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Scanning Electron Microscopic Studies on Surface Pattern of the Pollen Loads from *Apis cerana* in Jajarkot District

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Abstract

Over nineteen species of pollen flora belonging to thirteen families were recovered from four pollen load samples from honey bee *Apis cerana* collected in Jajarkot district, mid-western region, Nepal. The pollen morphology was investigated using light microscopy along with scanning electron microscopy for correct identification of pollen to its lower taxonomic level and to contribute to melissopalynological studies originating from the native apiflora. The palynological assemblage identified to the generic and some up to species level belong to *Alnus* sp., *Artemisia* sp., *Brassica* sp., *Cornus* sp., *Diploknema* sp., *Fraxinus* sp., *Ilex* sp., *Jasminum* sp., *Justicia* sp., *Ligustrum* sp., *Myrica esculenta*, *Salix* sp., *Strobilanthes* sp., and *Urtica* sp. Some of the pollen grains identified to only family level, belong to, Acanthanceae, Compositae, Lamiaceae and Rosaceae. The identified pollens clearly reflect the botanical and geographical origins of the pollen load samples. Palynomorphological investigation included the description of pollen symmetry, polarity, ornamentation, aperture, shape and size. The results for the pollen assemblages and nectariferous plant sources of Jajarkot district are discussed.

Key words: pollen flora, palynomorphology, botanical origin, nectar source, honey bee

Introduction

The study of pollen composition is an effective tool for studying the interaction between honey bees and vegetation, and has also been stressed its importance in establishing apiculture-based honey industries. Analysis of pollen of different species of the floral nectar sources gathered by honeybees to produce honey enable the botanical origin of honey and pollen load specimens to be identified (Dustman & von der Ohe 1993), its type and quality (Seethalakshmi 1980, Attri 2010) from different countries (Zander 1941). The honey bees frequently make use of the resources available. In the growth and development of honey bees, nectar is the source of carbohydrates, whereas proteins are provided by the pollen (Lin *et al.*1990). The honey bees frequently make use of the resources available close to the site of the hives and analyzing the proportional representation of different pollen types allows the characterization of honey from different regions, in terms of flora and vegetation. Therefore, assessment of the botanical taxa that are the source of honey is of high practical and scientific importance (Maurizio 1951, Louveaux *et al.* 1978, Molan 1998, Terrab *et al.* 2003) in the food control (Lieux 1975, Louveaux *et al.*1978, Moar 1985). For this reason, it is necessary to protect consumers from the fraudulent mislabeling of inferior honeys (Dustmann & Bote 1985, 1987, Dustman & von der Ohe 1993).

Both light microscopic (LM) and scanning electron microscopic (SEM) observations of pollen loads from a particular locality reflects the geographical origin of a particular type of honey, since its pollen spectrum reflects the floral situation of the place where that particular honey was produced (Louveaux *et al.* 1978). This helps the beekeepers in the proper management of bee colonies to enhance honey production.

The bee flora of Nepal have been previously surveyed (Kafle 1984, 1992, Maskey 1989, 1992, Partap & Verma 1996, Partap 1997). However, pollen analytical studies of Nepali honeys are mostly fragmentary, although the honey and pollen load samples from different parts of Nepal have been melissopalynologically studied by some workers (ICIMOD 1996, Partap 1997, Joshi 1999) using LM to identify and interpret the pollens present in a particular honey. SEM studies on surface pattern of pollen loads of honey bees are rarely studied in Nepal. Therefore, SEM studies led us to investigate the possibility of identifying pollen loads of *A. cerana* bees to a lower taxonomic level from Jajarkot district. Because the district has considerable potentiality for bee keeping ventures for the production of good quality honey, the present study is aimed to understand and identify the composition of vegetation especially nectariferous plants around the bee hives of the *A. cerana* bees of Jajarkot district through SEM observation of pollen loads.

Study Area

Jajarkot, one of the district of Bheri Zone in mid-western region of Nepal with geographical coordinates 28°42'0"N, 82°14'0"E, is situated 190 kilometers north west (295°) of the approximate center of Nepal and 321 kilometers west of the capital Kathmandu at an average elevation of 1642 meters above sea level.

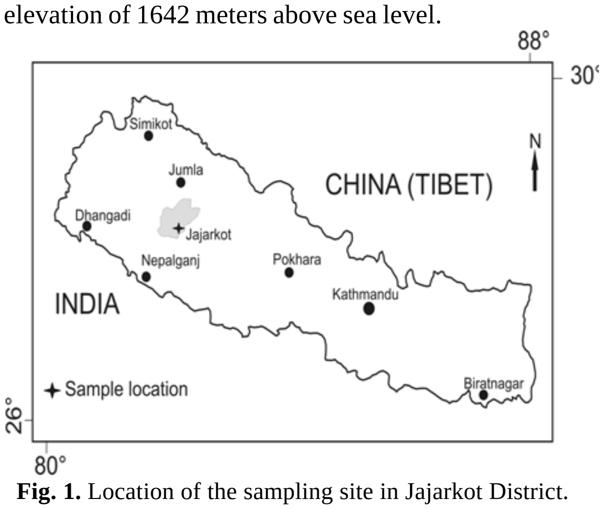

Fig. 1. Location of the sampling site in Jajarkot District.

Methodology

The study was comprised of analysis of four pollen load samples from *A. cerana* honeybee. The pollen loads were obtained from four randomly selected locations from Jarjakot district in 2001 and *taken to the* palynology section of the Institute of Palaeontology, Vienna University, Austria *in* sealed vials during the same year for scanning electron microscope (SEM) observation. In order to collect the pollen, the samples were diluted with distilled water and centrifuged at 3000 rpm (rotation per minute) for 3 minutes. The samples were washed with glacial acetic acid two times and proceeded for acetolysis to remove the cellulose and cell content in the pollen grains. This included the treatment with a solution containing acetic anhydride $(CH_3CO)_2O$ and concentrated sulphuric acid (H_2SO_4) in a ratio of 9:1 (Erdtman 1954). The samples were then kept in water bath for 5 minutes and washed with glacial acetic acid and water respectively. At the end, the samples were mounted in glycerin jelly and proceeded for light microscopic (LM) study.

After examining the pollen grain, under light microscope (LM) and taking LM photographs, the same pollen grains were brought to the edge of the glycerin using specially adapted needles with a human hair glued at the tip (Zetter 1989). With the help of needles the pollens were transferred to a SEM stub to which a drop of absolute ethanol (C_2H_5OH) had been applied with a pipette simultaneously. The SEM stub was kept under a binocular microscope at the required magnification. The pollen grains were then coated with gold in a BIORAD Sputter Coater for four minutes. The samples then followed by examination of the pollen with Jeol JSM 6400 SEM at 10 kV at different magnifications and orientation. SEM photographs were taken with the camera attached to the microscope using AGFA APX 100 (100 ASA) black and white film. This was followed by development of the film and photographs in the dark room.

Pollen grains were generally identified according to their physical appearance. The criteria of identification used according to the position and number of apertures, the shape and size of the pollen grains as a whole, fine structures (ornamentation) on the sexine, and the sculptured exine. There are mainly three types of apertures; (i) *porate*, having isodiametric pores and (ii) colpate, having pores that are long, boat shaped with pointed ends. Sometimes the pore and colpus in a pollen grains combined together to form (iii) *colporate* apertures. If arranged equidistantly round the equator of the pollen grain; they are assigned the prefix; *zono-*. If scattered all over, the prefix *panto-*. The number of apertures were also indicated by prefixes; *mono-* for one aperture, *di-* for two apertures, *tri-* for three apertures, tetra for four and *penta* or *poly-* for numerous apertures. Morphological classes of pollen grains on the basis of aperture and symmetry could be viewed from equatorial as well as polar axis. Each pollen grain varied in their ultra-structures at the species level which helps for accurate taxonomical groupings (Erdtman 1952). The SEM study is very helpful to identify the pollen to lower taxonomical level as the details in the tectum can be measured in microns in high magnification (Ferguson *et al.* 2007).

Results

Palynological assemblages

A series of pollen articles and manuals (Huang 1972, 1981, Waha 1982, Valdes *et al.* 1986, Gupta & Sharma 1986, Iwanami *et al.* 1988, Scotland 1991, Tryon & Lugardon 1991, Moore *et al.*1991, Harley 1991, Uffelen 1993, Fuhsiung *et al.* 1995, Jones *et al.* 1995, Schneider 1996, Unfried 1997, Carine & Scotland 2000, Kapp *et al.* 2000, Shrestha *et al.* 2005) were found to be very useful for comparison and identification of pollen grains. The taxonomy and the morphological details of palynological assemblages are described in the following paragraphs. The descriptive terminology was followed after Punt *et al.* (2007).

Since, SEM investigation of pollen is poorly done in Nepal, in present study the pollen morphology was investigated using light microscope along with scanning electron microscopy for correct identification of pollen to its lower taxonomic level and to contribute melissopalynological studies originating from the native apiflora. Thus, a binomial was only given to the pollen when it could be confirmed by examining well identified comparative material from herbarium specimens. In present study, a diverse spectrum of 19 pollen types belonging to 13 families was recovered from four pollen load samples, collected from the Jajarkot district, mid- western region, Nepal (Table 1). The pollen assemblages were belonging to angiosperms only. Palynomorphological identification included the description of pollen symmetry, polarity, ornamentation, aperture, shape and size of the pollens to the generic and some up to species level belong to *Alnus* sp., *Artemisia* sp., *Brassica* sp., *Cornus* sp., *Diploknema* sp., *Fraxinus* sp., *Ilex* sp., *Jasminum* sp., *Justicia* sp., *Ligustrum* sp., *Myrica esculenta*, *Salix* sp., *Strobilanthes* sp., *Urtica* sp. etc. In the present study, only the generic name was used. Some of the pollen could not be identified beyond the family level. The LM and SEM photographs pollen taxa are shown in Figs. 2-5.

Table 1. Floral diversity of the pollen taxa in four pollen loads from Jajarkot district

Families	Name of the identified genera	Identified as gen. et spec. indet.	Total genera
Acanthaceae	*Strobilanthes* sp. (1), *Strobilanthes* sp. (2)	2	4
Aquifoliaceae	*Ilex* sp.		1
Betulaceae	*Alnus* sp.		1
Brassicaceae	*Brassica* sp.		1
Compositae		2	2
Cornaceae	*Cornus* sp.		1
Lamiaceae		1	1
Myricaceae	*Myrica* sp.		1
Oleaceae	*Fraxinus* sp., *Jasminum* sp.		2
Rosaceae		2	2
Salicaceae	*Salix* sp.		1
Sapotaceae	*Diploknema* sp.		1
Urticaceae	*Urtica* sp.		1
Total	12	7	19

Morphological description of individual palynomorphs
Angiosperms
Family Acanthaceae
Strobilanthes sp. (1)

Fig. 2 (1-3)
Shape: Prolate.
Size: Polar axis 80 µm, equatorial axis 36 µm.
Aperture: Tricolporate, colpi shallow and distinct.
Exine: 4 µm, sexine thicker than nexine, pseudocolpi uniformly present throughout the grain dividing the sexine into longitudinal strips, sexine bireticulate, each longitudinal sexine strip having an uneven coarse reticulum with a depressed reticulated tectum.

Strobilanthes sp. (2)
Fig. 2 (4-6)
Shape: Prolate.
Size: Polar axis 85µm, equatorial axis 29 µm.
Aperture: Tricolporate.
Exine: 5 µm, sexine thicker than nexine, sexine divided into longitudinal stripes, sexine bireticulate, each longitudinal sexine strip having an uneven coarse reticulum with a depressed reticulated tectum.

Acanthaceae gen. et spec. indet. (1)
Fig. 2 (7-9)
Shape: Prolate.
Size: Polar axis 76 µm, equatorial axis 29 µm.
Aperture: Tricolporate.
Exine: 5 µm, sexine thicker than nexine, sexine divided into longitudinal stripes, sexine reticulated. The sizes of reticulation are different in stripes. Some stripes are without reticulum having randomly distributed granules on the surface.

Acanthaceae gen. et spec. indet. (2)
Fig. 2 (10-12)
Shape: Prolate.
Size: Polar axis 60 µm, equatorial axis 27 µm.
Aperture: Tricolporate.
Exine: 3 µm, sexine thicker than nexine, sexine divided into longitudinal but unequal stripes, sexine reticulated.

Family Aquifoliaceae
Ilex sp.
Fig. 3 (13-15)
Shape: Prolate, sub-circular in polar view.
Size: Polar axis 36 µm, equatorial axis 24 µm.
Aperture: Tricolporate.
Exine: 3 µm, atectate, clavate and pilate, pila with fine striate ornamentation.

Family Betulaceae
Alnus sp.
Fig. 3 (16-18)
Shape: Oblate, penta-angular in polar view.
Size: 17-20 µm
Aperture: Pentaporate, pori vestibulum type, neighbouring pori connected by archs or bands of nexinous thickening.
Exine: 1.5 µm, tectum consists of irregular rugulae with very small spinules (microechinate). Sexine slightly thicker than nexine.

Family Brassicaceae
Brassica sp.
Fig. 3 (19-21)
Shape: Prolate, circular in polar view.
Size: Polar axis 30 µm, equatorial axis 20 µm.
Aperture: Tricolpate
Exine: 1.3 µm, tectum uniformaly reticulate, the colpi area is granulate.

Family Composiate
Artemisia sp.
Fig. 3 (22-24)
Shape: Prolate, circular in polar view, lobate.
Size: Equatorial axis 20 µm.
Aperture: Tricolporate, colpi nearly as long as polar axis.
Exine: 3 µm, sexine much thicker than nexine, distinctly stratified, sexine in mesocolpium thicker than in the colpi area forming a margo, sexine

microechinate and granulate, granules uniformly distributed between the spinules.

Compositae gen. et spec. indet. (1)
Fig. 4 (25-27)
Shape: Sub-prolate to prolate
Size: 28 µm
Aperture: Tricolporate.
Exine: 6-7 µm (with spines), sexine thick, perforate and echinate, the basal part of spine broadly perforate with perforations of various shape and sizes, surface granulate, spines 2-3 µm in length.

Compositae gen. et spec. indet. (2)
Fig. 4 (28-30)
Shape: Prolate to spheroidal, lobate.
Size: 25 µm.
Aperture: Tricolporate.
Exine: 1 µm, sexine as thick as nexine, spiny and granulate, tectum and spines with only very few perforations, surface granulate.

Family Cornaceae
Cornus sp.
Fig. 4 (31-33)
Shape: Prolate.
Size: Polar axis 20 µm.
Aperture: Tricolporate
Exine: 1.5-2 µm, tectate, tectum micro-echinate, colpi nearly as long as polar axis.

Family Labiatae
Labiatae gen. indet.
Fig. 4 (34-36)
Shape: Oblate, semicircular in polar view.
Size: Equatorial axis 34-40 µm.
Aperture: Hexacolpate, colpi long and 6-7 µm wide.
Exine: 1.5 µm, sexine slightly thicker than nexine, sexine suprareticulate, muri less than 1 µm, lumina large up to 2 µm.

Family Myricaceae
Myrica sp.
Fig. 5 (37-39)
Shape: Oblate, triangular in polar view.
Size: 20 µm.
Aperture: Triporate.
Exine: 1 µm, sexine is thicker than nexine near and around pori, sexine regularly microechinate.

Family Oleaceae
Fraxinus sp.
Fig. 5 (40-42)
Shape: Prolate, circular in polar view.
Size: Polar axis 18 µm, equatorial axis 15 µm.
Aperture: Tricolporate, colpi are long with small circular endoapertures.
Exine: 1 µm, sexine thicker than nexine, tectum reticulate, lumina are heterobrochate, triangular to polygonal in shape.

Jasminum sp.
Fig. 5 (43-45)
Shape: Prolate, circular in polar view.
Size: Equatorial axis 39 µm.
Aperture: Tricolporate, colpi are long, small circular endoapertures.
Exine: 1 µm, sexine thicker than nexine, tectum reticulate, lumina are heterobrochate, triangular to polygonal in shape

Family Rosaceae
Rosaceae gen. indet. (1)
Fig. 5 (46-48)
Shape: Prolate, circular in polar view.
Size: Polar axis 37µm, equatorial axis 27 µm.
Aperture: Tricolporate, colpi long.
Exine: 1-1.5 µm, sexine is thicker than nexine, striate, striations sometimes joined with each other and variable in size, striations granulated.

Rosaceae gen. indet. (2)
Fig. 6 (49-51)
Shape: Prolate, circular in polar view.
Size: Polar axis 36 µm, equatorial axis 30 µm.
Aperture: Tricolporate, colpi long.
Exine: 1-1.5 µm, sexine is thicker than nexine, striate, striations sometimes joined with each other and variable in size.

Family Salicaceae
Salix sp.
Fig. 6 (52-54)
Shape: Prolate, circular in polar view.
Size: Equatorial axis 11 µm, polar axis 23µm
Aperture: Tricolporate, colpi long broad reaching nearly to poles.

Exine: 2 µm, reticulated, lumina broad in mesocolpium, smaller in apertural area.

Family Sapotaceae
Diploknema sp.
Fig. 6 (55-57)
Shape: Prolate.
Size: Polar axis 49 µm, equatorial axis 33 µm.
Aperture: Tetracolporate, colpi long and gradually broaden towards the poles, end of colpi rounded.
Exine: 1.5 µm, sexine as thick as nexine, sexine micro-rugulate, perforated and covered by irregularly distributed nano-granules.

Family Urticaceae
Urtica sp.
Fig. 6 (58-60)
Shape: Oblate.
Size: Equatorial axis 15-20 µm.
Aperture: Tricolporate, colpi short and barrow.
Exine: 1.5 µm, sexine as thick as nexine, sexine micro-echinate, perforated and covered by irregularly distributed nano-granules. Microechinae fused to form clusters

Discussion

The honey from different regions has a specific pollen spectrum depending on the floristic composition of the region. As a result of the pollen analysis of four pollen load samples from the Jajarkot district, the mid-western region of Nepal, the important apiflora have been identified and the plant species used by *A. cerana* as nectar source assessed. They are: *Strobilanthes* sp. (Acanthaceae), *Ilex* sp. (Aquifoliaceae), *Alnus* sp. (Betulaceae), *Brassica* sp. (Brassicaceae), *Artemisia* sp. (Composiate), *Cornus* sp. (Cornaceae), *Myrica* sp. (Myricaceae), *Fraxinus* sp. and *Jasminum* sp. (Oleaceae), *Salix* sp. (Salicaceae), *Diploknema* sp. (Sapotacea), *Urtica* sp. (Urticaceae) including Labiatae and Rosaceae.

Joshi (1999) identified total 16 pollen types in honey samples from *A. cerana* in the Jajarkot district using LM. Whereas, the present study of pollen loads of the same honeybee in Jajarkot district using LM as well as SEM revealed 19 species of pollen flora belonging to 13 families. The pollen composition was mostly from the plants growing in subtropical climate zones. Most of the pollen in the palynological assemblages are derived from entomophilous plants, however, the honey samples also contained a significant number of airborne (anemophilous) pollens from some plant such as *Alnus*, *Salix* and *Myrica*.

Beekeeping is based on vegetation and generates multi-pronged employment through small and rural scale enterprises. It has the potential of preventing the out flow of rural folk from villages towards cities. Jajarkot is one of the districts in Nepal that has a rich potential of indigenous beekeeping. Therefore, this study not only reflects the palynological composition, botanical origins of the pollen loads of the particular area, but also provides knowledge of floral assemblage of the area where the *A. cerana* honey bees are foddering, and which may be useful for the local beekeepers in bee management and in promoting beekeeping development.

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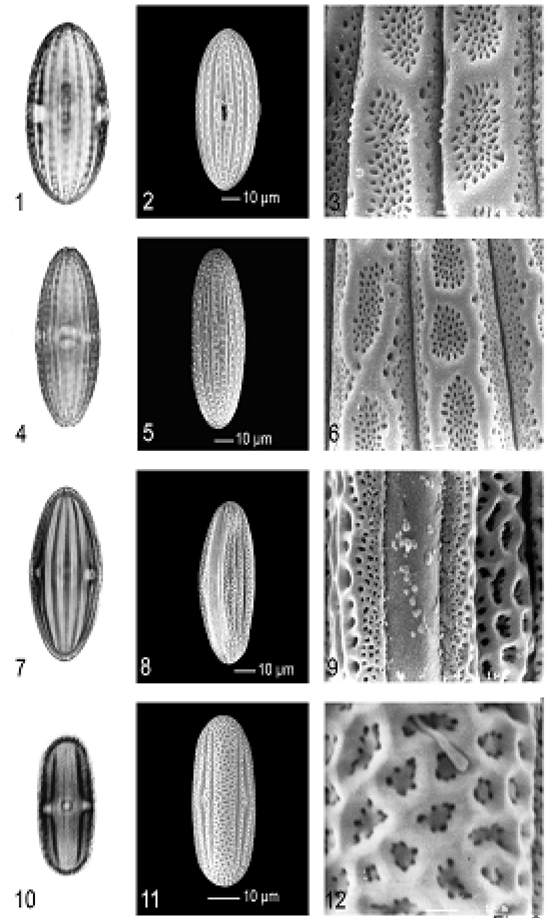

Fig. 2

Family Acanthaceae (1-12)

1. *Strobilanthes* sp. (1), equatorial view, LM x 850
2. *Strobilanthes* sp. (1), equatorial view, SEM x 1,000
3. *Strobolanthes* sp. (1), details of the tectum x 10,000
4. *Strobilanthes* sp. (2), equatorial view, LM x 850
5. *Strobilanthes* sp. (2), equatorial view, SEM x 900
6. *Strobolanthes* sp. (2), details of the tectum x 8,500
7. Acanthaceae gen. et spec. indet. (1), equatorial view, LM x 850
8. Acanthaceae gen. et spec. indet. (1), equatorial view, SEM x 1,000
9. Acanthaceae gen. et spec. indet. (1), details of the tectum x 8,500
10. Acanthaceae gen. et spec. indet. (2), equatorial view, LM x 850
11. Acanthaceae gen. et spec. indet. (2), equatorial view, SEM x 1,600
12. Acanthaceae gen. et spec. indet. (2), details of the tectum x 15,000

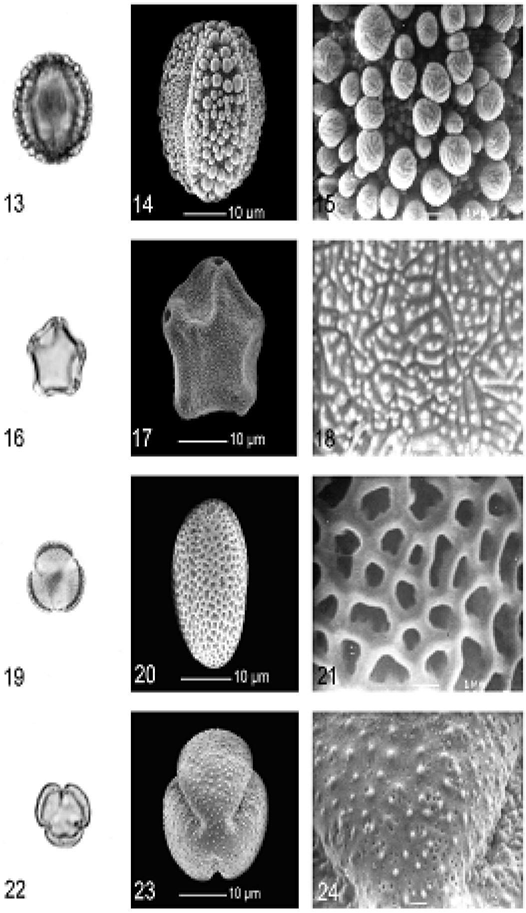

Fig. 3

Family Aquifoliaceae (13-15), Betulaceae (16-18), Brassicaceae (19-21), Compositae (22-24)

13. *Ilex* sp., equatorial view, LM x 850
14. *Ilex* sp., equatorial view, SEM x 2,000
15. *Ilex* sp., details of the tectum x 8,500
16. *Alnus* sp., polar view, LM x 850
17. *Alnus* sp., polar view, SEM x 2,500
18. *Alnus* sp., details of the tectum x 8,500
19. *Brassica* sp., polar view, LM x 850
20. *Brassica* sp., Equatorial view, SEM x 2,500
21. *Brassica* sp., details of the tectum, SEM x 13,500
22. *Artemisia* sp., polar view, LM x 850
23. *Artemisia* sp., polar view, SEM x 2,700
24. *Artemisia* sp., details of the tectum SEM x 9,000

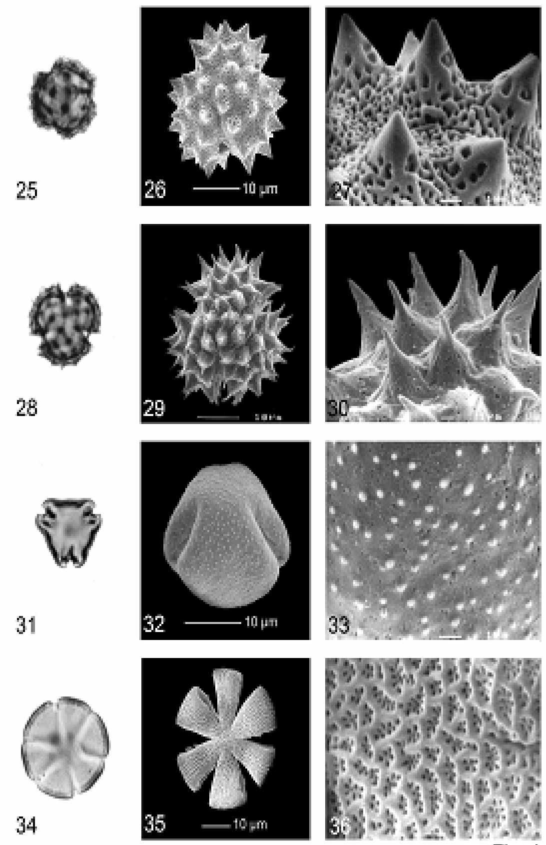

Fig. 4

Family Compositae (25-30), Cornaceae (31-33), Lamiaceae (34-36)

25. Compositae gen. et spec. indet., polar view, LM x 850
26. Compositae gen. et spec. indet., polar view, SEM x 2,300
27. Compositae gen. et spec. indet., details of the tectum, SEM x 10,000
28. Compositae gen. et spec. indet., polar view, LM x 850
29. Compositae gen. et spec. indet., polar view, SEM x 2,200
30. Compositae gen. et spec. indet., details of the tectum, SEM x 6,000
31. *Cornus* sp., polar view, LM x 850
32. *Cornus* sp., oblique view, SEM x 3,000
33. *Cornus* sp., details of the tectum, SEM x 10,000
34. Lamiaceae gen. et spec. indet., polar view, LM x 850
35. Lamiaceae gen. et spec. indet., polar view, SEM x 1,500
36. Lamiaceae gen. et spec. indet., details of the tectum SEM x 13,000

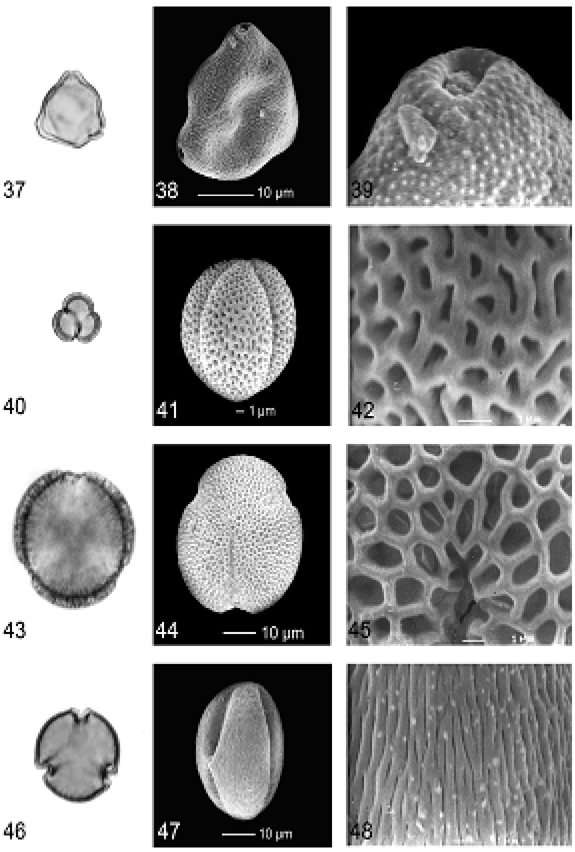

Fig. 5

Family Myricaceae (37-39), Oleaceae (40-45), Rosaceae (46-48)

37. *Myrica* sp., polar view, LM x 850
38. *Myrica* sp., polar view, SEM x 2,700
39. *Myrica* sp., details of the tectum, SEM x 11,000
40. *Fraxinus* sp., polar view, LM x 850
41. *Fraxinus* sp., equatorial view, SEM x 4,000
42. *Fraxinus* sp., details of the tectum, SEM x 15,000
43. *Jasminum* sp., polar view, LM x 850
44. *Jasminum* sp., Polar view, SEM x 1,300
45. *Jasminum* sp., details of the tectum, SEM x 10,000
46. Rosaceae gen. et spec. indet., polar view, LM x 850
47. Rosaceae gen. et spec. indet., equatorial view, SEM x 1,000
48. Rosaceae gen. et spec. indet., SEM x 10,000

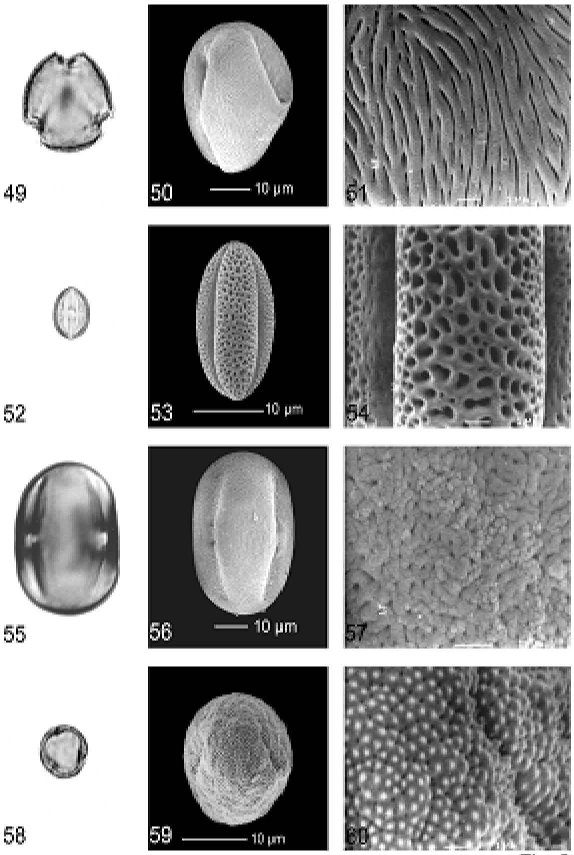

Fig. 6

Family Rosaceae (49-51), Salicaceae (52-54), Sapotaceae (55-57), Urticaceae (58-60)

49. Rosaceae gen. et spec. indet., polar view, LM x 850
50. Rosaceae gen. et spec. indet., equatorial view, SEM x 1,500
51. Rosaceae gen. et spec. indet., SEM x 10,000
52. *Salix* sp., equatorial view, LM x 850
53. *Salix* sp., equatorial view, SEM x 3,500
54. *Salix* sp., details of the tectum, SEM x 11,000
55. *Diploknema* sp., equatorial view, LM x 850
56. *Diploknema* sp., equatorial view, SEM x 1,300
57. *Diploknema* sp., details of the tectum, SEM x 8,000
58. *Urtica* sp., polar view, LM x 850
59. *Urtica* sp., Polar view, SEM x 3,500
60. *Urtica* sp., details of the tectum, SEM x 14,000

References

Attri, P.K. 2010. Melissopalynological studies of *Apis cerana* summer honey from Chamba district of the Himachal pradesh. *Asian J. Exp. Biol. Sci.* **1(4):** 930-939.

Carine, M.A. and R.W. Scotland. 2000. 68 taxa and 32 characters: resolving species relationships using morphological data. In M. M. Harley, C. M. Morton and Blackmore (Eds.). Pollen and spores: Morphology and biology, Royal Botanical Garden, Kew. 365-384 pp.

Dustmann, J.H. and K. Bote. 1985. Raster-elektronenmikroskopische studien an pollen aus bienenhonig. I. Vergleich verschiedener präparationsmethoden. *Apidologie*. 16 (3): 331-340 (in German).

Dustmann, J.H. and K. Bote. 1987. Raster-elektronenmikroskopische studien an pollen aus bienenhonig. II. Vergleich der exine-strukturen trockener und in honig expandierter pollen bei verschiedenen apfel- bzw. Zierapfelsorten. *Gartenbauwissenschaft.* 52: 271-278 (in German).

Dustmann, J.H. and von der Ohe. 1993. Scanning electron microscopic studies on pollen from honey. IV. Surface pattern of pollen of *Sapium sebiferum* and *Euphorbia* spp (Euphorbiaceae) *Apidologie.* 24:59-66.

Erdtman, G. 1952. Pollen morphology and plant taxonomy Angiosperm (An introduction to palynology, vol. 1). Almqvist and Wiksell. Stockholm.

Erdtman, G. 1954. An introduction to pollen analysis. Chronica Botanica Company, Waltham, Mass., USA. 239 p.

Ferguson, D.K., R. Zetter and K.N. Paudayal, 2007. The need for the SEM in palaeopalynology. *Comptes Rendus Palevol.,* **6 (6-7):** 423-430.

Fuhsiung, W.C., N.Z. Yulong and Y. Huiqiu. 1995. Pollen flora of China. Institute of Botany, *Academia Sinica.* 461 p.

Gupta, H.P. and C. Sharma. 1986. Pollen flora of North-West Himalaya. Indian Association of Palynostratigraphers, Janki Bhawan, Narhi, Lucknow, India. 181p.

Harley, M.M. 1991. The pollen morphology of the Sapotaceae. *Kew bulletin.* **46(39):** 379-491.

Huang,Tseng-Ching. 1972. Pollen flora of Taiwan. National Taiwan University, Botany Department press. 297 p.

Huang, Tseng-Ching. 1981. Spore flora of Taiwan. National Taiwan University, Botany Department press, 112 p.

ICIMOD. 1996. Promotion and development of beekeeping through preservation of Iidigenous *Apis cerana*. Annual Project Progress Report Submitted to the Federal Chancellory of Austria.

Iwanami, Y., T. Sasakuma and H. Yamada. 1988. Pollen: Illustrations and scanning electronmicrographs. Springer-Verlag. 198 p.

Jones, G.D., V.M. Bryant Jr., H. L. Meredith, S. D. Jones and P. D. Lingren. 1995. Pollen of the southern United States: with emphasis on melissopalynology and entomopalynology. AASP contribution series number 30, the American Association of Stratigraphic Palynologists Foundation. 104 p.

Jones, G.D. and J.R. Coppedge. 1998. Pollen analyses of the boll weevil exoskeleton. In V. M. Bryant, Jr and J.H. Wrenn (Eds). New developments in palynomorph sampling, extraction, and analysis American Association of Stratigraphic Palynologists Foundation, Dallas, Texas. 121–125 pp.

Joshi, S.R. 1999. Physico-chemical and melissopalynological characteristics of Nepalese honey. Ph.D. Thesis, University of Agricultural Sciences, Vienna , Austria.

Kafle, G.P. 1984. A general survey of bee flora in and around Kathmandu valley. *Nepalese Journal of Agriculture*. **15:** 89-99.

Kafle, G.P. 1992. Salient features of beekeeping in Nepal. In Verma, L.R. (ed), Honeybees in Mountain Agriculture, New Delhi: Oxford and IBH Publishing Co. Pvt. Ltd. 155-162 pp.

Kapp, O.R., O.K. Davis and J.E. King. 2000. Ronald O. Kapp's pollen and spores. The American Association of Stratigraphic Palynologists Foundation. 279 p.

Lieux, M.H. 1975. Dominant pollen types recovered from commercial Louisiana honeys. *Econ. Bot.* **29:** 78-96.

Lin, X.L., L.Q. Zhu, Y.Y. Yuan and L.M. Li. 1990. Morphological changes in aged canine prostatic hyperplasia treated with bee pollen. *Chinese Traditional and Herbal Drugs.* **21:** 164-166.

Louveaux, J., A. Maurizio and G. Vorwohl. 1978. Methods of melissopalynology. *Bee World.* **59(4):** 139-157.

Maskey, M. 1989. Inter-relationship between bees and flowers in Kathmandu valley. Paper presented at the fourth international conference on apiculture in tropical climates, Cairo, Egypt.

Maskey, M. 1992. Mountain women and beekeeping in Nepal. In Verma, L.R. (ed), Honeybees in Mountain agriculture, pp 119-132. New Delhi: Oxford and IBH Publishing Co. Pvt. Ltd.

Maurizio, A. 1951. Pollen analysis of honey. *Bee World*. **32:** 1-5.

Moar, N.T. 1985. Pollen analysis of New Zealand honey. *New Zealand Jrnl. Apic. Res.* **28:** 39-70.

Moar, N.T. 1993. Pollen grains of New Zealand dicotyledonous plants. Lincoln, Canterb. N.Z. Manaaki Whenua Press.

Molan, P.C. 1998. The limitations of the methods of identifying the floral source of honeys. *Bee World.* **79:** 59-68.

Moore, P.D., J.A. Webb and M.E. Collinson. 1991. Pollen analysis. 2nd ed. London: Blackwell Sci. Publ. 216 p.

Partap, U. and L.R. Verma. 1996. Impact of food resource availability on growth and development of *Apis cerana* colonies in the Kathmandu valley of Nepal. *Ecoprint.* **3:**21-29.

Partap, U. 1997. Bee Flora of the hindu Kush-Himalayas: Inventory and management. International Centre for Integrated Mountain Development, Kathmandu, Nepal.

Punt, W., P.P. Hoen, S. Blackmore, S. Nilsson and A. Le Thomas. 2007. Glossary of pollen and spore terminology. *Review of Palaeobotany and Palynology.* **143:**1-81.

Schneider, W. 1996. Zur pollenmorphologie ausgewählter rezenter und fossiler vertreter der Oleaceae. Diplomarbeit zur erlangung des akademischen grades magistra der naturwissenschaften an der Formal- und naturwissenschaftlichen fakultät der Uni Wien (unpublished M. Sc. thesis). 66 p (in German).

Scotland, R.W. 1991. A systematic analysis of pollen morphology of Acanthaceae genera with contorted corollas. In Blackmore S. and Barnes, S. H. (Ed.). Pollen and Spores, Pattern and Diversification. Systematics Association. 269-289 pp.

Seethalakshmi, T.S. 1980. Melittopalynological investigation on some Indian honeys. Proc. 2 Int. Conf. Apic. Trop. Clim. Delhi, India. 609-621pp.

Shrestha, K.K., K.N. Paudayal and D.K. Ferguson. 2005. Pollen morphology of the Himalayan *Viola* (Violaceae). *Nepal Journal of Science and Technology.* **5:** 73-80.

Terrab, A., M.J. Diez and F.J. Heredia. 2003. Palynological, physicochemical and colour characterisation of Moroccan honeys. I. River Red Gum (*Eucalyptus camaldulensis* Dehnl.) Honey. *International Journal of Food Science and Technology.* **38:** 379-386.

Tryon, A.F. and B. Lugardon. 1991. Spores of the pteridophyta: surface, wall structure and diversity based on electron microscope studies. Springer-Verlag. 648p.

Uffelen, G.A. Van. 1993. Morphogenesis and evolution of the spore wall of Polypodiaceae (Filicales). Rijksherbarium/ Hortus Botanicus, Leiden. 180 p.

Unfried S. 1997. Zur pollenmorphologie rezenter und fossiler vertreter der gattung *Rhododendron*. diplomarbeit zur erlangung des akademischen grades magistra der naturwissenschaften an der Formal- und naturwissenschaftlichen fakultät der Uni Wien (unpublished M. Sc. thesis). 64 p (in German).

Valdes, B., M.J. Diez and Fernandez, I. 1986. Atlas polinico de Andalucia Occidental, instituto de Desarrollo Regional N° 43, Universidad de Sevilla, Excma. Diputacion de Cadiz. 451p (in Spanish).

Waha, M. 1982. Zur ultrastruktur und funktion pollenverbindender fäden bei angiospermen, insbesondere der vscinfäden bei ericaceae. Dissertation zur erlangung des doktorgrades an der formal- und naturwissenschaftlichen fakultät der Uni Wien (unpublished PhD dissertation). 190p (in German).

Zander, E. 1941. Beiträge zur herkunftsbestimmung bei honig. III. Pollengestaltung und herkunftsbestimmung bei blütenhonig. Verlag Liedloff, Loth und Michaelis, Leipzig (in German).

Zetter, R. 1989. Methodik und bedeutung einer routinemäßig kombinierten lichtmikros-kopischen und rasterelektronenmikroskopischen untersuchung fossiler mikrofloren. Cour. Forsch.-Inst. Senckenberg, Frankfurt am Main. 109: 41-50 (in German).