

POLLEN STUDIES IN THE *PRIMULA* SPECIES (*PRIMULACEAE*) FOUND IN PAKISTAN WITH SPECIAL REFERENCE TO TAXONOMY*

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Abstract

Three basic pollen types, namely auricula, farinosa and veris are found in *Primula*. The species occurring in Pakistan are categorised and the relationship between various species discussed. SEM for the majority of Himalayan taxa has been attempted for the first time.

Introduction

In the mountainous northern regions of Pakistan, the genus *Primula* is represented by 22 species distributed in 4 subgenera and 10 sections (Wendelbo, 1961a). Several earlier works, notably that by Spanowsky (1962), have pointed out the need for a revision of the genus utilising pollen morphological characters. For the Indo-Pakistan subcontinent no comprehensive work on the palynology of the genus exists. What one can find on the subject is scattered in various diverse literature. Table 1 shows less than 23% of primulaceous taxa in Pakistan that have been studied for pollen, with 41% for *Primula*. Work of Spanowsky (1962) deals with 117 *Primula* species found in the Asian mountains when only 9 these are found in Kashmir. Punt *et al* (1974) did not include any Himalayan species of *Primula* and Nair (1965) briefly described the pollen of 6 W. Himalayan primulas. The reports of Wendelbo (1961b) on the palynology of *Primula* appears to be considerable but all of his work has not been published (Dr. K. Faegri informs me that he studied between 200-250 species).

In the present study an attempt has been made to utilise pollen characters using light microscopy (LM) and the stereoscan(SEM) as an aid in studying the morphology and taxonomy of the genus.

Material and Methods

Pollen material of 21 species of *Primula* were examined by the light microscope and SEM. Pollen samples were collected from herbarium specimens for most of the taxa, for some fresh material was also obtained. For SEM, the pollen samples were transferred to a double-sided adhesive tape on a metal stub, then coated with gold in vacuum, examined and photographed using a JEOL JSM 1 (at Kew, England) or a JEOL JSM 35 R Scanner (at Reading University, England) at 15-30 Kv. Only a few taxa prior to scanning

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Table 1. Palynological studies of the family Primulaceae from Pakistan.

Genera	Species	Pollen morphology of the relevant taxa studied by:			
		Wendelbo (1961b)	Spanowsky (1962)	Nair (1965)	Punt <i>et al</i> (1974)
<i>Primula</i>	22	?	9	6	—
<i>Dionysia</i>	2	2	—	—	—
<i>Androsace</i>	25	10	—	2	1
<i>Cortusa</i>	2	2	1	—	—
<i>Lysimachia</i>	7	—	—	—	1
<i>Glaux</i>	1	—	—	—	1
<i>Samolus</i>	1	—	—	—	1
Total	61	14	10	8	4

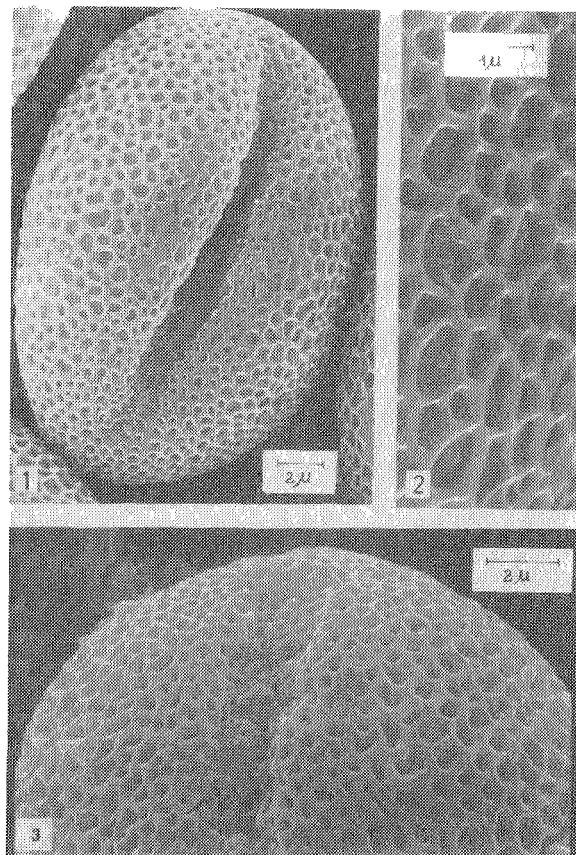
were acetolysed by the method of Erdtmann (1966). For light microscopy, pollen grains were mounted in glycerine jelly, stained with safranin and sealed with paraffin wax. The slides were examined using an Erma Standard Microscope KL-Bi-1 type.

Polar length (P), equatorial diameter (E), aperture(-s), size, exine thickness, diameter of lumina, P/E ratio and the Polar Area Index (PAI) of each pollen was made. Twenty to fifty pollen grains, sometimes fewer due to lack of sufficient material, per 1-3 herbarium specimens for each taxon were studied. Slides of all the pollen samples are deposited at the National Herbarium (RAW). Abbreviations used are: l.s. = long styled; s.s. = short styled; e.v. = equatorial view; p.v. = polar view; p.p. = pro parte. The terminology generally follows Erdtmann (1966). Definition of the exine structure is after Praglowski & Punt (1973).

Results

Following Wendelbo (1961 b) and Spanowsky (1962), a pollen study of the *Primula* species shows that the taxa can be grouped in three categories, namely the *Primula auricula*, *P. farinosa* and *P. veris* type pollen (Tables 2 & 3).

Pollen grains 3 (-4) colpate or more or less colpoidate, prolate-spheroidal, p/e 1.10-1.39, dimorphic due to heterostyly (s.s. flowers have larger pollen than l.s. flowers), subspherical and slightly compressed along polar axis (e.v.), circular with usually open colpi (p.v.) Ectocolpium narrow, grooved. Endoaperture indistinct. Exine 1-1.3 μ m thick. Sexine equal to or less than nexine, microreticulate to reticulate. Polar area small, from 0.21-0.50



Figs. 1-3. Scanning electron micrographs of *P. auricula* type pollen, 1: *P. floribunda* (R.R. Stewart 17148), Equatorial view $\times 4800$; 2: sexine detail $\times 15452$; 3: (Parker, s.n. in 1920). Partial equatorial view showing colpus detail $\times 7500$.

The *P. auricula* type pollen is characterised by more or less indistinct endoaperture, circular pollen outline in p.v., with narrow and sunken colpi and small polar area. Wendeblo (1961b) mentions that this type of pollen also occurs in other primulaceous genera as *Bryocarpum* Hook. f. & Thoms., *Dodecatheon* L. (p.p.), *Hottonia* L. and *Omphalogramma* Franch. (p.p.) (Figs. 1-3).

Pollen grains 3(-4) syncolpate or parasyncolpate, oblate to oblate-spheroidal, p/e 0.52-0.92. Amb pear-shaped or transversely elliptic with the long sides concave (e.v.), triangular (p.v.) or square in 4-apertured forms. Colpi narrow, grooved, not readily observable in e.v., fusing at the poles to form a triangular apocolpial field. Endoaperture absent. Exine 1.3-1.5 μm thick. Sexine thicker or equal to nexine, microreticulate or reticulate. Polar area absent.

A key to 15 species included in this type based on pollen material from s.s. flowers is given below:

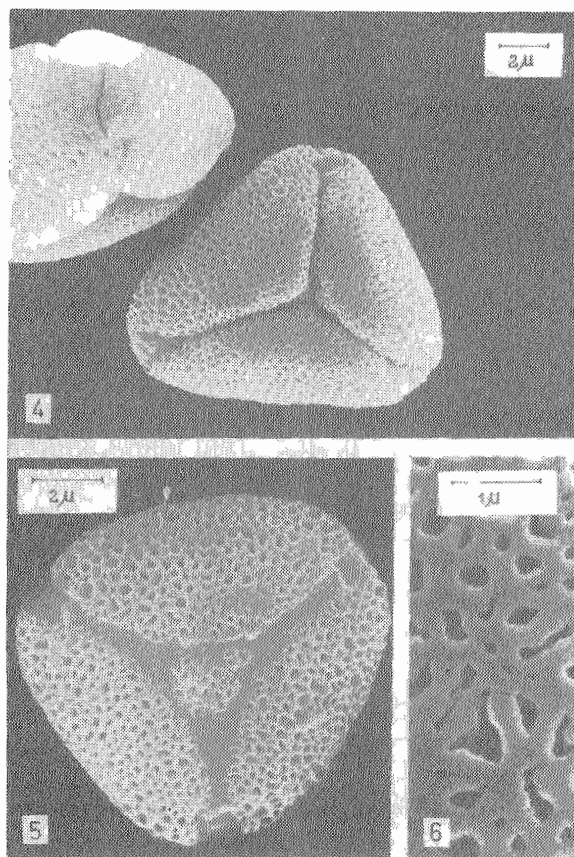
1. Sexine microreticulate. Lumina 0.2-0.5 μ broad in any diameter *P. macrophylla*
P. duthieana
- Sexine reticulate. Lumina 0.6-1.9 μ broad in any diameter 2
2. Maximum pollen diameter 21.2-23.9 μ *P. pulchra*
P. obtusifolia
- Maximum pollen diameter 13.2-17.8 (-21.4) μ *P. clarkei*
P. rosea
P. warshenewskiana
P. elliptica
P. inayatii
P. denticulata
P. denticuloides
P. hazarica
P. capitellata
P. minutissima
P. reidii

The *P. farinosa* type pollen is characterised by oblate 3-syncolpate or parasyncolpate pollen, indistinct colpi (e.v.). In several of the species studied (e.g., *P. denticulata* Sm., *P. pulchra* Watt, *P. duthieana* Balf. f. & Sm., *P. macrophylla* D. Don, *P. minutissima* Jacquem.), the pollen was 4-aperturate with a square amb. According to Wendelbo (1961b) the genera *Cortusa* L., *Dodecatheon* (p.p.) and *Omphalogramma* (p.p.) have a similar pollen (Figs. 4-6).

Pollen grains (4-) 5-10 colpate, suboblate to spheroidal, p/e 0.76-0.99, sometimes subprolate (1.29). Amb circular to slightly compressed along the polar axis (e.v.), circular with more or less intruding colpi. Colpi usually shallow, margins distinct or not. Exine 1.3-1.4 μ thick, reticulate, sexine more than nexine in thickness, lumina irregular. PAI relatively large, 0.35-0.59.

A key to 3 species based on pollen from s.s. flowers is given below:

- + Pollen grains 26 x 20.5 μ . Colpi 4-5 in number,
11.4-14.0 μ long *P. involucrata*
- Pollen grains 20 x 17.5 μ . Colpi 3 or 5 in number,
8-10.5 μ long *P. pamirica*
P. nutans

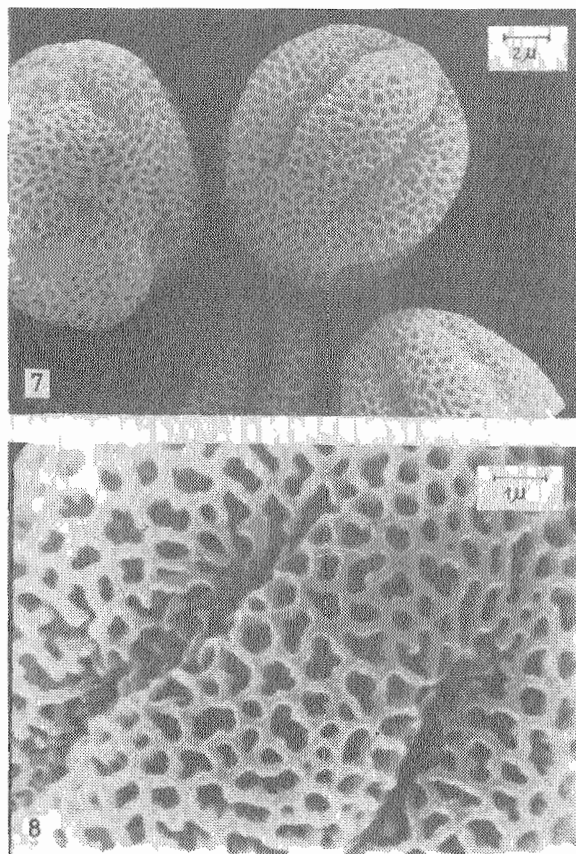


Figs. 4-6. Scanning electron micrographs of *P. farinosa* type pollen, 4: *P. inayatii* (Greenfield s.n. in Feb. 1920). Pollen grains in both polar and oblique equatorial view (lower grain), x 5068; 5: *P. warshenewskiana* (M.A. Siddiqi and A. Rahman 26764), Polar view, note swollen colpi membrane, x 7391; 6: *P. hazarica* (Inayat 19916a), Cracks in the tectum, x 18494.

The *P. veris* type pollen is characterised by the larger number of colpi (5-10), which are usually shallow, spherical shape and relatively large PAI. The genus *Dionysia* Fenzl and *Sredinskya* (Stein.) Fed., have similar pollen (fide Wendelbo, 1961b) (Figs. 7-10).

Discussion

Apart from the three basic and distinct pollen types in *Primula*, variability in the pollen may occur within the same species and even sometimes the same pollen sample. In 5 photomicrographs of *P. incisa* Franch., taken from a single pollen sample (China, Rock 17474, US, courtesy of Dr. J. Nowicke) variation was observed in size, shape and presence or absence of a bridge or partial bridge. According to Spanowsky (1962) the

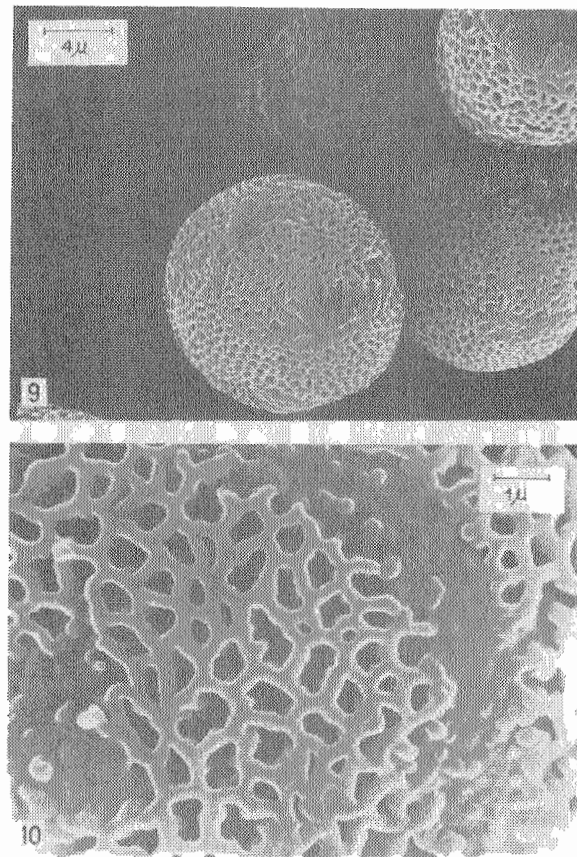


Figs. 7-8. Scanning electron micrographs of *P. veris* type pollen, 7: *P. pamirica* (Bowes Lyon 999). Pollen grain, $\times 4560$; 8: surface detail, 10500.

pollen shape may vary up to 20% within various taxa of Primulaceae. This variability, however, must be considered within the text of the basic pollen types where each may show some variation.

Pollen variability in *Primula* is also influenced by heterostyly. Darwin (1877) recognised that s.s. flowers of the genus have larger pollen associated with smaller stigmatic papillae as compared to l.s. flowers with smaller grains (also more transparent when dry) and larger papillae. It is now known that s.s. flowers also have pollen with larger lumina.

All of the species studied for pollen were found to be heterostylous; in the appendix therefore the nature of the voucher specimen has been indicated (i.e. whether the plant has s.s. or l.s. flowers).



Figs. 9-10. Scanning electron micrographs of *P. veris* type pollen, 9: *P. nutans* (W. Koelz 2075), Pollen grain, x 3590; 10: surface detail, note shallow colpi, x 11180.

As compared to other primulaceous taxa, the pollen in *Primula* exhibited a maximum amount of differentiation. The *P. farinosa* type was found to be the commonest, occurring in 15 of the species studied. In the sect. *Crystallophlomis* Ruprecht., *P. obtusifolia* Royle differed from *P. macrophylla* and *P. duthieana* in the larger lumina (0.9 vs. 0.5 μ). The other species representing various sections could be grouped together on the basis of pollen size, where the width could be used to distinguish *P. pulchra* and *P. obtusifolia* from the other species (see key to *P. farinosa* type pollen). Although *P. inayatii* Duthie is unique in both its vegetative morphology and cytology (Smith & Fletcher, 1943; Bruun, 1932), pollen does not support this contention. The sect. *minutissimae* Pax and sect. *Aleuritia* Duby were found to be heterogenous in their pollen content as both *farinosa* and *veris* pollen types were observed in each case (Table 3).

In taxa with *P. farinosa* type pollen, 4-aperture grains were observed in several of the species, giving the pollen a square appearance. In two species, *P. clarkei* Watt and *P. hazarica* Duthie the tectum had fine cracks (Fig. 6); this may only be an artifact caused by too little coating of the pollen or the pollen sample may have taken up moisture. Pollen material for *P. denticuloides* Y. Nasir was not available as the specimen was in fruit; the withered flowers from the type specimen (at GH) are l.s. and some of the pollen scrapped from the interior of the corolla tube revealed oblate, 3-syncolpate grains as those found in *P. denticulata*.

The *P. auricula* type like the *P. farinosa* type pollen, was present in different sections of the genus, namely sect. *Minutissimae* (*P. reptans* Hook. f. ex Watt) and sect. *Aleuritia* (*P. algida* Adams and *P. schlagintweitiana* Pax). On the basis of pollen and lumina size and nature of colpi (i.e. intruding or open) *P. floribunda* Wall. could be distinguished from the other two species. Wendelbo (1961a) has mentioned several primitive vegetative features in *P. floribunda*, which is also supported by the primitive type auricular pollen (Spanowsky, 1962). Some differences in the size of the lumina was noted between *P. schlagintweitiana* (up to 0.3 μ wide) and *P. algida* up to 0.6 μ wide) in the pollen studied, but this may be of no significance.

The *P. veris* type pollen is characteristic of *P. involucrata* Wall. ex Duby, *P. pamirica* Fed. and *P. nutans* Georgi. The larger pollen in *P. involucrata* is undoubtedly due to tetraploidy, Bruun (1932) records a chromosome count of $2x = 44$ for this taxon, as compared to *P. nutans* (*P. sibirica* Jacquem.) with $2x = 22$. The double chromosome number in *P. involucrata* also correlates with a more robust habit and a heavier seed. Smith & Fletcher (1943) had suggested that this group (i.e. subsect. *Sibirica* Smith & Fletcher of the sect. *Farinosae* Pax) was fairly distinct from other members of the section. The restricted pollen type here reinforces this view. In a recent paper Nasir (1986) raised the subsect. *Sibirica* to sectional rank.

Several species differing in vegetative characters can have similar pollen and vice versa; for instance the *P. farinosa* type pollen is found in 8 sections and 15 species of *Primula*. The *P. auricula* type is found in the sect. *Minutissimae*, sect. *Sphondylia* and sect. *Aleuritia*. This trend is not confined to *Primula* only but related genera may have a similar pollen. The similarity of pollen of different taxa must be due to parallel development since there is no similarity in gross morphology. The heterogeneity of some sections of the genus as regards the *P. farinosa* and *P. veris* type suggests that they must have arisen several times independently from the primitive *P. auricula* type pollen.

Acknowledgements

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Appendix: List of voucher specimens studied for their pollen morphology (SEM or LM) in *Primula*.

Species	Voucher specimen
<i>P. algida</i>	Turkey, <i>Albury et al.</i> , 3072 (K), s.s.
<i>P. clarkei</i>	Alpine House, Dr. <i>Stoker</i> s.n. in 1938 (E), s.s.
<i>P. capitellata</i>	Band-i-Amir, Afghanistan, <i>R. & L. Gibbons</i> 725 (K), s.s.
<i>P. denticulata</i>	Changla Gali, <i>M.A. Siddiqi & Y. Nasir</i> 6651 (RAW), s.s.
"	Arkaso, above Kel, <i>F. Schmid</i> s.n. in May 1954 (RAW), l.s.
<i>P. duthieana</i>	Burzil Pass, <i>R.R. & I.D. Stewart</i> 19050 (RAW), s.s.
"	Saiful Maluk, <i>F. Schmid</i> 332 (RAW), s.s.
"	Burzil, <i>R.R. Stewart</i> 22070 (RAW), l.s.
<i>P. elliptica</i>	Pahlgam, <i>R.R. Stewart</i> 7812 (RAW), s.s.
"	Gangabal lakes, <i>R.R. & I.D. Stewart</i> 4447 (RAW), s.s.
"	Kamri Pass, <i>R.R. Stewart & E. Nasir</i> 22681 (K), l.s.
"	Babusar-Gilgit, <i>M. Qaiser & Kamal</i> 451 (KUH), s.s.
<i>P. floribunda</i>	Landour, <i>R.R. Stewart</i> 17148 (RAW), s.s.
"	Mandi, Poonch, <i>R.R. Stewart</i> 12072 (RAW), l.s.
"	Chamba, Kashmir, <i>Parker</i> s.n. in 1920 (K), s.s.
<i>P. hazarica</i>	Dadar, Hazara, <i>Inayat</i> 21976a (K), l.s.
"	Shinkiari, Hazara, <i>Inayat</i> in 1899 (E), l.s.
"	Siran vy., Hazara, <i>Inayat</i> 19916a (E), l.s.
<i>P. inayatii</i>	Uri, 400', Kashmir, <i>Coventry</i> 140 (K), l.s.
"	Uri, <i>Greenfield</i> s.n. in Feb. 1920 (RAW), s.s.
"	Dunga Bela, Hazara, <i>Inayat</i> s.n. (US), l.s.
<i>P. involucrata</i>	Silrundi, Chamba, <i>R.R. & I.D. Stewart</i> 2527 (RAW), s.s.
<i>P. macrophylla</i>	Chogolisa glacier, Karakoram, <i>E. Nasir & G.L. Webster</i> 6252 (RAW), s.s.
"	Gittidas, <i>Ch. Shaukat Ali</i> 94 (RAW), s.s.
"	Rotang Pass, Lahul, <i>M. Nath</i> in 1935 (RAW), s.s.
"	Burzil Pass, <i>R.R. & I.D. Stewart</i> 22068a (RAW), l.s.
"	above Kyelang, Lahul, <i>J.R. Drummond</i> 8943 (K), l.s.
<i>P. minutissima</i>	Keylang, Lahul, <i>W. Koelz</i> 2069 (K), s.s.
<i>P. nutans</i>	Serchu, Lahul, <i>W. Koelz</i> 2075 (RAW), s.s.
<i>P. obtusifolia</i>	Baspa vy., Simla, <i>Sheriff</i> 7344 (BM), s.s.
<i>P. pulchra</i>	Tatbhaiya, Nepal, <i>Williams</i> 805 (BM), s.s.
"	Bhut Bakhri, Nepal, <i>Dwoj</i> 474 (E), s.s.
<i>P. pamirica</i>	Yarkhun, Chitral, <i>Bowes-Lyon</i> 999 (RAW), s.s.
<i>P. rosea</i>	Gumrat, Dir, <i>Farrukh Hussain</i> 7179 (RAW), s.s.
"	Mt. Makra, Kagan, <i>M.A. Siddiqi</i> 4454 (RAW), s.s.
"	Utrot, Swat, <i>S.A. Khan</i> s.n. (PPFI-B), l.s.

<i>P. reidii</i>	Palang Gadh, Kumaon, <i>Duthie</i> 5746 (K), s.s.
<i>P. reptans</i>	Mt. Makra, Kagan, <i>Inayat</i> s.n. in 1899 (K), s.s.
<i>P. schlagintweitiana</i>	Ghondokoro glacier, <i>E. Nasir & G.L. Webster</i> 6024 (RAW) s.s.
”	Ulter nala, Hunza, <i>F. Schmid</i> 1882 (RAW), l.s.
”	Karambar glacier, <i>Bowes Lyon</i> 8214 (RAW), s.s.
”	above Hushe, Karakoram, <i>E. Nasir & G.L. Webster</i> 6054 (K), s.s.
<i>P. warshenewskiana</i>	Bombrait, Chitral, <i>M.A. Siddiqi & A. Rahman</i> 26764 (RAW), s.s.
”	Rawat-Yasin, <i>F. Schmid</i> 2366 (RAW), l.s.
”	Ayun, Chitral, <i>Y. Nasir</i> 4980 (RAW), s.s.
”	Sol Laspur, Chitral, <i>E.J. Ecker</i> 7578 (RAW), s.s.