SEM INVESTIGATION OF POLLEN TAXA IN HONEYS FROM AUTOCHTONE *APIS CERANA* IN GODAVARI, LALITPUR DISTRICT, NEPAL

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ABSTRACT
Pollen analysis of 8 multifloral honey samples collected from 4 locations of Godavari, Lalitpur district, Nepal was performed using Scanning Electron Microscope (SEM). In this investigation, a wide range of foraging plant sources for *Apis cerana* honey bees was identified which demonstrates the adequate potential for expanding and sustaining beekeeping in this area. The palynological assemblage of a total of 44 species of pollen flora representing 28 families was identified to the generic and some up to species level. Some of the pollen grains identified to only families, belong to Acanthaceae, Apiaceae, Araliaceae, Chenopodiaceae, Compositae, Lamiaceae, Loranthaceae, Meliaceae, Poaceae, Rosaceae, Rutaceae and Pteridaceae. The pollen assemblages in honeys were mostly belonging to angiosperms while the gymnosperm pollen was completely absent. One pteridophyte spore belonging to family Pteridaceae recovered. In this paper the morphology of the pollen grains based on SEM observation are described and the importance of the systematic documentation of various bee flora are discussed.

Key words: pollen analysis, multifloral honey, autochtone honey bee, SEM, Kathmandu Valley

INTRODUCTION
Honey is natural complex food product produced by bees from nectar of plants and also from honeydew. Honey contains a significant quantity of pollen which is the only proteic food within the bee hive and used for feeding the larvae and the young bees. The combination of wind and insect-pollinated taxa found in a honey sample often produce a pollen spectrum that is a valuable tool for identifying the geographical origin of the honeys for the presence of a combination of pollen that is typical only to that particular location (Louveaux et al. 1978) and to identify the genera of the plants the honeys bees visited. In Nepal, bee-keeping is practiced in many areas, characterized by a remarkable richness of bee floras. Information gained from a given honey sample is useful when substantiating claims of a particular honey source and is also of great importance for quality control and helps to ascertain whether honey is adulterated or not (Maurizio 1951, Molan 1998, Louveaux et al. 1978, Terrab et al. 2003). The element content of bees and bee products recognized as useful indicator of the presence of specific minerals within their forage area (Crane 1984). Honey may also contain enzymes of plant origin from nectar or honeydew, and possibly from pollen (Crane 1981). Therefore, a pollen analysis of honey not only gives the palynological composition itself but also provides a broad knowledge of floral assemblage of an area where the bees are foddering.

Light microscopy (LM) in melissopalynology is used to identify and interpret the pollen spectrum of a particular sample. Whereas several workers used Scanning Electron Microscopy (SEM) for morphological comparisons and taxonomy (Skvarla and Larson 1965, Ridgeway and Skvarla
to create new terminology for describing pollen ornamentation (Rowley et al. 1988, Vezey et al. 1991), developing a numerical approach to pollen sculpturing (Vezey et al. 1991), and even computer analysis of the exine (Vezey and Skvarla 1990). There are several reports available on pollen morphology from various parts of the world such as SEM micrographs and some atlases (Ogden et al. 1974, Moore et al. 1991, Nilsson et al. 1977, Bassett et al. 1978, Moar, 1993, Qiao 2004) and few SEM micrographs for the pollen presentation (Adams and Morton 1972, 1974, 1976, 1979, Bambara and Leidy 1991, Jones et al. 1995, Wei et al. 2003); however, the majority focuses only on LM (Herrera and Urrego 1996, Beug 2004). Most entomopalynological studies use LM for pollen analyses (Jones and Coppedge 1998). However, SEM is useful in melissopalynology (Van Laere et al. 1969) with its use in honey study.

The earliest research on the pollen analysis of honey dates back to 1895 by Pfister with examination of pollen contents of various Swiss, French, and other European honeys. He was able to identify many of the pollen grains he found because of earlier studies of pollen morphology, structure, and identification of European pollen types by botanists including Guillemin in 1825, Fritsche in 1832, Mohl in 1834, and Fischer in 1890 (Woodehouse 1935). Further, there were several works carried out on melissopalynology (Fehlman 1911, Armbruster 1929, 1934-35, Griebel 1931, Fossel 2000, Köppler et al. 2007) including the most authoritative five-volume work of Zander (1935, 1937, 1941, 1949, 1951), which laid the foundation for melissopalynology research in Europe. The first melissopalynological investigations in the US honey was carried out by Young (1908) and Swiss honey by Fehlman (1911) (Maurizio 1951). Works of Betts (1923, 1925) and Allen (1928) on English honey, made other research advancements in melissopalynology. Todd and Vansell in the US (1942) investigated the relationship and importance of pollen in honey. Chen and Shen (1990) used SEM to examine the pollen of Formosan honey. The first melissopalynological study in Africa is reported by Smith in 1956 (Sowunmi 1976). In India, Sen and Banerjee (1956) analyzed the pollen content of honey samples obtained from a garden near Calcutta and observed an over abundance of anemophilous pollen grains. Vishnu-Mitre (1958) examined the pollen contents of ten samples of Indian honey. Other melissopalynological works from different parts of India were also carried out (Sen and Banerjee 1956, Vishnu-Mitre 1958, Nair 1964, Sharma and Nair 1965, Suryanarayana and Thakar 1966, Suryanarayana et al. 1977, Chanda and Ganguly 1981, Chaturvedi 1983, 1989).


There is some information available on the honey plant resources in Nepal. The sources of informations on bee plants in Nepal are field surveys and melissopalynology (Partap 1997). The bee flora of Nepal have been previously surveyed by Kafle (1984, 1992), Maskey (1989, 1992), Partap and Verma (1996). A list of 156 (Kafle 1984) and 113 (Partap and Verma 1996) bee flora of Kathmandu valley was recorded. Partap (1997) has also reported some bee
plants from Nepal. Diverse bee floras reported from different agro-ecological zones of Nepal represent various agricultural, horticultural, and forage crops, ornamental plants, avenue trees, wild plants, and forest trees (Partap 1997). Further, SEM study of pollen morphology of the Himalayan Viola had been carried out by Shrestha et al. (2005). In spite of elucidation of the melissopalynological advancement in the world very few works have been carried out in Nepal. Nevertheless, the melissopalynological studies of A. cerana honey from Jumla, Nepal recorded the occurrence of about 103 plant species constituting the bee forage sources of that region (ICIMOD 1996). The detail investigation on pollen spectrum of Nepali honeys carried out by Joshi (1999). He identified total 51 pollen types in Apis dorsata, A. cerana and A. mellifera honeys from Chitwan district, 50 pollen types in A. cerana and A. mellifera honeys from Kathmandu valley, 16 pollen types in A. cerana honeys from Jajarkot district, 49 pollen types in A. cerana honeys from Dadeldhura district, 43 pollen types in A. cerana honeys from Jumla district, and 25 pollen types in A. cerana honeys from Langtang. His study represents the major findings in melissopalynology in Nepal indicating the ecological origins of the honeys produced by Apis dorsata, A. cerana and A. mellifera and provided important contributions to the knowledge of the wild and cultivated bee forage. In addition, over nineteen species of pollen flora belonging to thirteen families were recovered from four pollen load samples from honey bee A. cerana collected in Jajarkot district, mid-western region, Nepal using light microscopy along with scanning electron microscopy for the first by Paudayal and Gautam (2011a) and eight pollen types representing seven families of foraging plant sources for the same autochtone species of honey bee in Bajhang district, west Nepal (Paudayal and Gautam 2011b). The previous melissopalynological investigations conducted on Nepali honeys limited to light microscopy (LM) to identify and interpret the pollen spectrum of a particular honey sample. Therefore, the present investigation deals with the SEM description of the pollens of autochtone honey bee A. cerana honeys collected from Godavari area, southern part of the Kathmandu Valley, Nepal. The morphological characters of the pollen of 44 species belonging to 28 families described in this paper are certainly not possible with a single LM study.

STUDY AREA

Godavari, in Lalitpur district, in the Bagmati Zone of central Nepal, with a latitude of 27.6 (27° 36’ 0 N) and a longitude of 85.4 (85° 24’ 0 E), is situated around at an altitude of 1,1455m to 2,765 m above the mean sea level. Godavari lies at the foot of Phulchowki mountain, offers rich flora and fauna and provides access to Nepal’s famous Botanical garden which has a large collection of plants. A marble quarry that has operated in the region has caused mass deforestation, exposing the Phulchowki mountain.

MATERIALS AND METHODS

The bee hives examined were either those of exotic A. mellifera or autochtone A. cerana bee colonies. The honey samples produced by A. cerana were obtained from sealed honey combs from four randomly selected locations of Godavari area. The study comprised the analysis of 8 honey samples. The honey samples from A. cerana were taken to the palynology section of the Institute of Palaeontology, Vienna University, Austria in sealed plastic jars for Scanning Electron Microscope (SEM) observation. The samples were diluted with distilled water and
centrifuged at 3000 rpm (rotation per minute) for 3 minutes to collect the pollen. This process was repeated for several times to collect a significant amount of pollen. The samples were washed with glacial acetic acid two times and then proceeded for acetolysis to remove the cellulose and cell content in the pollen grains. This included the treatment with a solution containing acetic anhydride \((\text{CH}_3\text{CO})_2\text{O}\) and concentrated sulphuric acid \((\text{H}_2\text{SO}_4)\) 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, samples were kept in glycerin and proceeded for Light Microscopic (LM) study.

After examining the pollen grain under LM and taking LM photographs the same pollen grain was brought to the edge of the glycerin on the glass slide using the specially adapted needle with a human hair glued at the tip (Zetter 1989, Ferguson et al. 2007). With the help of needle the pollen was transferred to a SEM stub to which a drop of absolute ethanol \((\text{C}_2\text{H}_5\text{OH})\) was applied with a pipette simultaneously. The SEM stub was kept under a binocular microscope at the required magnification. Care was taken while dropping the absolute ethanol (99% pure), not to wash the pollen grain off the stub. The pollen grains were then coated with gold in a BIORAD Sputter Coater for four minutes. It was then followed by the examination of the pollen with Jeol JSM 6400 Scanning Electron Microscope at 10 kV at different magnification 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 grain as a whole, and the elaborate, fine structure (ornamentation) on the sexine; the sculptured exine. There are mainly three types of apertures; porate, having isodiametric pores and colpate, having apertures that are long, boat shaped with pointed ends. Sometimes the pore and colpus in a pollen grains combined together to form 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 aperture is 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 can be viewed from equatorial as well as polar axis. Each pollen grain varies in their ultra-structures in the species level which helps for accurate taxonomical groupings (Erdtman et al. 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).
Fig. 1. Location of the sample area indicated by arrow.
RESULTS AND DISCUSSION

The melissopalynological investigation of 8 *A. cerana* honey samples from Godavari area revealed 44 species of plant taxa belonging to 28 families (Table 1). The palynological assemblages identified to the generic and some up to species level belong to *Aesandra* sp., *Alnus* sp., *Artemisia* sp., *Brassica* sp., *Cornus* sp., *Corylus* sp., *Fagopyrum* sp., *Fraxinus* sp., *Grevillea* sp., *Impatiens* sp., *Jasminum* sp., *Justicia* sp., *Ligustrum* sp., *Melia azederach*, *Myrica esculenta*, *Polygonum* sp., *Quercus lanata*, *Rhododendron* sp., *Salix* sp., *Sarcococca* sp., *Syzygium* sp., and *Urtica* sp. etc. Some of the pollen grains identified to only families, belong to, *Acanthaceae*, *Apiaceae*, *Araliaceae*, *Chenopodiaceae*, *Compositae*, *Lamiaceae*, *Loranthaceae*, *Meliaceae*, *Poaceae*, *Rosaceae*, *Rutaceae* and *Pteridaceae*. The pollen assemblages were mostly belonging to angiosperms while the gymnosperm pollen was completely absent. One pteridophyte spore belonging to family *Pteridaceae* recovered from the sample.

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<td>Lamiaceae</td>
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A. ANGIOSPERMS

Family Acanthaceae

Acanthaceae gen. et spec. indet. (1)
Plate I, Figs. 1-2
Shape: Prolate.
Size: Polar axis 45 µm, equatorial axis 22 µm.
Aperture: Colporate.
Exine: 3 µm, sexine much thicker than nexine, sexine reticulate, the reticulations are smaller in mesocolpium while wider near the aperture area.

Justicia sp.
Plate I, Figs. 3-4
Shape: Prolate.
Size: Polar axis 40 µm, equatorial axis 28 µm.
Aperture: Colporate.
Exine: 1.5 µm, sexine as thick as nexine, sexine reticulate, lumina microreticulate, sexine around aperture rugulate, perforate and granulate.

Acanthaceae gen. et spec. indet. (2)
Plate I, Figs. 5-6
Shape: Prolate.
Size: Polar axis 27 µm, equatorial axis 15 µm.
Aperture: Tricolporate.
Exine: 1.5 µm, sexine as thick as nexine, sexine reticulate, rugulate, granulate and perforate near aperture, lumina wider near the aperture area compared to mesocolpium. Aperture wide and distinct.

Acanthaceae gen. et spec. indet. (3)
Plate I, Figs. 7-8
Shape: Prolate.
Size: Polar axis 25 µm, equatorial axis 13 µm.
Aperture: Colporate.
Exine: 1.5 µm, sexine as thick as nexine, sexine reticulate, lumina microreticulate, area around the aperture perforate and granulate, muri sometimes granulate.
Family Apiaceae

Apiaceae gen. et spec. indet.
Plate II, Figs. 9-10
Shape: Prolate.
Size: Polar axis 26 μm, equatorial axis 12 μm.
Aperture: Tricolporate, endoaperture lalongate.
Exine: 1 μm, sexine shortly striate, striae up to 7 μm long and sometimes crisscrossed, granulate.

Family Araliaceae

Araliaceae gen. et spec. indet. (1)
Plate II, Figs. 11-12
Shape: Prolate, triangular in polar view.
Size: Equatorial axis 18 μm.
Aperture: Tricolporate, endopore is lalongate and distinct.
Exine: 1.5-2 μm, tectate, tectum micro-reticulate to reticulate, lumina heterobrochate, broader in mesocolpium than in aperture areas, sexine as thick as nexine but slightly thicker in the polar area.

Araliaceae gen. et spec. indet. (2)
Plate II, Figs. 13-14
Shape: Prolate.
Size: Polar axis 18 μm.
Aperture: Tricolporate
Exine: 1.5-2 μm, tectate, tectum micro-reticulate to reticulate, lumina heterobrochate, broader in mesocolpium than in aperture areas. Colpi nearly as long as polar axis.

Family Balsaminaceae

Impatiens sp.
Plate II, Figs. 15-16
Shape: Oblate, rectangular in polar view.
Size: Equatorial axis 33 μm.
Aperture: Tetracolpate, colpi short.
Exine: 1.5 μm, sexine as thick as nexine, reticulate, rudimentary columellae are present in lumina.
Family Betulaceae

*Alnus* sp.

Plate III, Figs. 17-18

Shape: Oblate, penta-angular in polar view.

Size: 15-20 μm

Aperture: Pentaporate, pori vestibulum type, neighbouring pori connected by archs or bands of nexinuous thickening.

Exine: 1.5 μm, tectum consists of irregular rugulae with very small spinules (microechinate). Sexine slightly thicker than nexine.

Family Brassicaceae

*Brassica* sp.

Plate III, Figs. 19-20

Shape: Slightly prolate, circular in polar view.

Aperture: Tricolpate

Exine: 1.3 μm, tectum uniformaly reticulate, the colpi area is granulate.

Family Buxaceae

*Sarcococca* sp.

Plate III, Figs. 21-22

Shape: Spheroidal.

Size: 24-25 μm.

Aperture: Pantoporate, pori small and devoid of annulus.

Exine: 2 μm, sexine much thicker than nexine, tectum reticulate (retipilate), muri formed by rows of pila.

Family Chenopodiaceae

*Chenopodiaceae* gen. et spec. indet.

Plate III, Figs. 23-24

Shape: Spheroidal.

Size: 18 - 27 μm.

Aperture: Pantoporate, pore circular.

Exine: 1 μm, sexine thicker than nexine, sexine spinulate, perforate, mesoporium raised above pori, pore membrane spinulate, granulate.
Family Compositae (Asteraceae)

Artemisia sp.
Plate IV, Figs. 25-26
Shape: Prolate, circular in polar view, lobate.
Size: Polar 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.

Tubiflorae Compositae gen. et spec. indet. (1)
Plate IV, Figs. 27-28
Shape: Prolate, circular in equatorial view.
Size: Polar axis 29 μm, equatorial axis 26 μm.
Aperture: Tricolporate, colpi long.
Exine: 2 μm (without spines), sexine as thick as nexine, spiny, perforate, spines >7 μm in length, with a broad perforated base and long apices.

Tubiflorae Compositae gen. et spec. indet. (2)
Plate IV, Figs. 29-30
Shape: Prolate, in equatorial view elliptical, in polar view circular, lobate.
Size: Polar axis 24 μm, equatorial axis 20 μm.
Aperture: Tricolporate.
Exine: 4 μm, sexine very thick, micro perforate, granulate, spiny, lumina of the perforations larger in size at the base of the spines.

Tubiflorae Compositae gen. et spec. indet. (3)
Plate IV, Figs. 31-32
Shape: Spheroidal to oblate, in polar view circular, lobate.
Size: Equatorial axis 21 μm.
Aperture: Tricolporate.
Exine: 6 μm, sexine much thicker in mesocolpium than in the colpi area, perforate, spiny and occasionally granulate, spines with broad base, short (1-2 μm) and extremely perforated.
**Tubiflorae Compositae** gen. et spec. indet. (4)

Plate V, Figs. 33-34

Shape: Prolate to spheroidal, lobate.

Size: 19 μm.

Aperture: Tricolporate.

Exine: 1 μm, sexine as thick as nexine, spiny and granulate, tectum and spines with only very few perforations.

**Family Cornaceae**

*Cornus* sp.

Plate V, Figs. 35-36

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 Corylaceae**

*Corylus* sp.

Plate V, Figs. 37-38

Shape: Oblate, triangular in polar view.

Size: 26-28 μm.

Aperture: Triporate.

Exine: 1.5 μm, sexine is slightly thicker than nexine, nexine is poorly developed near pori, surface sculpture scabrate and slightly regulate and microechinate.

**Family Ericaceae**

*Rhododendron* sp.

Plate V, Figs. 39-40

Shape: Tetrahedral tetrad.

Size: 26-30 μm.

Aperture: Monads tricolporate.

Exine: 1.5 μm, sexine as thick as nexine, sexine rugulate in mesocolpium areas and perforate to foveolate in distal pole areas, irregularly distributed granules.
Family Fagaceae

Quercus lanata Smith

Plate VI, Figs. 41-42

Shape: Prolate.
Size: Polar axis 22 μm, equatorial axis 16 μm.
Aperture: Tricolporate.
Exine: 1-1.3 μm, tectum consists of small (<1 μm) randomly oriented, sometimes bifurcated rods, which are not uniformly distributed. The rods sometimes accumulated to make clusters.

Family Labiatae (Lamiaceae)

Labiatae gen. et spec. indet.

Plate VI, Figs. 43-44

Shape: Oblate, semicircular in polar view.
Size: Equatorial axis 38-42 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 Loranthaceae

Loranthaceae gen. indet.

Plate VI, Figs. 45-46

Shape: Oblate, amb angular and lobate.
Size: Equatorial 30 μm.
Aperture: Tricolporate (syncolporate).
Exine: 1-2 μm, sexine finely granulate.

Family Meliaceae

Melia azederach

Plate VI, Figs. 47-48

Shape: Prolate
Size: Polar 40 μm, Equatorial 30 μm
Aperture: Tetracolporate
Exine: 1-2 μm, sexine regulate and granulate.
Meliaceae gen. et spec. indet
Plate VII, Figs. 49-50
Shape: Prolate
Size: Polar 22 μm
Aperture: Tetracolporate
Exine: 1-2 μm, sexine regulate and finely granulate.

Family Myricaceae
Myrica esculenta Buch.-Ham. ex D. Don
Plate VII, Figs. 51-52
Shape: Oblate, triangular in polar view.
Size: 19 μm.
Aperture: Triporate.
Exine: 1 μm, sexine is thicker than nexine near and around pori, sexine regularly microechinate.

Family Myrtaceae
Syzygium sp.
Plate VII, Figs. 53-54
Shape: Oblate, triangular and lobed in polar view.
Size: 20-21 μm.
Aperture: Trisyncolporate.
Exine: 1 μm, sexine as thick as nexine, tectum is coarsely rugulate, perforate.

Family Oleaceae
Fraxinus sp. (1)
Plate VII, Figs. 55-56
Shape: Prolate, in equatorial view circular and lobate.
Size: 25 μm.
Aperture: Tricolporate.
Exine: 1 μm, sexine is thicker than nexine, reticulate, lumina homobrochate and polygonal in shape, muri are distinctly granulate.
**Fraxinus** sp. (2)
Plate VIII, Figs. 57-58
Shape: Prolate, circular in polar view.
Size: Polar axis 16 μm, equatorial axis 13 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.

**Jasminum** sp.
Plate VIII, Figs. 59-60
Shape: Prolate, circular in polar view.
Size: Polar axis 40 μm, equatorial axis 33 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.

**Ligustrum** sp.
Plate VIII, Figs. 61-62
Shape: Sub prolate.
Size: Polar axis 37 μm, equatorial axis 29 μm.
Aperture: Tricolporate.
Exine: 1.2 μm, sexine is thicker than nexine, reticulate, heterobrochate, muri are smooth, lumina with rudimentary columellae.

**Family Poaceae**
**Poaceae** gen. indet.
Plate VIII, Figs. 63-64
Shape: Spheroidal.
Size: 35 μm.
Aperture: Ulcerate, pore annulate, diameter of the pore 2-3 μm.
Exine: 1 μm, sexine as thick as nexine, with small spiny elements throughout the sexine.
Family Polygonaceae

*Fagopyrum* sp.
Plate IX, Figs. 65-66
Shape: Sub prolate.
Size: Polar axis 60 μm, equatorial axis 30μm.
Aperture: Tricolporate.
Exine: 1.2 μm, sexine is thicker than nexine, reticulate, heterobrochate, muri are smooth and with slopes, lumina are narrow.

*Polygonum* sp.
Plate IX, Figs. 67-68
Shape: Spheroidal.
Size: 42 μm.
Aperture: Pantoporate.
Exine: 4 μm, sexine much thicker than nexine, reticulate, columella broad, lumina 10 μm with numerous rudimentary columellae, width of muri 1 μm.

Family Proteaceae

*Grevillea* sp.
Plate IX, Figs. 69-70
Shape: Oblate, triangular in polar view
Size: 25 μm
Aperture: Triporate.
Exine: 1.5 μm, sexine as much thicker than nexine, nearly reticulate, regulate, Granulate.

Family Rosaceae

*Rosaceae* gen. et spec. indet. (1)
Plate IX, Figs. 71-72
Shape: Prolate, circular in polar view.
Size: Polar axis 30 μm, equatorial axis 20 μm.
Aperture: Tricolporate.
Exine: 1.5 μm, sexine slightly thicker than nexine, striate, striations more or less uniformly distributed, partly fused with one another. Colpi broad and granulate.
Rosaceae gen. et spec. indet. (2)
Plate X, Figs. 73-74
Shape: Prolate, circular in polar view.
Size: Equatorial axis 22 μm.
Aperture: Tricolporate.
Exine: 1.5 μm, sexine slightly thicker than nexine, striations are in the form of sloping rods, randomly oriented, sometimes fused with one another.

Rosaceae gen. et spec. indet. (3)
Plate X, Figs. 75-76
Shape: Prolate, circular in polar view.
Size: Equatorial axis 20 μm.
Aperture: Tricolporate.
Exine: 1.5 μm, sexine slightly thicker than nexine, striations are in the form of flat rods, randomly oriented, sometimes fused with one another. Tectum perforated.

Rosaceae gen. et spec. indet. (4)
Plate X, Figs. 77-78
Shape: Prolate, circular in polar view.
Size: Equatorial axis 25 μm.
Aperture: Tricolporate.
Exine: 1.5 μm, sexine slightly thicker than nexine, striations are thin and fused, Colporate aperture are broad and distinct.

Family Rutaceae
Citrus sp.
Plate X, Figs. 79-80
Shape: Oblate, circular in polar view.
Size: Equatorial axis 25-30 μm.
Aperture: Tetracolporate.
Exine: 2 μm, reticulated, lumina rounded broad in mesocolpium while small in apocolpium.
Family Salicaceae

*Salix* sp.
Plate XI, Figs. 81-82

Shape: Prolate, circular in polar view.
Size: Equatorial axis 16 μm, polar axis 25 μm
Aperture: Tricolporate, colpi long broad reaching nearly to poles.
Exine: 2 μm, reticulated, lumina broad in mesocolpium, smaller in apertural area.

Family Sapotaceae

*Aesandra* sp.
Plate XI, Figs. 83-84

Shape: Prolate.
Size: Polar axis 50 μm, equatorial axis 35 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.
Plate XI, Figs. 85-86

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.

B. PTERIDOPHYTE

Pteridaceae gen. et spec. indet.
Plate XI, Figs. 87-88

Shape: Sub-triangular.
Size: 35 μm.
Aperture: Trilete.
Exospore: Rugulate in distal pole, rugulae often fused to each other to form irregular depressions (channels) in between them.
EXPLANATION OF PLATES

PLATE-I
Figs. 1-8, Family Acanthaceae
1. Acanthaceae gen. et spec. indet. (1)
2. Details of the tectum of Acanthaceae gen. et spec. indet. (1)
3. Justicia sp.
4. Details of the tectum of Justicia sp.
5. Acanthaceae gen. et spec. indet. (2)
6. Details of the tectum of Acanthaceae gen. et spec. indet. (2)
7. Acanthaceae gen. et spec. indet. (3)
8. Details of the tectum of Acanthaceae gen. et spec. indet. (3)

PLATE-II
Figs. 9-10, Family Apiaceae; 11-14, Family Araliaceae; 15-16, Family Balsaminaceae
10. Details of the tectum of Apiaceae gen. et spec. indet.
11. Araliaceae gen. et spec. indet. (1)
12. Details of the tectum of Araliaceae gen. et spec. indet. (1)
13. Araliaceae gen. et spec. indet. (2)
14. Details of the tectum of Araliaceae gen. et spec. indet. (2)
15. Impatiens sp.
16. Details of the tectum of Impatiens sp.

PLATE-III
Figs. 17-18, Family Betulaceae; 19-20, Family Brassicaceae; 21-22, Family Buxaceae; 23-24, Family Chenopodiaceae
17. Alnus sp.
18. Details of the tectum of Alnus sp.
20. Details of the tectum of Brassica sp.
21. Sarcococca sp.
22. Details of the tectum of Sarcococca sp.
24. Details of the tectum of Chenopodiaceae gen. et spec. indet.
PLATE-IV

Figs. 25-32, Family Compositae

25. Artemisia sp.
26. Details of the tectum of Artemisia sp.
27. Tubiflora Compositae gen. et spec. indet. (1)
28. Details of the tectum of Compositae gen. et spec. indet. (1)
29. Tubiflora Compositae gen. et spec. indet. (2)
30. Details of the tectum of Compositae gen. et spec. indet. (2)
31. Tubiflora Compositae gen. et spec. indet. (3)
32. Details of the tectum of Compositae gen. et spec. indet. (3)

PLATE-V

Figs. 33-34, Family Compositae, 35-36, Family Cornaceae, 37-38, Family Corylaceae, 39-40, Family Ericaceae

33. Compositae gen. et spec. indet. (4)
34. Details of the tectum of Compositae gen. et spec. indet. (4)
35. Cornus sp.
36. Details of the tectum of Cornus sp.
37. Corylus sp.
38. Details of the tectum of Corylus sp.
39. Rhododendron sp.
40. Details of the tectum of Rhododendron sp.

PLATE-VI

Figs. 41-42, Family Fagaceae; 43-44, Family Lamiaceae; 45-46, Family Loranthaceae; 47-48, Family Meliaceae

41. Quercus lanata
42. Details of the tectum of Quercus lanata
43. Lamiaceae gen. et spec. indet.
44. Details of the tectum of Lamiaceae gen. et spec. indet.
45. Loranthaceae gen. et spec. indet.
46. Light Microscope picture of Loranthaceae gen. et spec. indet.
47. Melia azederach
48. Details of the tectum of Melia azederach
PLATE-VII
Figs. 49-50, Family Meliaceae; 51-52, Family Myricaceae; 53-54, Family Myrtaceae; 55-56, Family Oleaceae
49. Meliaceae gen. et spec. indet
50. Details of the tectum of Meliaceae gen. et spec. indet.
51. Myrica sp.
52. Details of the tectum of Myrica sp.
53. Syzygium sp.
54. Details of the tectum of Syzygium sp.
55. Fraxinus sp. (1)
56. Details of the tectum of Fraxinus sp. (1)

PLATE-VIII
Figs. 57-62, Family Oleaceae; 63-64, Family Poaceae
57. Fraxinus sp. (2)
58. Details of the tectum of Fraxinus sp. (2)
59. Jasminum sp.
60. Details of the tectum of Jasminum sp.
61. Ligustrum sp.
62. Details of the tectum of Ligustrum sp.
63. Poaceae gen. et spec. indet.
64. Details of the tectum of Poaceae gen. et spec. indet.

PLATE-IX
Figs. 65-68, Family Polygonaceae; 69-70, Family Proteaceae; 71-72, Family Rosaceae
65. Fagopyrum sp.
66. Details of the tectum of Fagopyrum sp.
67. Polygonum sp.
68. Details of the tectum of Polygonum sp.
69. Grevillea sp.
70. Details of the tectum of Grevillea sp.
71. Rosaceae gen. et spec. indet. (1)
72. Details of the tectum of Rosaceae gen. et spec. indet. (1)
PLATE-X

Figs. 73-78, Family Rosaceae; 79-80, Family Rutaceae

73. Rosaceae gen. et spec. indet. (2)
74. Details of the tectum of Rosaceae gen. et spec. indet. (2)
75. Rosaceae gen. et spec. indet. (3)
76. Details of the tectum of Rosaceae gen. et spec. indet. (3)
77. Rosaceae gen. et spec. indet. (4)
78. Details of the tectum of Rosaceae gen. et spec. indet. (4)
79. *Citrus* sp.
80. Details of the tectum of *Citrus* sp.

PLATE-XI

Figs. 81-82, Family Salicaceae; 83-84, Family Sapotaceae; 85-86, Family Urticaceae; 87-88, Family Pteridaceae

81. *Salix* sp.
82. Details of the tectum of *Salix* sp.
83. *Aesandra* sp.
84. Details of the tectum of *Aesandra* sp.
85. *Urtica* sp.
86. Details of the tectum of *Urtica* sp.
87. Pteridaceae (Pteridophyte spore)
88. Details of the exospore of Pteridaceae spore.

Identification of the pollen up to species level needs a well-identified pollen data bank along with abundant pollen literature of the study area. SEM investigation of honey pollen is not widely recorded in Nepal. Thus, a binomial was only given to the pollen when it could be confirmed by observing a well identified comparative material from herbarium specimens. In other cases only the generic name was used. Some of the pollen could not be identified beyond the family level. A series of pollen articles and manuals (Huang 1972, Nilsson 1973, Huang 1981, Waha 1982, Valdes *et al.* 1986, Gupta and Sharma 1986, Iwanami *et al.* 1988, Scotland 1991, Tryon and Lugardon 1991, Moore *et al.* 1991, Harley 1991, Uffelen 1993, Tissot *et al.* 1994, Fuhsiung *et al.* 1995, Jones *et al.* 1995, Schneider 1996, Unfried 1997, Carine and Scotland 2000, Kapp *et al.* 2000, Shrestha *et al.* 2005, Paudayal and Gautam 2011a, 2011b) 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).
In beekeeping, honey bees play a role as micro-manipulators of flowers to produce honey and other hive products. Identification of various bee plants representing potential sources of nectar, pollen, for the honey bees, and their relative importance, is an important pre-requisite for implementing management strategies in particular area to maintain the strength of the colonies and to maximize honey yields. This study dealing with SEM investigation of pollen spectrum of A. cerana honeys from Godavari area of Kathmandu Valley provided a unique opportunity to understand the diversity of autochtoone bee flora and reliable description regarding the floral types which serve as major or minor nectar and/or pollen sources. The pollens described in this paper are also an expression of its geographical origin of a honey produced by A. cerana. The multifloral honey samples contain 44 species of pollen flora belonging to 28 families. The pollen composition was mostly from the plants growing in tropical-subtropical climate zones. Most of the pollen in the palynological assemblages derived from entomophilous plants however the honey sample also contain a significant numbers of anemophilous pollens such as Alnus, Quercus, Corylus, Salix, Chenompoideae, Poaceae, etc. Further, this study highlights and signifies the systematic documentation of various nectariferous floras from different parts of the country, thereby helping the beekeepers in the proper management and conservation of autochtoone bee colonies.

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