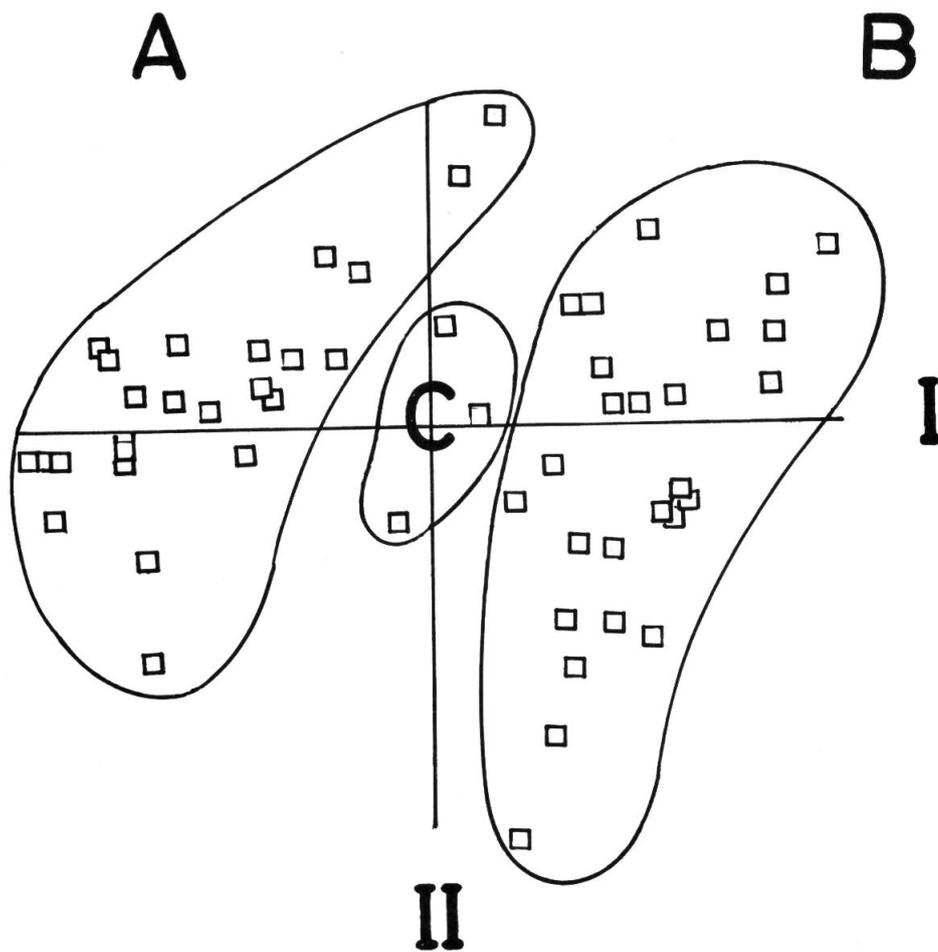


Fig. 1. — A biplot showing the distribution of OTUs on the first two principal components. The groups marked are **A** = *Euphrasia paucifolia*, **B** = *E. pseudopaucifolia*; **C** = intermediate.

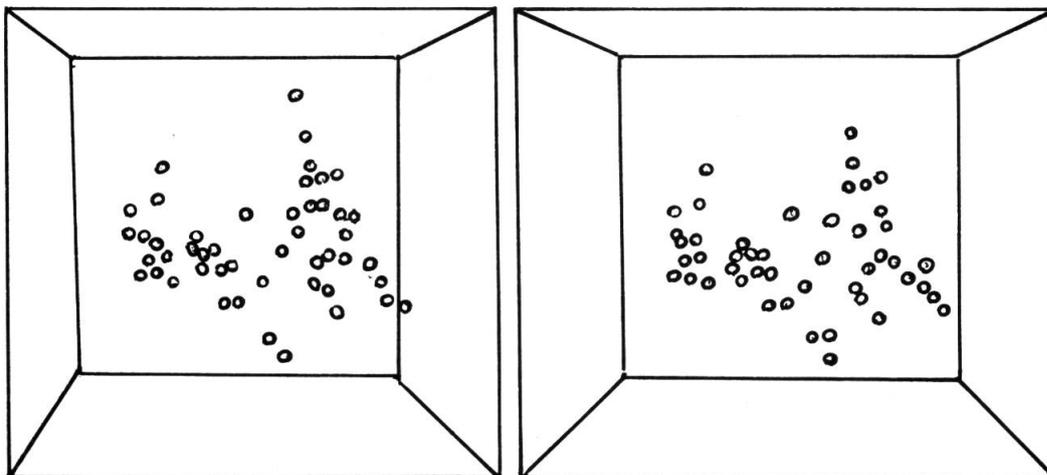


Fig. 2. — A stereogram showing the distribution of OTUs on the first three principal components.

Component	Eigenvalue	% Variance explained	% Cumulative variance	First 5 ranked eigenvector elements	Associated variable
1.	0.8993	25.442	25.442	-0.4549 -0.4523 -0.3856 0.3122 0.2909	BLC DBI DKI ULL BB
2.	0.4551	12.8747	38.316	0.4309 0.3431 0.3414 0.3249 0.3196	TKL KLL DKI BL BB
3.	0.3406	9.6375	47.954	0.6368 0.5518 0.3679 -0.2476 0.1560	BIT TKL ECL BB
4.	0.3014	8.527	56.481	0.5946 0.3988 -0.3014 0.2924 -0.2784	ECL TCL TKL CTL KTL

Table 2. — Results of principal component analysis of 53 OTUs by 20 characters. Eigenvalues and ranked eigenvector coefficients (first five) together with the associated variables for the first four principal components are presented. (For symbols refer to Table 1).

Character		$\bar{X}$	SE $\pm$	SD	C.V. %	t value	P
BL	A	5.629	0.178	0.9260	16.450	1.34	n.s.
	B	5.25	0.229	1.168	22.253		
BB	A	4.351	0.2148	1.116	25.6492	2.5	0.05
	B	3.596	0.229	1.168	22.253		
TKL	A	4.370	0.178	0.926	21.803	0.732	n.s.
	B	4.211	0.1276	1.208	15.450		
KTL	A	2.296	0.1140	0.5925	25.803	0.410	n.s.
	B	2.2307	0.092	0.4739	21244		
KLL	A	2.166	0.1193	0.6201	28.628	1.453	n.s.
	B	1.961	0.0780	0.3980	20.295		
KLB	A	0.638	0.042	0.222	34.877	0.620	n.s.
	B	0.596	0.055	0.2835	47.567		
TCL	A	7.166	0.215	1.308	18.252	1.062	n.s.
	B	6.826	0.243	1.240	18.170		
CTL	A	3.870	0.161	0.838	21.672	0.114	n.s.
	B	3.846	0.140	0.717	18.665		
ULL	A	3.370	0.195	1.015	30.118	2.824	0.01
	B	2.75	0.1009	0.514	18716		
LLL	A	3.796	0.1693	0.879	23.175	1.865	n.s.
	B	3.423	0.109	0.560	29.214		

Table 3. — Mean and SD for each variable in group A and B.

prevalence of two distinct group (delineated in Fig. 1) with only three OTUs occupying an intermediate position.

A comparison of quantitative attributes of groups A and B is given in Table 3. This table clearly indicates that only two out of ten characters showed significant difference between the two groups. Upper lip of corolla length ULL differed highly significantly ( $p < 0.01$ ) while bract breadth BB was also significantly different ( $p < 0.05$ ) between the two taxa. The intermediate group is not compared with the main groups because of small sample size.

WETTESTEIN (1896) has based his new species *Euphrasia paucifolia* Wettst. on the following 3 syntypes collected by Schlagintweit from India and high Asia viz. *Schlagintweit 5939, 8939, 8647*. The authors had an opportunity to examine one of his isosyntypes i.e. *Schlagintweit 5939* (PH!) and one more specimen, *Duthie 234* (K!) cited in his original publication. Both the specimens were included as OTUs in the present study. The isosyntype and Duthie's specimen fall within the group 'A' delineated by principal component analysis. Thus group 'A' represents the true *E. paucifolia* Wettst. However, in order to accommodate the group 'B', a new species *E. pseudopaucifolia* T. Siddiqui & Qaiser is described.

As far as the distribution of both the taxa is concerned there is a considerable overlapping (Fig. 5) and they are more or less sympatric. Since interspecific hybridization in the genus *Euphrasia* has been frequently reported earlier (YEO, 1956, 1966, 1978), it is likely that the intermediate OTUs emerging in the ordination diagram as Group 'C' represent a hybrid population. Which is also evident from its capsule which is smaller ( $2-3 \times 1-3$  mm) than *E. paucifolia* ( $3-6 \times 1.5-3.0$  mm) and *E. pseudopaucifolia* ( $3.5-4.5 \times 1.5-2.5$  mm) and lesser number of imperfectly developed seeds (2-4 seeds/capsule) while in *E. paucifolia* and *E. pseudopaucifolia* the seeds/capsule are 4-7 and 4-12 respectively. However, due to its small sample size no taxonomic rank is accorded. Nevertheless, key to both the species is given below.

- + Inflorescence dense and broad at the tip. Lobes of cauline leaves and bracts medianly incurved, densely long hisrute all over. Bracts ovate to broadly ovate

**1. *E. pseudopaucifolia***

- Inflorescence neither dense nor broad. Lobes of cauline leaves and bracts not medianly incurved, sparsely hirsute with short hairs on the margins only. Bracts elliptic

**2. *E. paucifolia***

**1. *Euphrasia pseudopaucifolia* T. Siddiqui & Qaiser, spec. nov.** (Fig. 3, A-F).

**Holotypus:** Kashmir: Sorus above Pahlgam, R. R. Stewart, 21606 (PH!), **isotypus** (K!, NA!, NY!).

Herba annua, 3.5-15 cm alta, ramorum paribus 0-3 erectis, flexuosis, brevibus vel longis, pilis eglandulosis et glanduliferis obsita. Internodia basalia 2-3 cm, media 1.0-1.5 cm, inflorescentiae 0.5-1 cm longa vel subnulla. Inflorescentia ad nodum binerum vel tertium incipiens. Folia bractee calycesque pilis longis eglandulosis et glanduliferis hirsuta; pili glanduliferi capite glanduloso ultra 6-plo longiores. Folia caulina  $4-7 \times 2-9$  mm, ovata ad late ovata, rarissime elliptica, 4-5-loba; lobi terminales et laterales obtusi vel acuti, medio incurvi; pili margine et ad lobos frequentes. Inflorescentia superne densa et lata. Flores 5.5-10 mm longi. Pedicelli quam 1 mm breviores vel subnulli. Bractee  $4-9.5 \times 3-9.5$  mm, ovatae vel late ovatae, 4-5-lobi terminales et laterales, medio incurvae. Calyx 3-7 mm longus, 4-lobus; lobi aequales vel inaequales,  $1.5-3.5 \times 0.5-1$  mm, lanceolati aristulati; tubus calycinus 1.5-3 mm longus. Corolla alba, violaceo-striata, fauce flava, dorso 5-9 mm longa; tubus 2-5 mm longus, extus imprimis ad labium superius dense hirsutus; labium superius 2-4 mm longum, bilobum, lobis aequalibus, deflexo-patulis, erosis vel leviter marginatis; labium inferius 3.0-6.0 mm longum, trilobum, lobis obovatis-oblongis, obcordatis, deflexo-patulis, inaequalibus, lobo mediano maximo  $1.5-3 \times 1-3.5$  mm, sinistro  $1.5-3 \times 1-3$  mm, dextro  $1.5-2.5 \times 1-3$  mm. Capsula  $3.5-4.5 \times 1.5-2.5$  mm, oblonga, retusa, apice obtusa, distaliter tenuiter ciliata, quam calyx brevior. Semina 4-12 per capsulam, ca.  $1 \times 0.5$  mm, oblonga, acuta, costis tenuibus pilosis.

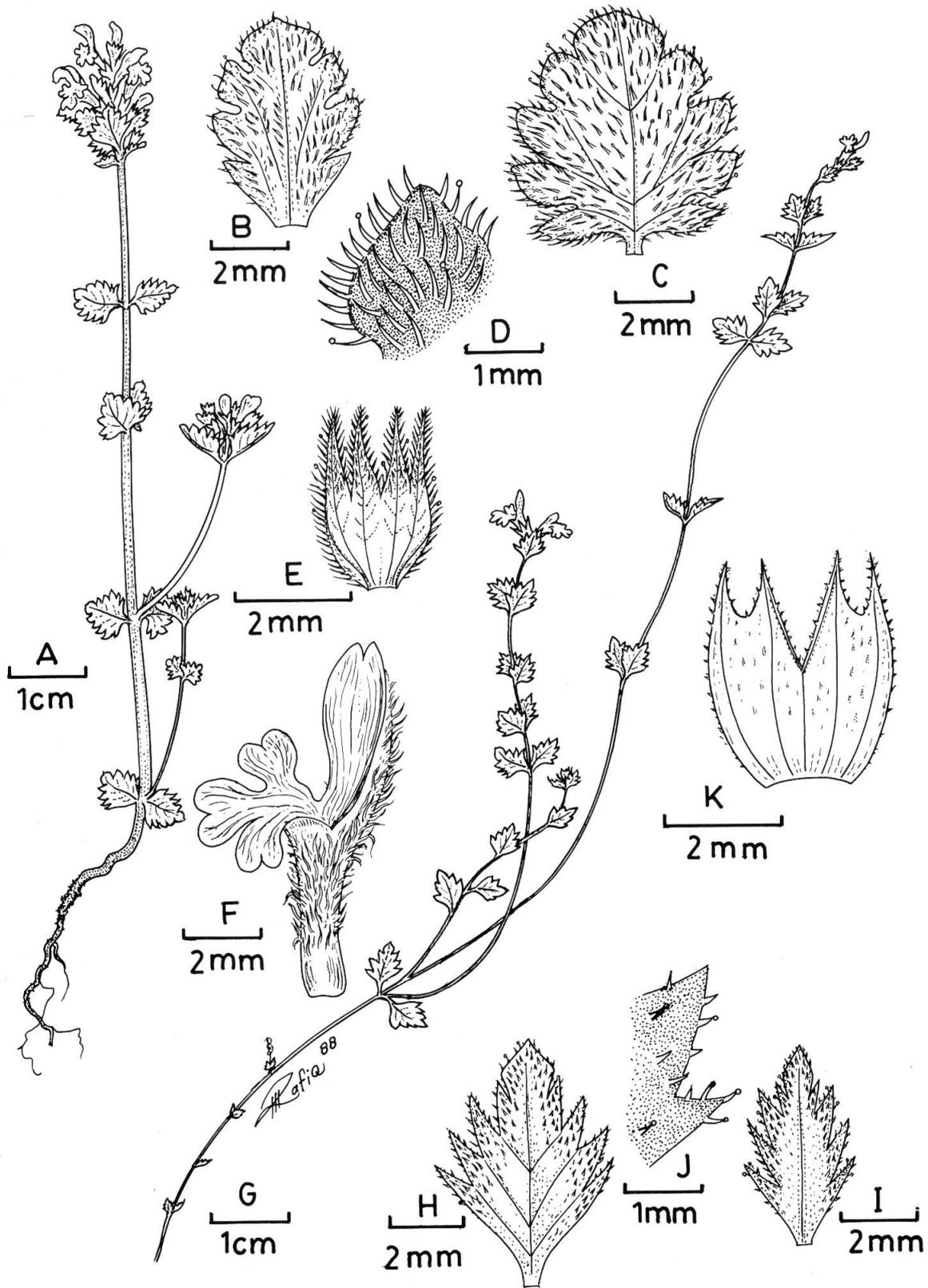


Fig. 3. — *Euphrasia pseudopaucifolia*: A, habit; B, leaf; C, bract; D, bract lobe magnified; E, calyx; F, corolla (all from Stewart, 21606).  
*Euphrasia paucifolia*: G, habit; H, bract; I, leaf; J, bract lobe magnified; K, calyx (all from Stewart 22696).

Annual herb, 3.5-15.0 cm tall, with 0-3 pairs of erect, flexous short-long branches, villose, hairs long, eglandular and glandular both. Internodal distance at the base 2.0-3.0 cm, at the middle 1.0-1.5 cm, at the floral axis 0.5-1.0 cm, or even absent. Flowering begins at node 2-3. Leaves, bracts and calyx surface covered with long hirsute eglandular rarely glandular hairs, stalks of glandular hairs more than 6 times as long as the heads. Cauline leaves 4.0-7.0 × 2.0-9.0 mm, ovate-broadly ovate very rarely elliptic, 4-5 lobed, terminal and lateral lobes obtuse-acute, medianally incurved, hairs frequent at the margin and at the lobes. Inflorescence, dense and broad at the upper portion. Flower 5.5-10.0 mm long, pedicels less than 1.0 mm or even absent. Bracts 4.0-9.5 × 3.0-9.5 mm, ovate-broadly ovate, 4-5 lobed, terminal and lateral lobes obtuse-acute, medianally incurved. Calyx 3.0-7.0 mm long, 4 lobed, lobes equal or unequal, 1.5-3.5 × 0.5-1.0 mm, lanceolate, aristulate, calyx tube 1.5-3.0 mm. Corolla white with violet streaks, throat yellow, dorsally 5.0-9.0 mm, long, corolla tube 2.0-5.0 mm, externally densely hirsute especially on the upper lip; upper lip 2.0-4.0 mm long, 2 lobed, lobes equal, deflexed-spreading, erose-slightly notched; lower lip 3.0-6.0 mm long, 3-lobed, lobes obovate-oblong, obcordate, deflexed-spreading, erose-slightly or widely notched, unequal, middle lobe largest 1.5-3.0 × 1.0-3.5 mm, left lobe 1.5-3.0 × 1.0-3.0 mm, right lobe 1.5-2.5 × 1.0-3.0 mm. Capsule 3.5-4.5 × 1.5-2.5 mm, oblong, retuse, tip obtuse, distally finely ciliate, shorter than calyx. Seeds 4-12/capsule, ca. 1.0 × 0.5 mm, oblong, acute, with white thin low hairy ridges.

#### *Specimens examined*

**Pakistan:** Chitral, ca. 3 Km south of Garm Chashma on way to Kindjall and Avarik, 19.6.1987, *Ghafoor & Omer*, 2593 & 2593A (KUH); Bumboret Gol, Kafiristan ca. 1800 m, 30.6.1987, *Ghafoor & Omer* 3302 (KUH); Kaghan, Sarul Kagan, 13.8.1897, *Duthie s.n.* (K); Saiful Maluk, 31.7.1954, *Shaukat Ali* 26274 (BM); **Kashmir:** Karsha Zaskar, 19-21.7.1933, *Koelz*, 5769 (MICH, NA, PH); Kargia Zaskar, 12-15.7.1933, *Koelz*, 5535 (MICH); Sonamarg, 11.8.1921, *Stewart*, 6621 (K, PH, US); Sorus above Pahlgam, 27.7.1925, *Stewart* 8049 (NY, RAW); Vicinity of Kolahoi, about 50 road miles north of Islamabad, via Pahlgam and Aran, July-August 1927, *Dickason*, 52 (MICH); Vicinity of Pahlgam on East Liddar river, 27 road miles north of Islamabad, July-August 1927, *Dickason*, 1638 (MICH); Vicinity of Sonamarg on the Sind river, 50 road miles east-northeast of Srinagar, July-August, 1928, *Dickason*, 53 (MICH); Turnik Nallah Baltistan 1928, *Ludlow*, 368 (BM); Above Gulmarg., 12.8.1929, *Stewart*, 10418 (PH); Sat Sar, 11.8.1939, *Stewart & Stewart*, 18279 (NY, PH-p.p., RAW); Upper Bringhi, 23.8.1943, *Ludlow & Sherriff*, 9347 (BM); **India:** Kulu-Lahaul, 18.6.1988, *Drummond*, 23292 (K); Kulu-Lahaul, 24.6.1888, *Drummond*, 23291 (K); Sissu, 3.6.1941, *Bor*, 14609 (K); Gondala, 7.7.1941, *Bor*, 10253 (K).

The scanning electron microscopic studies of the leaves, bract, pollen and seeds exhibit the differences in *E. pseudopaucifolia* T. Siddiqui & Qaiser and *E. paucifolia* Wettst. In the former taxon the cauline leaves, bracts and calyx are densely hirsute with long hairs, all over while in the latter taxon the cauline leaves, bracts and calyx are sparsely hirsute with short hairs at the margin only (Fig. 4). The seed surface of *E. pseudopaucifolia* T. Siddiqui & Qaiser have fairly developed transverse ridges and areolae while in *E. paucifolia* Wettst., the ridges and areolae are not deeper. The pollen in *E. paucifolia* Wettst. are with narrow colpal membrane while in *E. pseudopaucifolia* T. Siddiqui & Qaiser the colpal membrane is broad with distinct foveolae (?) (Fig. 4). However, the tectum of both the taxa is more or less the same.

#### *Distribution*

Pakistan, Kashmir and India (Fig. 5).

## 2. *Euphrasia paucifolia* Wettst., Monog. 198, t. 12, fig. 4. 1896 (Fig. 3, G-K).

**Syntypus:** India & High Asia, *Schlagintweit*, 5939, 8939, 8647, **isotypus** (PH!).

#### *Specimens examined*

**Pakistan:** Chitral central Arkari Gol near middle school, 20.6.1987, *Ghafoor & Omer*, 2748 (KUH); Gilgit, Gilgit road, Chhachor pass, 7.8.1955, *Webster & Nasir*, 6406 (GH); **Kashmir:** Pahlgam, July-August 1927, *Dickason*, 54 (MICH); Sonamarg, 23.7.1928, *Stewart*, 9777 (PH);

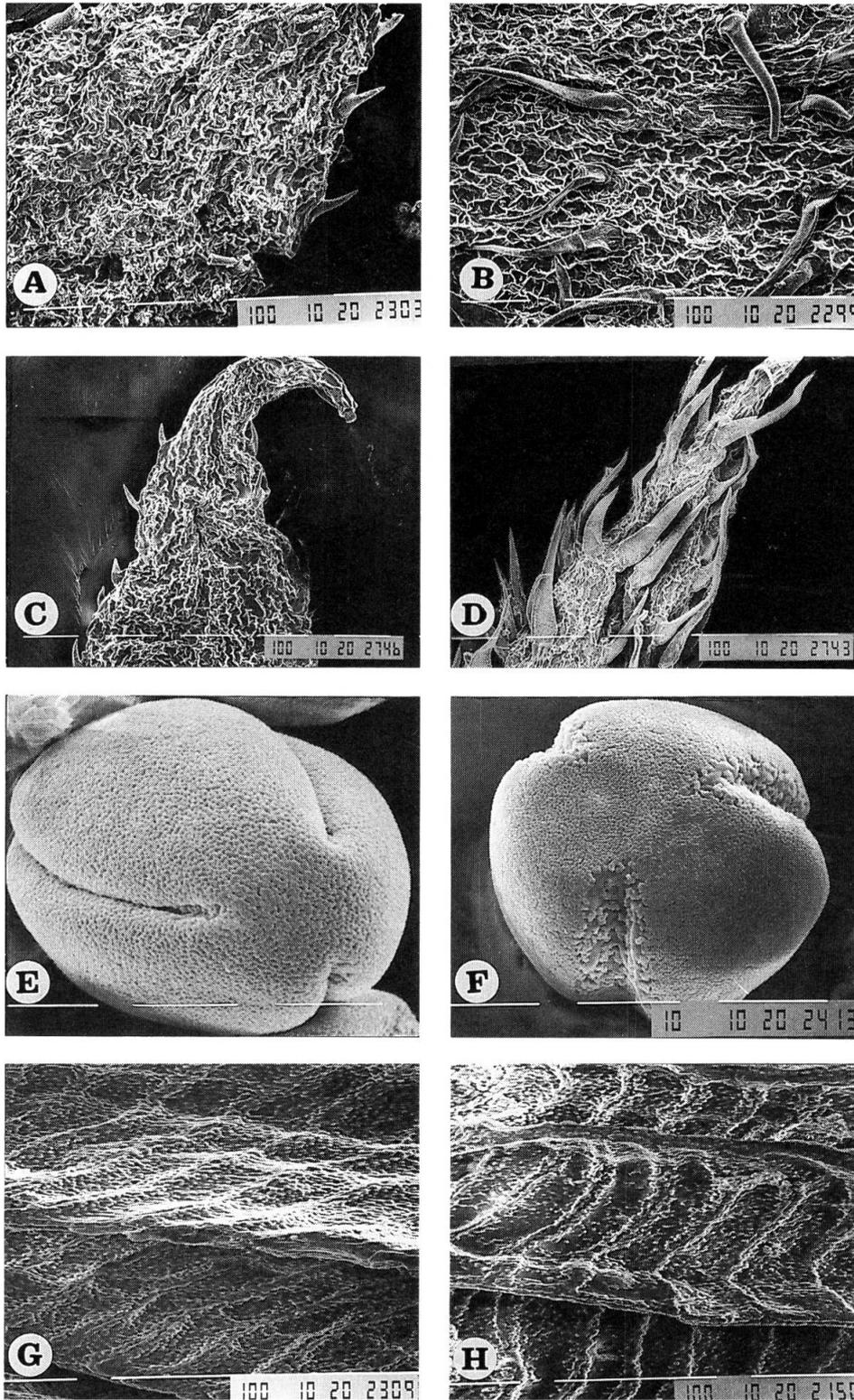


Fig. 4. — Scanning electron micrographs.

*Euphrasia pseudopaucifolia*: A, bract surface; C, portion of calyx lobe; E, pollen grain-polar view; G, seed surface (all from Stewart 8049).

*Euphrasia paucifolia*: B, bract surface; D, portion of calyx lobe; F, pollen grain-polar view; H, seed surface (all from Webster & Nasir 6406).

Fig. A-D, G-H white bar = 100 μm and E, F white bar = 10 μm.

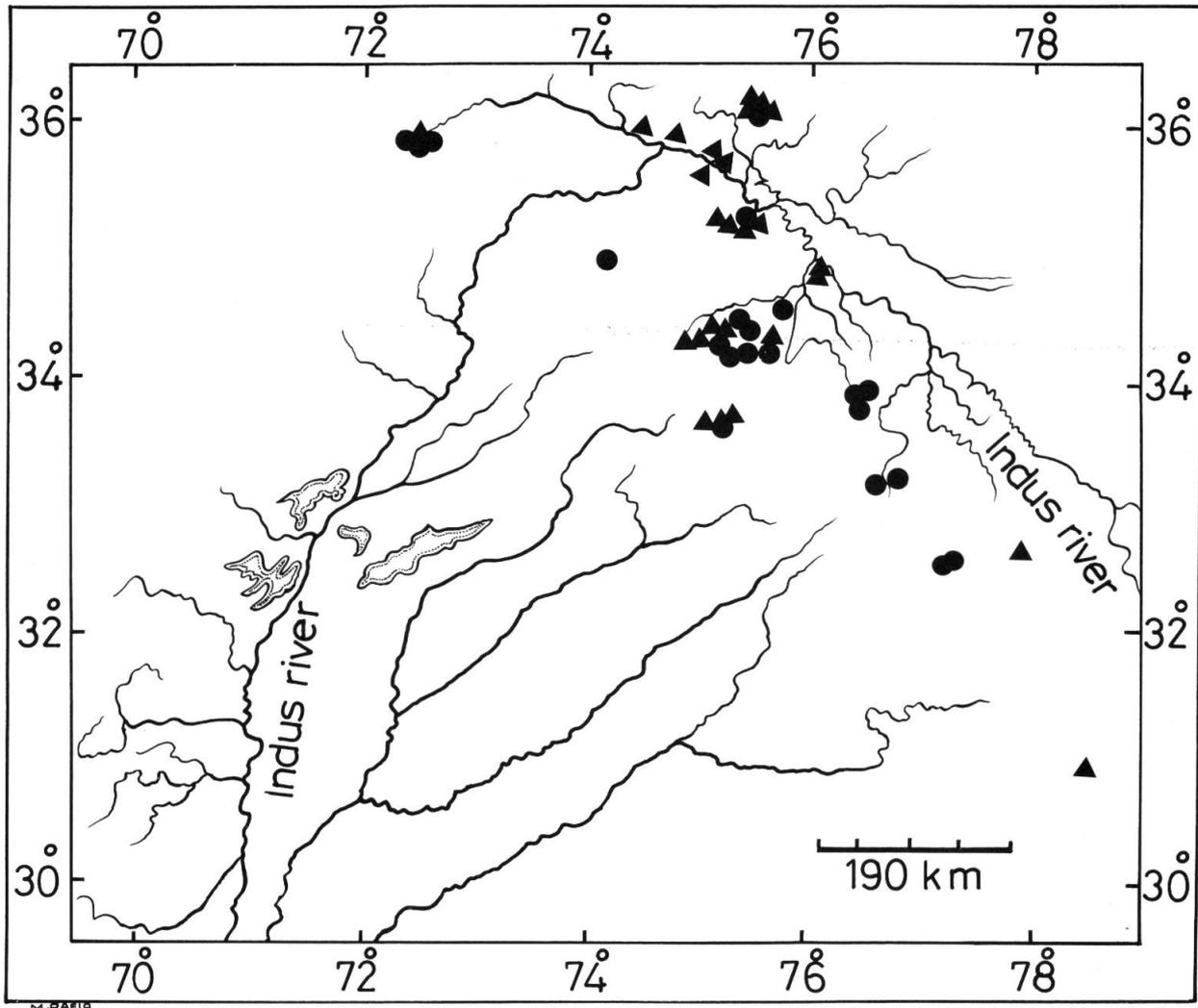


Fig. 5. — Distribution of *Euphrasia paucifolia* (▲) and *E. pseudopaucifolia* (●) in Pakistan and Kashmir.

Sonamarg, 9.8.1928, *Stewart*, 9870A (NY, PH-p.p., RAW); Luderwas Sonamarg, 11.8.1928, *Stewart*, 9378 (NY); Burzil pass ascent, 27.8.1939, *Stewart & Stewart*, 19059 (PH-p.p., RAW); Burzil pass, 28.8.1940, *Stewart* 19930 (NY, RAW); Deosai plains, 29.7.1940, *Stewart*, 20024 (NY, GH-p.p.); Deosai plains, 30.7.1940, *Stewart*, 20095 (NA, NY, PH); Kamri pass top, 14.7.1946, *Stewart*, 22741 (NA, NY, PH); Kamri pass top, 14.7.1946, *Stewart*, 22696 (NY, PH-p.p., US); Burzil pass north slope, 30.7.1946, *Stewart*, 22986 (K, NA, NY, PH-p.p.); Ladak Matayan, Aug, 1928, *Stewart*, 9957A (PH); Baltistan, Thalle La, 14.8.1940, *Stewart*, 20656 (GH-p.p., NA, NY, PH-p.p., RAW); Paskyuntreaty road, 4.6.1941, *Ludlow & Sherriff*, 8359 (A); **India:** Garhwal, July, 1883, *Duthie*, 234 (K).

#### *Distribution*

Pakistan, Kashmir and India (Fig. 5).

#### ACKNOWLEDGEMENTS

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