

## STUDIES IN THE POLLEN MORPHOLOGY OF *ARNEBIA* SPECIES (BORAGINACEAE) FROM PAKISTAN

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### Abstract

A palynological study of 9 species of the genus *Arnebia* from Pakistan was carried out. The pollen were characterized by having two endoapertures per ectoaperture and a well developed costae with rectangular to constructed rectangular equatorial outline and echinate tectum. Using Principle Component Analysis (PCA) and Cluster Analysis of various pollen characters a correlation of the style length and pollen size was worked out.

### Introduction

The genus *Arnebia* Forssk. with c. 20-25 species (Willis, 1973), belongs to the tribe Lithospermeae of Boraginaceae, is distributed from North Africa to Central Asia and Himalayas. In Pakistan it is represented by 13 taxa (Kazmi, 1971). Pollen of Boraginaceae have been studied by various workers (Singh, 1930; Erdtman, 1952; Avetisian, 1956; Nair, 1965; Grau & Leins, 1968; Gupta, 1971; Huynh, 1971; 1972; Nowicke & Ridgway, 1973; Nowicke & Skvarla, 1974; Clarke, 1977; Qureshi, 1985; Qureshi & Khan, 1985; Qureshi & Qaiser, 1987). Johnston (1952, 1953, 1954) used pollen morphology as a tool in the re-evaluation of the genera *Lithospermum* and *Arnebia* where characters of endoaperture were used. *Lithospermum* is characterized by the presence of endoapertures at equator while in *Arnebia* the endoapertures are present on both the poles. Ben Saad-Liman & Nabli (1982) have described ultra and fine structures of *Arnebia decumbens* pollen from Tunis. Huynh (1971) while studying the taxonomic position of *Macrotomia echioides* also studied the pollen of seven species of *Arnebia* with light microscopy.

Due to the absence of clearcut morphological differences in the genus *Arnebia* the specific delimitation is difficult. In order to provide additional micromorphological characters and due to scarcity of pollen morphological and fine structural data in the genus *Arnebia*, results of a palynological study is reported in this paper.

### Materials and Methods

Pollen samples obtained from Karachi University Herbarium (KUH) and National Herbarium Rawalpindi (RAW) were processed by the standard acetolysis method of

Table 1. Selected pollen morphological characters in the genus *Arnebia*

Taxa	Length Polar in Axis ( $\mu\text{m}$ )		Equatorial diameter ( $\mu\text{m}$ )		Ratio (P/E)	Equatorial outline	Polar outline	No. of Apertures	Shape	Tectum at	Colpi length ( $\mu\text{m}$ )		Exine thickness ( $\mu\text{m}$ )			
	Max.	Mean	Min.	Max.							Mean	Meso-		Apo-		
1. <i>A. benthamii</i>	(21.0-)	22.0	(-24.0)	(11.0-)	14.0	(-15.0)	1.57	Const. Rect.	5-lobed	5	Pr.	—	21.0	7.7	1.7	1.7
2. <i>A. enchroma</i>	(28.6-)	31.3	(-34.1)	(12.1-)	15.4	(-17.6)	2.03	Const. Rect.	5-lobed	5	Perpro.	Psi-rugulate	25.3	5.5	4.4	2.2
3. <i>A. hispidiissima</i>	(21.0-)	28.0	(-30.0)	(10.0-)	18.0	(-23.0)	1.55	Oval	6-lobed	6	Pr-Perpro.	Echinate	24.2	5.5	4.4	1.9
4. <i>A. guttata</i>	(24.0-)	27.0	(-30.0)	(14.0-)	17.0	(-19.0)	1.58	Const. Rect.	6-lobed	6	Pr.	Echinate	19.8	4.4	2.8	2.2
5. <i>A. decumbens</i>	(33.0-)	35.2	(-41.8)	(18.7-)	19.4	(-22.0)	1.81	Rect.	5-lobed	5	Pr.	Psi-scabrate	26.4	11.0	1.6	3.3
6. <i>A. fimbriopetalata</i>	(27.0-)	34.0	(-46.0)	(17.0-)	21.0	(-25.0)	1.61	Oval-Rect.	4-lobed	4	Pr.	Psi-rugulate	33.0	11.0	1.6	3.3
7. <i>A. griffithii</i>	(35.0-)	41.0	(-43.0)	(14.0-)	18.0	(-21.0)	2.20	Const. Oval	4-lobed	4	Perpro.	Psi-rugulate	31.0	6.6	1.8	1.1
8. <i>A. linearifolia</i>	(37.0-)	41.0	(-44.0)	(27.0-)	28.0	(-29.0)	1.46	Rect.	5-lobed	5	Pr.	Psi-rugulate	27.5	6.6	5.5	2.2
9. <i>A. inconspicua</i>	(40.7-)	50.9	(-55.0)	(24.2-)	2.8	(-28.6)	1.89	Const. Rect.	5-lobed	5	Pr.	—	49.5	12.1	2.2	3.3

Key to abbreviations: Const. Rect. = Constricted rectangular; Rect. = Rectangular; Pr. = prolate; Perpro. = perprolate; Psi. = psilate

Erdtman (1952) for LM and SEM. For LM the grains were mounted unstained in glycerine jelly and observations were made with an Ortholux-II (Lietz-Wetzler) microscope equipped with a 35mm Lieca-D camera. For the measurements of style length and pollen size 38 specimens belonging to 9 species of *Arnebia* were examined.

Samples for SEM studies were prepared according to the method outlined by Qureshi & Qaiser (1987) and the specimens were examined by Jeol JSM-T 200 SEM. Details of the specimens examined shown in Appendix-I, are present in KUH, unless otherwise stated.

The quantitative characters of the pollen of nine species in Table 1, were employed to perform sum of squares clustering and principal component ordination (Orloci & Kenkel, 1985). The Euclidean distance used for the computation of dissimilarity matrix is as follows:

$$D_{jk} = \left[ \sum_{i=1}^P (X_{ij} - X_{ik})^2 \right]^{1/2}$$

where  $D_{jk}$  = Euclidean distance between species  $j$  and  $k$  and  $X_{ij}$  is the observation on character  $i$  in species  $j$ . The sum of squares clustering minimizes the within group sums of squares. For PCA the correlation coefficient was used to construct R-type resemblance matrix.

## Results

All the pollen grains studied were prolate to prolate, c. 14-22 x 28-55  $\mu\text{m}$ ; equatorial outline constricted oval, oval, and constricted rectangular; polar outline lobed, circular and square; 4-6 (-7) colpi, two endoapertures per ectoaperture, colpi 21-45  $\mu\text{m}$  long, mesocolpia 4-12  $\mu\text{m}$ , apocolpia 1.7-5.5  $\mu\text{m}$ . Exine 1.1-3  $\mu\text{m}$  thick, sexine and nexine differentiation not clear, sexine echinate, costae well developed with echinate or psilate sculpturing. A detailed description of selected pollen characters of individual *Arnebia* species is given in Table 1.

## Discussion

Pollen of *Arnebia* show typical arrangement of endoaperture i.e. each colpi at its extremity contains an endoaperture, thus forming two rows of endoapertures at both the poles. The endoapertures are not distinct in all the species whereas in some species the appearance of large protuberances (operculum) at both the poles show the presence of two endoapertures per ectoaperture. Johnston (1952, 1953, 1954) while studying the genus also observed two rows of pores at each pole but he did not account the presence of colpi. The presence of two endoapertures per ectoaperture has also been observed in other

families, for instance in Didymelaceae and Euphorbiaceae (Erdtman, 1952); Myoporaceae, Primulaceae, Scrophulariaceae and Grossulariaceae (Verbeek-Reuvers, 1976, 1977).

There seems to be a good deal of discrepancy in the number of apertures in pollen of some of the species of *Arnebia* observed in the present study (Huynh, 1971; Ben Saad-Liman & Nabli, 1982). In the present studies 4, 6, 5 and 4 apertures have been observed respectively in *A. griffithii*, *A. hispidissima*, *A. euchroma* and *A. inconspicua* whereas Huynh (1971) has reported 6, 7, 4 and 5 apertures for the same species. Furthermore the presence of 5-colporate grains with 10-endoapertures in *A. decumbens* is in conformity with the results of Huynh (1971), whereas Ben Saad-Liman & Nabli (1982) reported 6-7 colporate grains with 12-14 endoapertures in this species.

There is a maximum concentration of the echinae in the colpal membrane. Ornamentation on the costae is quite variable. Pollen of *A. euchroma*, *A. fimbriopetala*, *A. griffithii* and *A. linearifolia* (Fig. 1, A-F) are characterized by having psilate-rugulate costae, usually psilate towards the poles and rugulate in the constricted mesocolpal area. Psilate-scabrate costae i.e. psilate at poles and scabrate at the mesocolpia, are found in pollen of *A. decumbens* (Fig. 2, A-B). Echinata costae are characteristics of the grains of *A. guttata* and *A. hispidissima* (Fig. 2, C-F). However, considerable overlapping is found in all the three groups.

All the species of *Arnebia* are annual, biennial or perennial. There seems to be a good correlation within the size of the grains and habit. In all the annual species viz., *A. decumbens*, *A. griffithii*, *A. fimbriopetala*, *A. hispidissima* and *A. linearifolia* the pollen are larger as compared to the perennial species viz., *A. inconspicua*, *A. euchroma*, *A. hispidissima* and *A. guttata*. However, few exceptions are found in *A. hispidissima* having small pollen with annual habit and *A. inconspicua* with perennial habit and very large grains.

The above contention is also supported by Clustering Analysis. Fig. 3 shows the dendrogram derived from sum of squares (Clustering Analysis) of nine species which clearly indicates that three groups of pollen types can be recognized. Group 'A' includes all the annual species and in group 'B' perennial species are present while group 'C' includes *A. inconspicua*.

Fig. 4 shows PCA ordination of the pollen grains of nine *Arnebia* species. The groups obtained from cluster analysis are superimposed on the ordination, although the same three groups can be seen. It is obvious that there is a continuous variation in the pollen structure and discrete groups do not exist. In particular groups 'A' and 'B' are closely related and merge together. It is interesting to note that the grouping obtained by the exine ornamentation do not correspond with the Cluster Analysis or PCA ordination.

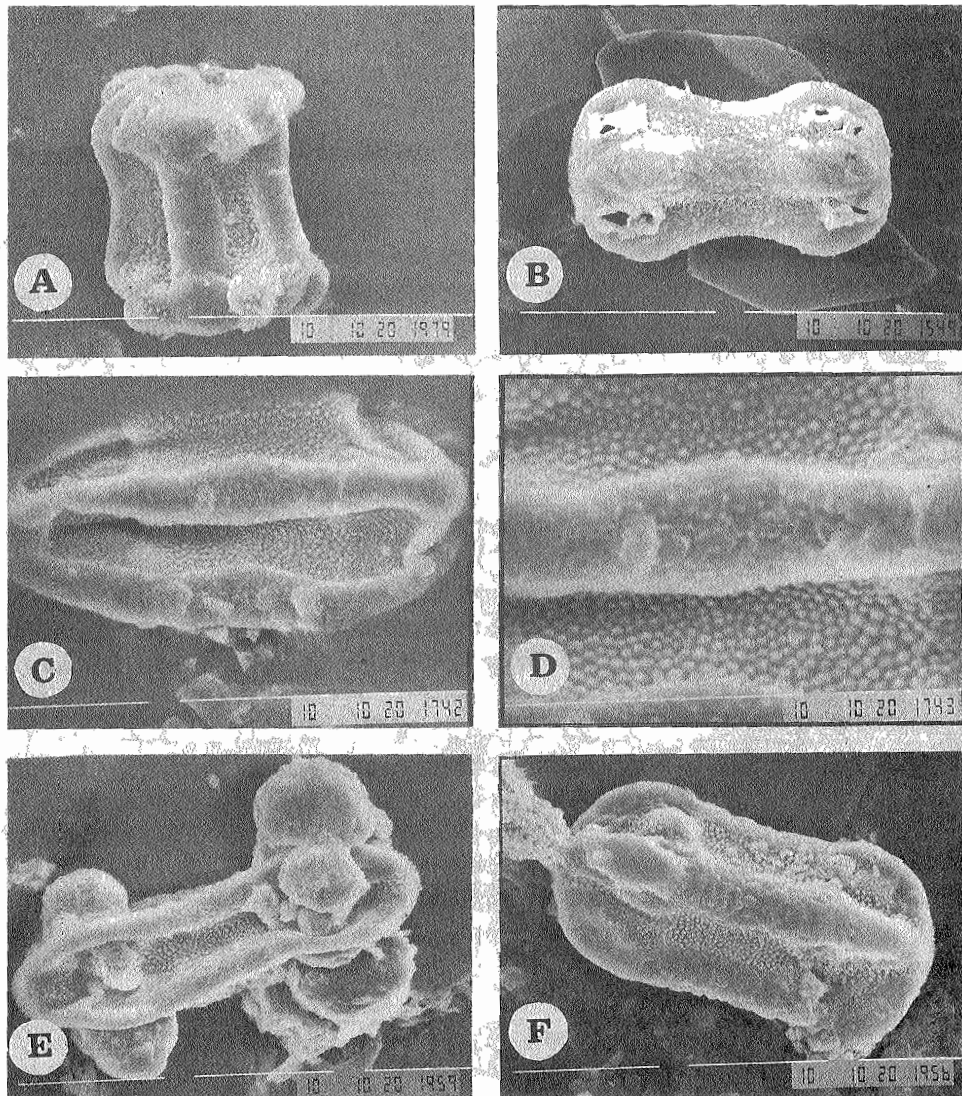


Fig. 1. Scanning electron micrographs showing pollen shape and surface sculpturing in *Arnebia* species. A, B. *A. euchroma*; C, D. *A. fimbriopetala*; E, F. *A. griffithii*. (white bar = 10  $\mu$ m).

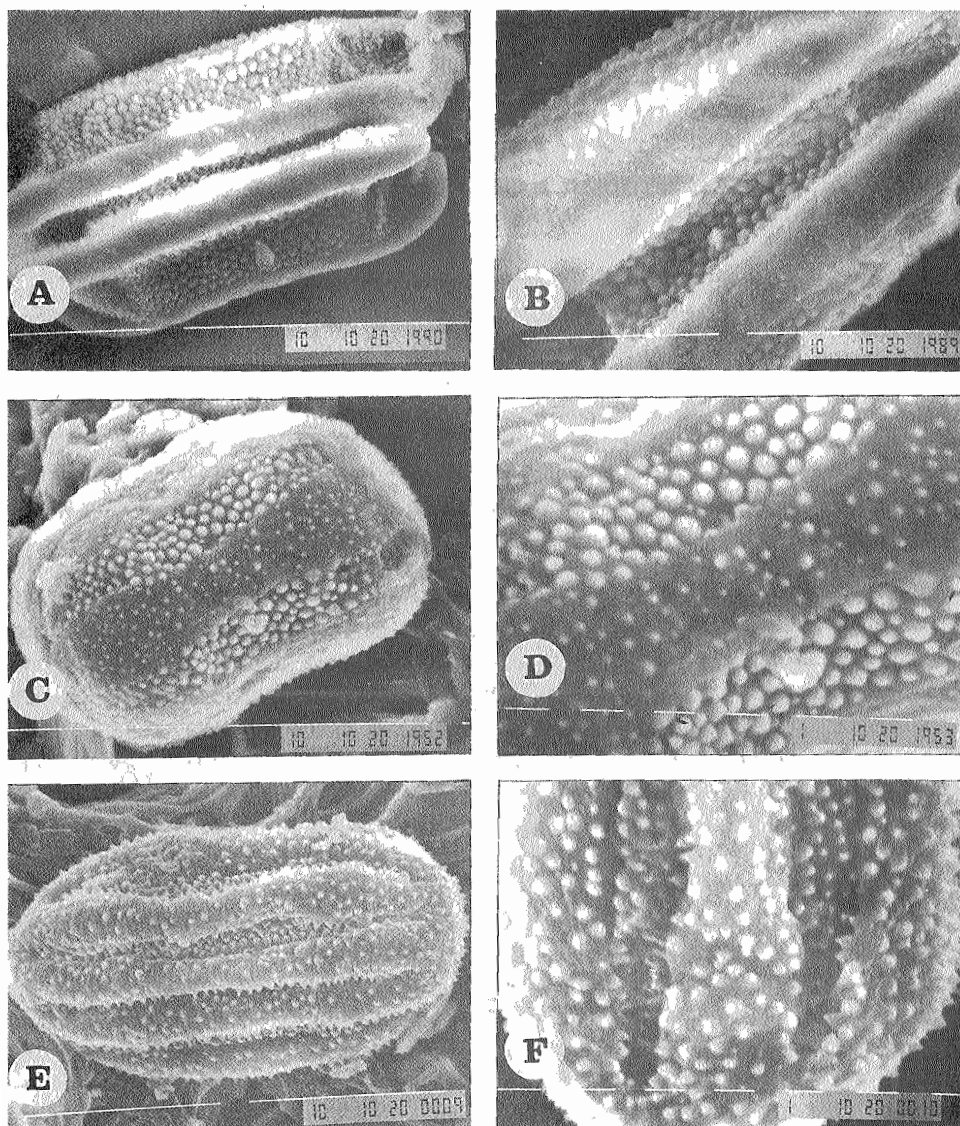


Fig. 2. Scanning electron micrographs showing pollen shape and surface sculpturing in *Arnebia* species. A, B. *A. decumbens*; C, D. *A. guttata*; E, F. *A. hispidissima* (white bar A, B, C, E = 10  $\mu$ m and DF = 1  $\mu$ m).

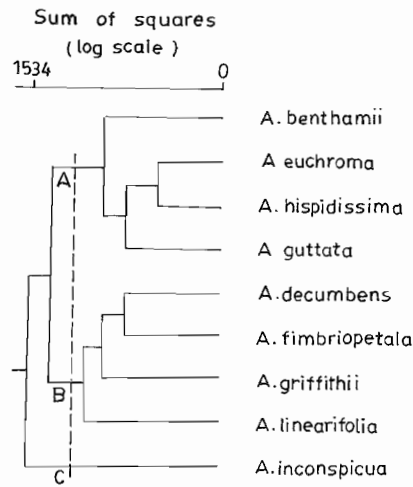


Fig. 3. Dendrogram derived from sum of squares clustering of the pollen characteristics of nine *Arnebia* species.

All the species of *Arnebia* are heterostylous. Johnston (1954) indicated an inverse correlation with the size of grains i.e., all long styled flowers had conspicuously smaller grains as compared to the short styled flowers. However, present findings did not accord the above contention. It is obvious from Table 2 that out of 6 species studied, 3 showed non-significant correlation while 3 taxa had a significant correlation. In *A. guttata* and *A. decumbens* long styled flowers had larger grains and short styled had smaller ones, while in *A. hispidissima* flowers with long style had smaller pollen and short styled flowers had large grains.

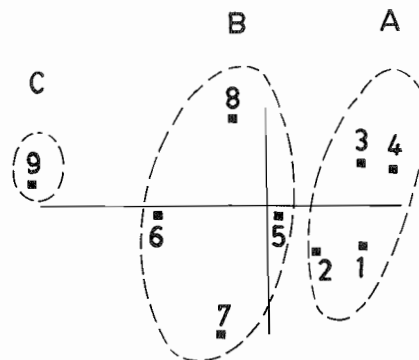


Fig. 4. Principal component analysis (PCA) ordination of the pollen characteristics of nine *Arnebia* species. Numbers 1 to 9 correspond to species indicated in Table 1.

Table 2. Pollen size in relation to style length for six species of *Arnebia*

Taxon	Pollen size ( $\pm$ S.E.) associated with		t-value	Significance
	Long style	Short style		
<i>Arnebia guttata</i>	21.125 $\pm$ 0.125	18.9 $\pm$ 0.404	5.226	p < 0.01
<i>Arnebia griffithii</i>	30.150 $\pm$ 0.75	32.266 $\pm$ 0.886	1.4672	n.s.
<i>Arnebia euchroma</i>	18.9 $\pm$ .4041	20.287 $\pm$ 1.344	0.7053	n.s.
<i>Arnebia fimbriopetala</i>	28.7 $\pm$ .4041	29.6 $\pm$ 1.430	0.605	n.s.
<i>Arnebia decumbens</i>	44.24 $\pm$ 1.959	34.75 $\pm$ 1.726	3.45	p < 0.01
<i>Arnebia hispidissima</i>	23.8 $\pm$ .808	31.66 $\pm$ .2867	10.65	p < 0.001

## References

- Avetisian, E.M. 1956. The morphology of microspores in the Boraginaceae. *Tr. Bot. Inst. Akad. Nauk. Arm. S.S.R.*, 10: 7-66.
- Ben Saad-Liman, S. and M.A. Nabli. 1982. Ultrastructure de l'exine de l' *Arnebia decumbens* (Vent.) Coss. et Kral. et du *Cerithe major* L. (Boraginaceae). *Pollen et Spores*, 24: 9-19.
- Clarke, G.C.S. 1977. The Northwest European Pollen Flora, 10 "Boraginaceae". *Rev. Palaeobot. Palynol.*, 24: 59-101.
- Erdtman, G. 1952. *Pollen morphology and plant taxonomy - Angiosperms*. Stockholm & Waltham, Mass.
- Grau, J. and P. Leins. 1968. Pollenkorntypen und Sektionsgliederung der Gattung *Myosotis*. *Ber. Dtsch. Bot. Ges.*, 81: 107-115.
- Gupta, H.P. 1971. Studies of Indian Pollen Grains-IV. Boraginaceae. *Geophytology*, 1: 127-134.
- Huynh, K. L. 1971. Le pollen du genre *Arnebia* Forssk. et du genre *Macrotomia* DC. (Boraginaceae), et la position taxonomique particuliere du *M. echioides* (L.) Boiss. *Candollea*, 26: 165-171.
- Huynh, K.L. 1972. The original position of the generative nucleus in the pollen tetrads of *Agropyron*, *Itea*, *Limnanthes* and *Onosma*, and its phylogenetic significance in the Angiosperms. *Grana*, 12: 105-112.
- Johnston, I.M. 1952. Studies in the Boraginaceae, XXIII. A survey of the genus *Lithospermum*. *J. Arnold Arbor.*, 33: 299-366.
- Johnston, I.M. 1953. Studies in the Boraginaceae, XXIV. Three genera segregated from *Lithospermum*. *J. Arnold Arbor.*, 34: 1-16.
- Johnston, I.M. 1954. Studies in the Boraginaceae, XXVI. Further re-evaluations of the genera of the Lithospermeae. *J. Arnold Arbor.*, 35: 1-81.



- Kazmi, S.M.A. 1971. A revision of the Boraginaceae of West Pakistan and Kashmir. *J. Arnold Arbor.*, 52: 359-363, 486-502.
- Nair, P.K.K. 1965. *Pollen grains of Western Himalayan plants*. Bombay.
- Nowicke J.W. and J.E. Ridgway. 1973. Pollen studies in the genus *Cordia* (Boraginaceae). *Amer. J. Bot.*, 60: 584-591.
- Nowicke J.W. and J.J. Skvarla. 1974. A palynological investigation of the genus *Tournefortia* (Boraginaceae). *Amer. J. Bot.*, 61: 1021-1036.
- Orloci, L. and N.C. Kenkel. 1985. *An introduction to Data Analysis: With examples from population and community ecology*. International Co-operative Publ., Fairland, Maryland.
- Qureshi, U.S. 1985. Studies on the pollen morphology of the genus *Heliotropium* L. from Pakistan. *Pak. J. Bot.*, 17: 107-114.
- Qureshi, U.S. and K.M. Khan. 1985. Anomaly in pollen apertures of *Coldenia procumbens* (Boraginaceae). *Pak. J. Bot.*, 17: 115-117.
- Qureshi, U.S. and M. Qaiser. 1987. Palynological study of *Onosma* (Boraginaceae) from Pakistan. *Pak. J. Bot.*, 19: 99-105.
- Singh, T.C.N. 1930. Studies in the morphology of pollen grains 1(a) Boraginaceae. *Jour. Ind. Bot. Soc.*, 10: 38-42.
- Verbeek-Reuvers, A.A.M.L. 1976. On the number of endoapertures per ectoaperture in colpate pollen grains. *Pollenet spores*, 18: 337-384.
- Verbeek-Reuvers, A.A.M.L. 1977. The Northwest European Pollen Flora, 12. Grossulariaceae. *Rev. Palaeobot. Palynol.*, 24: 107-116.
- Willis, J.C. 1973. *A dictionary of the flowering plants and ferns*. Univ. Press, Cambridge.

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## APPENDIX I – LIST OF VOUCHER SPECIMENS

- Arnebia benthamii*, Kashmir: Bedori, *L.D. Kapoor* s.n.
- Arnebia benthamii*, Baluchistan: Kalat, Punjgur to surat, Basima near jangal, *Jennifer Lamond* 662.
- Arnebia benthamii*, Between Quetta & Ziarat, stony ground, *Jafri & Akbar* 2025.
- Arnebia benthamii*, 29 mile sfrom Quetta on way to Chaman, *Sultan ul Abedin* 4853.
- Arnebia benthamii*, Rest house Nag, *S. Abedin & Abrar Hussain* 6071.
- Arnebia euchroma*, Baltal, Kashmir, *R.R. Stewart* 1912-13.
- Arnebia euchroma*, Kashmir; Mitsahoi, Ladak Rd. Matayan, *R.R. Stewart* 10004.
- Arnebia euchroma*, Satpra nullah above Skardu, *R.R. Stewart* 20289.
- Arnebia euchroma*, Saiful Maluk, *Ch. Shaukat Ali* 128.
- Arnebia euchroma*, Mitsahoi to zoji pass, *R.R. Stewart* 30.7.1940.
- Arnebia fimbriopetala*, 18 miles from Sunster on way to Gawader, *M. Qaiser, Asad Raza & Abrar* 1019.
- Arnebia fimbriopetala*, Baluchistan: Makran; Pasni to Kappar, *Jennifer Lamond* 456.
- Arnebia griffithii*, Peshawar to Cheerat, *Jennifer Lamond* 1952.
- Arnebia griffithii*, Peshawar, *Mohindar Nath* 25.4.1936.
- Arnebia griffithii*, To Parachinar; Kurram Vy., *R.R. Stewart* 28934.
- Arnebia griffithii*, Peshawar Cant., *Baldev Raj Khosla* 15.7.1941.
- Arnebia griffithii*, Attock Dist., Punjab, *Dev Raj Paggi*, May 1933.
- Arnebia griffithii*, Near Landhi Kotal, Khyber Agency, *M. Qaiser & S. Abedin* 6096.
- Arnebia griffithii*, Kaish Manda, 20 miles from Muslim Bagh on way to Qilla Saifullah, *S. Nazimuddin & S. Abedin* 852.
- Arnebia guttata*, Tikse, after Leh, Indus valley, Ladak, *F. Billiet et J. Leonard* 6845.
- Arnebia guttata*, Doghani to Kuru; Skyok Vy., *R.R. Stewart* 20853.
- Arnebia guttata*, Skardu, *R.R. Stewart* 20392.
- Arnebia guttata*, c. 35 miles from Gilgit on way to Skardu, *S. Omer, S. Nazimuddin & A. Wahid* 842.
- Arnebia guttata*, c. 1 mile from Lasht on way to Roshgol, *Kamal A. Malik & S. Nazimuddin* 1669.
- Arnebia guttata*, c. 14 miles from Skardu on way to Gol, *S. Omer, S. Nazimuddin & A. Wahid* 904.
- Arnebia guttata*, Skardu, Baltistan, *Saood Omer* 390.
- Arnebia guttata*, Nyenro, Ladak, *R.R. Stewart* 1912-13.
- Arnebia hispidissima*, 28 miles from Karachi on way to Thatta, *Sultan ul Abedin* 5210.
- Arnebia hispidissima*, Marine base, Jiwani, *M. Qaiser & D. Khan* 7144.
- Arnebia hispidissima*, Lahore, *R.R. Stewart* 7091.
- Arnebia hispidissima*, Karachi University Campus, Karachi, *Shafia Bano* s.n. 1962.
- Arnebia hispidissima*, 2 miles from Hub on way to Sonmiani in dry nullah, *S.I. Ali, S. Abedin & A. Ghafoor* 1271.
- Arnebia hispidissima*, Karachi, Malir, *Ruqayya Islam* 10.10.1956.
- Arnebia inconspicua*, Baluchistan: Maslakh near Sultan, *R.R. Stewart* 28293 (RAW).
- Arnebia linearifolia*, Makran; Hoshab to Panjgur, c. 20-30 km. from Panjgur, *Jennifer Lamond* 566.
- Arnebia linearifolia*, Kalat, Mastung, near Kohi-maran, *Jafri & Akbar* 2001.
- Arnebia linearifolia*, near Kach on way to Ziarat, *M. Qaiser & A. Ghafoor* 1399.
- Arnebia linearifolia*, 30 miles from Quetta on way to Ziarat, *S. Abedin* 4723.