Materials & Methods
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MATERIALS: The pollen samples and other necessary parts and organs were collected from authentic specimens of different herbaria: BH, BSI, CAL, FRC, HSHCH, JCB, K, MH and PCM (see Collection Data). Herbarium specimens were critically studied and properly identified. Photographs of 34 species are presented (Pl. 1 to 4).

The Indian floras (Clarke 1876, Hooker 1882, Cooke 1904, Gamble 1921) include 43 taxa of South Indian Vernonieae: Adenoon 1 sp., Centratherum 8 spp., Elephantopus 1 sp., Lampracteum 1 sp. and Vernonia 32 spp.. Of which four species of Vernonia (V.meleoidii W.W.Sm., V.pulneyensis Gamble, V.recurva Bedd. and V.setigera Arn.) are not included due to their unavailability. But two new species not mentioned in these floras (Centratherum sengaltherianum Naray. and Vernonia anaimudica Shetty & Vivek.) have been included in this study.

Of the 145 specimens from various herbaria examined, 92 pollen specimens (41/45 taxa) were prepared for light microscopic observation. The corallaceous trichomes, the pappus types, the achenial morphology and trichomes of these 41 species and the pericarpic crystals of 38 species have also been investigated. Scanning electron microscopy has been employed for the observation of pollen grains of 14 species of Vernonieae and 2 species of Cichorieae and achenes of 13 species of Vernonieae.
Pollen ultrastructure of 3 taxa, (Centratherum reticulatum Benth., Vernonie anthelmintica Willd. and V. travancorica Hk.f.) was investigated with transmission electron microscope.

METHODS:

FLORAL PARTS: Corollas and achenes were rehydrated in warm water before drafting camera lucida drawings. Floral parts were cleared by boiling in 1% NaOH and mounted in glycerine jelly for LM observation and microphotography. Two granules of plasticine used as cover glass supports, minimized the distorting effects of pressure by cover glass on softened and cleared floral parts in mountant. Microtome-cut sections of paraffin-embedded materials were also prepared for LM observation and microphotography.

METHODS OF POLLEN PREPARATION:

Light Microscopy: Various pollen rehydrants-distilled water, 1% NaOH, 10% KOH, Aerosol O.T., modified Hoyer's medium (H₂O = 15ml; Chloral hydrate = 50 gm; Aerosol O.T. = 5ml; Glycerine = 5ml - after Hardin, 1981), Alcian blue in HCl (pH 1.3) and different mountants - hydrophilic glycerine jelly, lactic acid or preservative- 10% Glutaraldehyde, HCl solution (pH 1.3) and KOH solution (pH 11.0) and hydrophobic Dow Corning silicone oil (Andersen, 1960) have been employed (see Graph 1).
Unacetolysed: Mature pollen grains after softening them by boiling in distilled water were mounted in glycerine jelly using plasticine granules as cover glass supports. Ethyl alcohol treatment before mounting was side-stepped (deviating from Wodehouse, 1935) which enabled the observation of the lipid globules on the pollen wall surfaces and their dispersion in the mounting medium (Pl. 5: 1, 2, 6 & 14).

Acetolysed and Chlorinated: Acetolysis of pollen and subsequent chlorination were carried out following Erdtman (1952). In accordance with Reitsma (1969) who underscored the importance of expansion of pollen before acetolysis, pollen grains were rehydrated with distilled water and then washed twice with glacial acetic acid prior to the addition of freshly prepared Erdtman's acetolysis mixture. As acetolysis at 90° - 100° C turned the echinolophate exines dark brown (unsuitable for microphotography), the acetolysis temperature was controlled at 70° - 80° C (duration 8 - 10 min.) so as to obtain golden brown exines. Plasticine\(^1\) granules were used as cover glass supports and sealed the preparations mounted in glycerine jelly with paraffin (B.P. 69° - 73° C, Merck Art. 7154).

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1 Used plasticine instead of modelling clay although Reitsma (1969, Rev. Palaeo. Paly. 9: 200) cautioned not to use synthetic material because of its melting point equivalent to paraffin.
Extraction of sub-fossilized exines from clayey peats: After deflocculation with distilled water and sieving, the centrifuged sediment was digested overnight in 49% HF. It was then washed in warm 10% HCl to remove all colloidal SiO₂ and silicofluorides. The residues were then heated with KOH (pH 12.5) at 80° C for 10 to 15 minutes. After thorough washing in distilled water, the centrifuged pollen residue was transferred to graduated vials to which was added a measured quantity of liquified Kaiser's glycerine jelly (Merck Art. 9242). Employing an Oxford sampler and disposable plastic tips, the residue, mixed with glycerine jelly was transferred to a 22 x 33 mm cover glass fixed to a rectangular slit (of similar dimensions) in a plastic frame (25 x 75 mm). An 18 x 18 mm cover glass was placed over the drop of glycerine jelly and its edges were sealed with D.P.X. (Distrene-Plasticizer-Xylene) or Glyceel (G.T.Gurr). This type of mounting between cover glasses facilitates the observation of both sides of fossilized exines by reversing the plastic frame under the light microscope.

Microtomy: Acetolysed exines or unacetolysed but rehydrated anthers fixed in F.A.A. were dehydrated with tertiary butyl alcohol and

2 "that everything that is buried sufficiently deeply and for long periods of time is a fossil. ..... that if the actual substance is still present in its original composition it is not a fossil, but a sub-fossil" (Faegri, 1971 : 257)
transferred to a mixture of equal parts of tertiary butyl alcohol
and paraffin-oil before infiltration (Johansen, 1940) with paraffin
(M. Point : 56 - 58° C, Merck Art, 7153). Thick sections (5 -
10 μm) were cut using platinum edged razor blades and JUNG microtome.
Deparaffinized sections were mounted in glycerine jelly like
whole grains.

Measurements and Statistics : Polar (P), Equatorial (E) axes and
Polar diameters (D1 & D2) of spherical or spheroidal grains were
measured in optical sections excluding the length of spin(ul)es.
Number of grains measured and preparation methods and mountants
used are indicated in TABLES. The arithmetic mean (X̄) and standard
deviation (σ) were calculated using a CASIO fx-82 Calculator :

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\text{Standard Deviation (σ) } = \sqrt{\frac{\sum x^2 - (\sum x)^2}{n - 1}}
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Pollen quantitative characters were measured with Wild M-20
light microscope calibrated with a Leitz micrometer scale (1 div.
= 10 μm) at x 500 and x 1000 (oil immersion).

Scanning Electron Microscopy : Dry pollen from dehisced anthers
or pollen rehydrated in Aerosol O.T. (Sodium sulphasuccinate)
following Webster & Lynch (1976) method, and acetolysed exines
were mounted, after routine dehydration, on aluminium stubs and
coated with evaporated gold, both as cement and anti-static coating.
The gold thickness was controlled at about 100 Å. The SEM pictures were taken by Dr. Stanley A.J. Pocock in an ETEC scanning electron microscope at the Esso Resources Canada Ltd., Calgary, Canada.

**Exine Fracturing**: Acetolysed pollen exines were fractured by the application of ultrasonic vibration. A small unit designed for dentistry, was employed for exine fracturing in order to observe the morphological details of the exine interior with SEM. Pollen grains of Vernonieae with complicated echinolophate exines were acetolysed at 80° C for 10 minutes. After cooling, the preparation tubes were transferred to the ultrasonic bath which was allowed to operate until the automatic cut-off turned the unit off at a temperature of about 60° C. Fractured exines were then processed for SEM observation.

**Transmission Electron Microscopy**: Pollen grains or anthers from mature flower buds were rehydrated in 1% Alcian blue in Hcl (pH 1.3) or in 1% Glutaraldehyde (GA) in Hcl-Cocodylate buffer at 100 mM (pH 6.9). Rehydrated materials were washed with 0.1% OsO₄ (Osmium tetroxide) in deionized double glass distilled water (pH 6, 20° C, 2 hrs). The osmium tetroxide solution was decanted and dehydrated in an acetone series and embedded in epon araldite. Sections were cut on a Porter-Blum MT - I microtome with a DuPont diamond knife. During microtomy, the knife reservoir was refilled
with double glass distilled water. Gray sections on gold or copper grids were prepared for TEM. Sections were stained with 4% aqueous uranyl acetate (UA) for 10 minutes at 20° C followed by lead citrate (PbC) for 10 minutes (Venable & Coggeshall, 1965). Micrographs were taken by Prof. Dr. John R. Rowley at the Botanical Institute, University of Stockholm, Sweden, with ZEISS EM - 9S and ZEISS EM IOA electron microscopes (Objective lens aperture 60 m), equipped with automatic film exposure devices that monitor the number of electrons striking the centre of the image screen just prior to film exposure. As a result, the general background density of negatives is highly uniform. The films were developed in a tank equipped with intermittent gaseous nitrogen burst agitation.