Development of pollinium in two epidendroid orchids

R. K. Bhanwra*, S. P. Vij, Vivek Chandel, Ravikant and Sunil Dutt

Department of Botany, Panjab University, Chandigarh 160 014, India

Development of pollinium has been studied in *Cymbidium aloifolium* (L.) Sw. (tribe Cymbidieae) and *Smitinandia micrantha* (Lindl.) Holtt. (tribe Vandeae) of the family Orchidaceae. The anther primordium develops two lobes oriented towards the labellum. Each lobe has a group of archesporial cells, which divide mitotically so as to form a spherical mass of sporogenous cells, in which there is differentiation of a dorsiventral plate of elongated sterile cells. In *C. aloifolium*, the sterile plate extends into about three-fourths of the fertile tissue, whereas in *S. micrantha* it is complete but asymmetrically oriented. Synchronous meiosis in the microsporocytes is followed by simultaneous cytokinesis so as to form microspore tetrads which organize into pollinia. Centripetal disintegration of the sterile plate during development of the male gametophyte forms two perforate (hollow) pollinia in *C. aloifolium*, but four unequal pollinia in *S. micrantha*. The anther wall is composed of an epidermis, an endothecium, two to three middle layers and the tapetum. The tapetum disorganizes during development of the male gametophyte, while the endothecium and adjacent middle layers develop fibrous thickenings.

Keywords: Anther, *Cymbidium*, Epidendroideae, Orchidaceae, pollinium, *Smitinandia*.

THE Orchidaceae with nearly 800 genera and 25,000 species represents one of the most highly evolved families of flowering plants. These are monocotyledonous perennial herbs with incredible range of diversity in size, shape, colour, orientation and longevity of the flowers. All these traits contribute towards a multimillion-dollar industry of cut-flowers and potted plants in countries like Thailand, Malaysia and USA.

A number of features of the stamens in orchids, e.g. their number, relative position on the column, number of pollinia, whether they are collateral or superposed within the anther, structure of pollinia and the associated caudicle, stipe and viscidium play a significant role in the classification of the family Orchidaceae[1]. Although the embryology of a number of orchids has been studied[2–5], detailed information regarding the early development of anther and differentiation of pollinia is lacking. Studies on some spiranthoid, orchidoid and epidendroid taxa have demonstrated that the anther initiates as a bisporangiate structure and each anther lobe with a single sporogenous mass forms two to four pollinia by insertion of a partial or complete septum of sterile cells[6–8]. The present study deals with development of the anther and male gametophyte in two epidendroid orchids *Cymbidium aloifolium* (L.) Sw. (tribe Cymbidieae) and *Smitinandia micrantha* (Lindl.) Holtt. (tribe Vandeae), with a view to adding to our knowledge regarding the development and variation in number and structure of pollinia in the family Orchidaceae.

C. aloifolium is an epiphytic herb distributed in Eastern Himalayas, Darjeeling and Khasia Hills at an elevation of 1500–2000 m. *S. micrantha* is also an epiphytic species widely distributed in the Himalayas from Garhwal to Arunanchal, at an altitude between 700 and 1200 m. For the present study, inflorescences of the above species at different stages of development were collected from plants grown in the Orchidarium, Botany Department, Panjab University, Chandigarh (India) and fixed in formalin–acetic acid–alcohol (90 ml of 50% ethyl alcohol : 5 ml acetic acid : 5 ml formalin). After usual procedure of dehydration in the ethyl alcohol–*tert*–butyl alcohol series and paraffin wax-embedding[9], sections cut from 8 to 10 μm were stained in Safranin-fast green combination.

The anther is incumbent in both the species studied here (Figure 1 *a*). The anther primordium initiates as an ovoid mass of parenchyma cells surrounded by a protoderm (Figures 1 *b* and 2 *a*). It develops two lobes oriented towards the labellum, each with a group of archesporial cells of uniform size. The archesporial cells undergo mitotic divisions so as to form six to eight concentric layers of sporogenous cells, which develop a dorsiventral strip of sterile cells (Figures 1 *c*, *d* and 2 *b*, *c*). This sterile septum (three to four cells wide) extends from the connective side in the form of an arc into three-fourths of the sporogenous mass in *C. aloifolium* (Figure 2 *d*). It, however, is oriented asymmetrically and runs through the entire width of the fertile tissue in *S. micrantha* (Figure 1 *e*). A similar mode of early anther development has been reported in some of the recently studied orchid taxa[6–8]. Whereas the dorsiventral partition of sterile cells is complete in many species, it is partial in *Eulophia hormusjii*[7] and *Rhynchostylis retusa*[8].

A meristematic activity in the wall layers forms an anther beak towards the rostellar surface (Figure 1 *g*). Epidendroid orchids frequently show the formation of a terminal beak[1].

The connective region comprises large, vacuolated cells with a procambium strand in the centre (Figures 1 *e*, *f* and 2 *b*, *c*). The hypodermal layer of cells undergoes periclinal and anticlinal divisions so as to form an anther wall consisting of two (*S. micrantha*) or three middle (*C. aloifolium*) layers and tapetum (Figures 1 *f* and 2 *e*, *f*). The anther wall is usually four-layered in the spiranthoid taxa, as there is only one middle layer[10–12]. It is five or six layered in cyperipedioid orchids[2,13] (two to three middle layers) while it may be four, five or even six-layered in epidendroid taxa[7,8,14,15] depending upon the number of middle layers.

*For correspondence. (e-mail: bhanwrark@yahoomail.co.in)

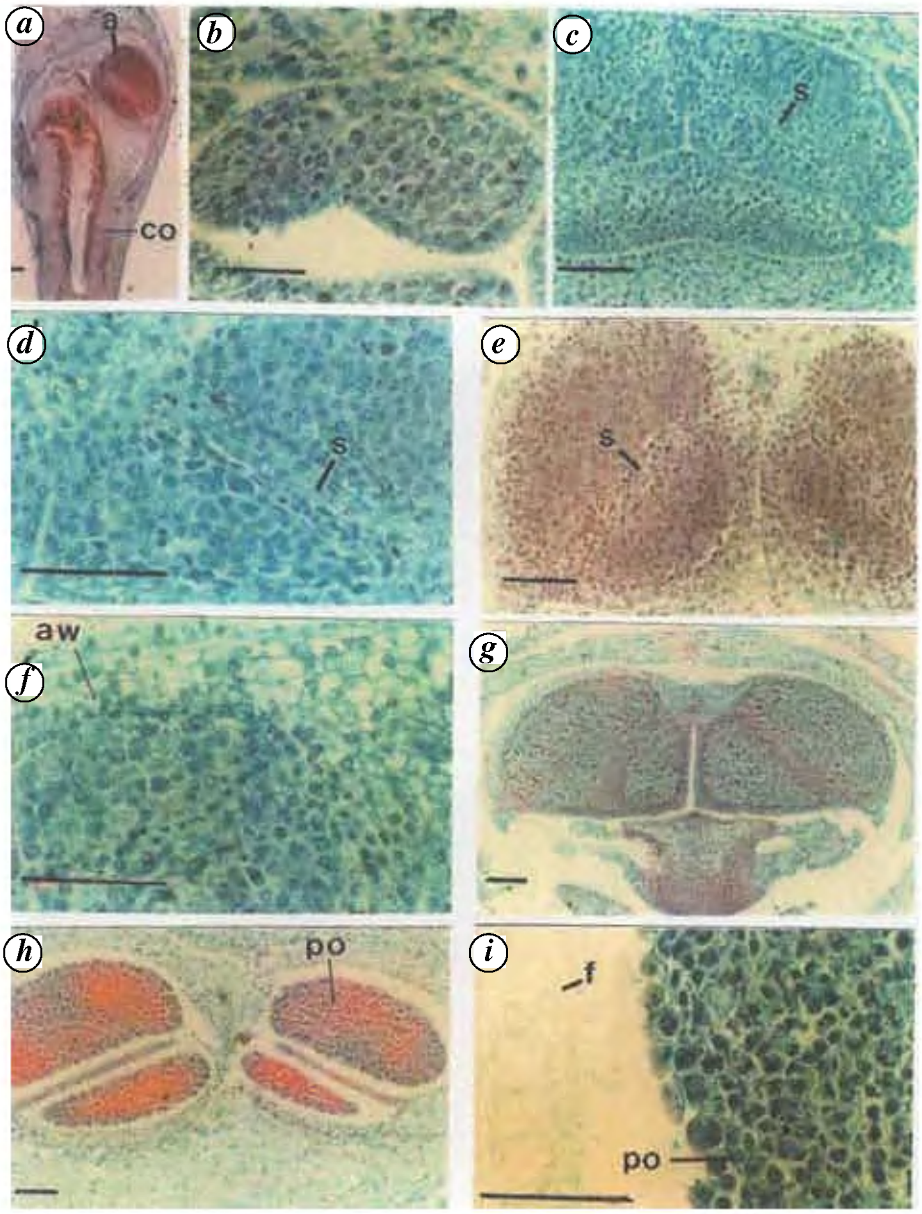

Figure 1. ***a–i***, Longitudinal and transverse sections showing development of anther in *Smitinandia micrantha*. ***a***, Column with incumbent anther; note anther cap. ***b***, Anther primordium with two lobes oriented towards the labellum. ***c–g***, Development of anther wall, sporogenous tissue and septum of sterile cells. ***h***, Mature anther with four unequal pollinia. ***i***, Fibrous endothecium and segment of pollinium. a, Anther; aw, Anther wall; co, Column; f, Fibrous endothecium; po, Pollinium; s, Septum of sterile cells (scale bar = 100 μm).

In *C. aloifolium*, the epidermal cells become papillate during development of the male gametophyte (Figure 2 *h*), while in *S. micrantha* they are stretched and flattened (Figure 1 *g*, *h*). The epidermal cells usually become stretched in other Orchidaceae[7,15]. Some notable exceptions, however, include *Zeuxine longilabris*[16,17] and *Epipactis gigantea*[5], where these cells become hypertrophied and densely cytoplasmic. Thus the epidermal cells may be concerned with nutrition besides their usual function of protection.

In both the orchids studied here, the endothecial cells in mature anthers become radially elongated and along with the adjacent middle layers develop oval, arc-shaped or simple rod-like thickenings (Figures 1 *h*, *i* and 3 *c*). Two to three endothecial layers have been reported in *Limodorum*[17], and *Epipactis gigantea*[5], whereas in *Cleisostoma racemifera* (= *Sarcanthine pallidus*)[17], *E. hormusjii*[7], *Rhynchostylis retusa*[8] and *Cyperipedium cordigerum*[13], only two are present. This feature is more frequent in epiphytic taxa compared to terrestrial ones.

The tapetal cells remain uninucleate but they do not form a conspicuous layer as the cell size is not large compared with other cells of the anther wall (Figures 1 *e–g*

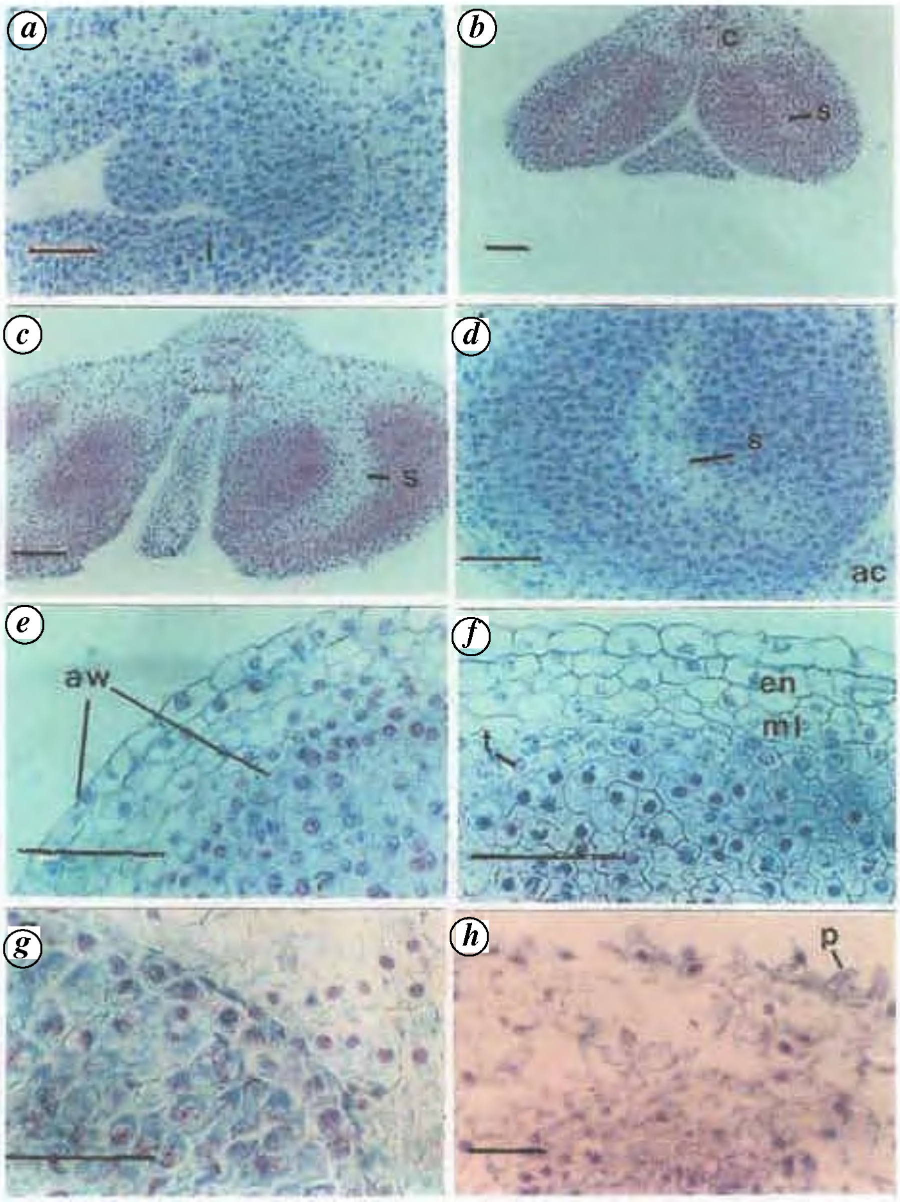

Figure 2. ***a–h***, Transverse sections showing development of anther in *Cymbidium aloifolium*. ***a***, Anther primordium with two lobes oriented towards the labellum. ***b–d***, Development of connective, sporogenous tissue and a septum of sterile cells. ***e–g***, Formation of anther wall with endothecial layer, three middle layers, tapetum and differentiation of sporogenous cells into microsporocytes. ***h***, Segment of anther wall showing papillate epidermal cells. ac, Anther cap; aw, Anther wall; c, Connective tissue; en, Endothecial layer; l, Labellum; ml, Middle layers; p, Papillate epidermis; s, Septum of sterile cells; t, Tapetum (scale bar = 100 µm).

and 2*f*, *g*). The tapetum is of glandular type, as the constituent cells disorganize *in situ* during development of the male gametophyte (Figure 3 *b*).

After several cycles of mitotic division, the sporogenous cells differentiate into microsporocytes (Figures 1 *e* and 2 *f*). Meiosis is synchronous and accompanied by simultaneous cytokinesis resulting in the formation of tetrahedral, isobilateral, decussate or rarely linear and T-shaped tetrads of microspores (Figure 3 *a*, *b*). Konta and Tsuji[18] classified tetrads into six general types: tetrahedral, isobilateral, decussate, rhomboidal, T-shaped and linear. All six types of pollen tetrads were reported in 30 out of 36 species studied. Decussate tetrads, however, were found to be most common. Microspore tetrads do not separate out of the tetrad configuration and the tetrads in turn form a coherent and compact mass commonly designated as the pollinium (Figures 1 *h*, *i* and 3 *d*). There are two pollinia in *C. aloifolium* which become hollow due to centripetal disintegration of the sterile plate of cells running obliquely from the connective side into about three-fourths of the microsporocytes (Figure 3 *d*). The sterile plate of cells in *S. micrantha* is laid in such a manner that there are four

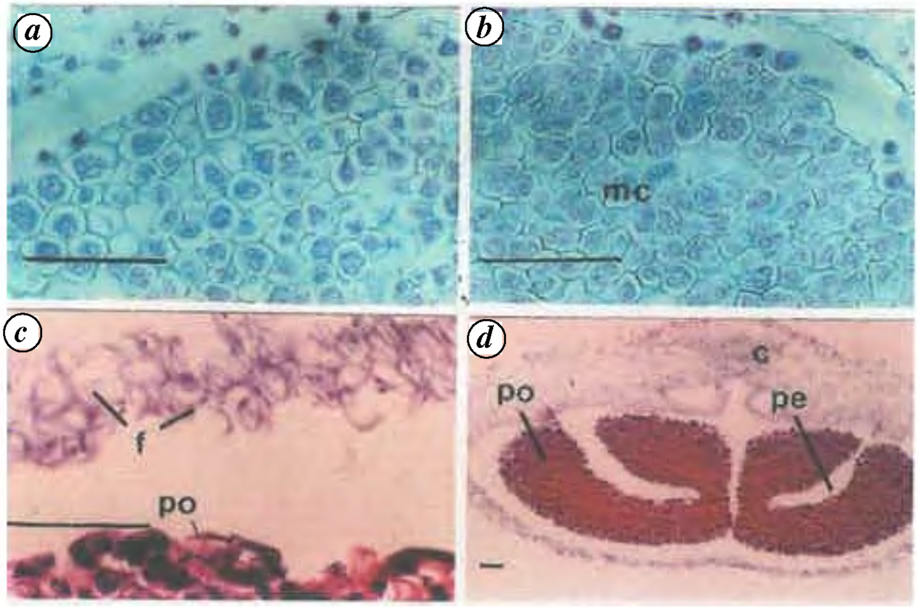

Figure 3. ***a–d***, Transverse sections of anther showing microsporogenesis and pollinia at maturity. ***a***, Segment of anther wall and microsporocyte at telophase of meiosis-I. ***b***, Simultaneous cytokinesis resulting in microspore tetrads. ***c***, Three-layered endothecium and segment of pollinium. ***d***, Two perforate pollinia. c, Connective tissue; f, Fibrous endothecium; mc, Microspore mother cells; pe, Perforation; po, Pollinium (scale bar = 100 µm).

pollinia in an anther, all of which are of different size (Figure 1 *g*) and oriented at an angle of 45° to the longitudinal axis of the column.

In Orchidaceae, the shedding units may be single grains, e.g. *Cyperipedium cordigerum*[13], *Paphiopedilum druryi*[2], compound grains, i.e. tetrads as in *Aa achalensis*[10], *Epipactis helleborine* & *E. veratrifolia*[14] and *E. latifolia*[4]. These may be sectile pollinia, e.g. *Zeuxine strateumatica*[11], species of *Habenaria*[12] or may be liberated as pollinia – most of the Epidendroideae. A good deal of variation occurs in the number of pollinia produced and their structural details in each anther in the epidendroid orchids studied thus far, e.g. there may be two hollow pollinia (*Eulophia streptopetala*[19], *E. hormusjii*[7] and *R. retusa*[8]), four superposed pollinia (*Pholidota imbricata*, *Corallorhiza maculata*)[19], four pollinia oriented at an angle of 45° to the long axis of the column (*Coelogyne lactea*, *Polystachya laxiflora*)[19], four pollinia: two large and two small (*Pelatanthera etenoglossum*)[19], four curved pollinia (*Sobralia micrantha*)[19], eight pollinia in four pairs (*Eria javonica*, *Thelasis pygmaea*)[6] or eight clavate pollinia (*Calanthe rubens*, *C. masuca*)[6].

In India, orchids are represented by 1141 species and 166 genera. They are distributed in the Himalayan, north-eastern and peninsular regions of the country. However, embryological data are available in only 68 species, primarily based on the development of the ovule and female gametophyte. Considering the importance of the features of the anther in taxonomy and phylogeny, little is known about the development of the anther and male gametophyte in Indian orchids. Therefore, a comparative study of anther and male gametophyte development in orchids of different habitats and taxonomic categories is likely to give deeper insight into the evolutionary trends in the anther wall layers, organization of pollen grains and the associated caudicle, stipe and viscidium.

1. Dressler, R. L., *Phylogeny and the Classification of the Orchid Family*, Timber Press, Portland, 1993.
2. Swamy, B. G. L., Embryological studies in Orchidaceae, I: Gametophytes. *Am. Midl. Nat.*, 1949, **41**, 184–201.
3. Yeung, E. C., Development of pollen and accessary structures in orchids. In *Orchid Biology: Reviews and Perspectives* (ed. Arditti, J.), Cornell University Press, Ithaca, New York, 1987, pp. 193–226.
4. Sood, S. K., Gametogenesis, seed development and pericarp in *Epipactis latifolia* L. *J. Indian Bot. Soc.*, 1997, **76**, 11–15.
5. Vij, S. P., Kaur, P. and Bhanwra, R. K., Embryological studies in *Epipactis gigantea* (Orchidaceae). *Lindleyana*, 1999, **14**, 160–167.
6. Freudenstein, J. V. and Rasmussen, H., Pollinium development and number in the Orchidaceae. *Am. J. Bot.*, 1996, **83**, 813–824.
7. Bhanwra, R. K. and Vij, S. P., The development of anther and male gametophyte in *Eulophia hormusjii* Duthie (Orchidaceae). *J. Orchid Soc. India*, 2003, **17**, 87–91.
8. Vij, S. P., Bhanwra, R. K., Dutt, S. and Nayyar, H., Development of anther and male gametophyte in *Rhynchostylis retusa* Bl. (Orchidaceae). *Phytomorphology*, 2005, **55**, 93–101.
9. Johansen, D. A., *Plant Microtechnique*, McGraw Hill, New York, 1940.
10. Coccuci, A. E., The life history of *Aa. achalensis* Schltr. (Orchidaceae). *Phytomorphology*, 1964, **14**, 588–594.
11. Vij, S. P., Sharma, M. and Shekhar, N., Embryological studies in Orchidaceae, III: *Zeuxine strateumatica* complex. *Phytomorphology*, 1982, **32**, 257–269.

12. Sharma, M. and Vij, S. P., Embryological studies in Orchidaceae, VI: *Habenaria* Willd. *Phytomorphology*, 1986, **37**, 327–335.
13. Sood, S. K., Occurrence of uninucleate tapetal cells in diandrous orchid *Cypripedium cordigerum* Don (Orchidaceae). *Ind. Bot. Cont.*, 1985, **2**, 65–66.
14. Vij, S. P. and Sharma, M., Embryological studies in Orchidaceae, V: *Epipactis* Adam. *Phytomorphology*, 1987, **37**, 81–86.
15. Sood, S. K. and Sham, N., Gametophytes, embryology and pericarp of *Rhynchostylis retusa* Bl. (Epidendreae, Orchidaceae). *Phytomorphology*, 1987, **34**, 307–316.
16. Karanth, A., Bhat, P. K. and Govindappa, D. A., Tapetum-like epidermis in *Zeuxine longilabris* (Lindl.) Benth. ex Hk., Orchidaceae. *Curr. Sci.*, 1979, **48**, 542–543.
17. Giugnard, L., Researches sur le development de l'anthere et di pollen des orchidees. *Ann. Sci. Nat.*, 1882, **14**, 26–45.
18. Konta, F. and Tsuji, M., The types of pollen tetrads and their formation observed in some species in Orchidaceae in Japan. *Acta Phytotaxon. Barcinonensia*, 1982, **33**, 327–336.
19. Freudenstein, J. V., Harris, E. M. and Rasmussen, H., The evolution of anther morphology in orchids: incumbent anthers, superposed pollinia and the vandoid complex. *Am. J. Bot.*, 2002, **89**, 1747–1755.

Received 19 November 2004; revised accepted 30 January 2006

Psychrophilic fungi from Schirmacher Oasis, East Antarctica

S. M. Singh[1,*], G. Puja[2] and D. J. Bhat[2]

[1]National Centre for Antarctic and Ocean Research, Headland Sada, Vasco-Da-Gama, Goa 403 804, India
[2]Department of Botany, Goa University, Goa 403 206, India

This communication presents results of a preliminary study on the fungal biodiversity of soils of Schirmacher Oasis, East Antarctica. Using 2% malt extract agar medium, serial dilution method was followed to recover the fungi in culture from the soil samples. Fungal colonies were visible in culture plates only when maintained at 2–5°C for up to 45 days. Several taxa of fungi were recovered.

Keywords: Psychrophilic fungi, Schirmacher Oasis, soils, serial dilution method.

PSYCHROPHILES thrive at very low temperatures. These include organisms living in deep sea (–1 to 4°C), Arctic and Antarctic (–1 to –35°C during wintertime), and glacial ice habitats (–5°C). Little is known on the biodiversity of such habitats, especially microfungal diversity. A few reported organisms from these habitats are gaining popularity in recent years with the advent of genomics and proteonomics[1–3]. Further, some of these, especially fungi and bacteria, are now known to produce unique enzymes and secondary metabolites of immense biotechnological potential.

The physiological and ecological mechanisms that help fungi to overcome and survive cold environmental conditions are well explained by Robinson[4]. He indicated that there is a predominance of sterile mycelia in the Antarctic soils and this could be a physiological adaptation to overcome the harshness of sub-zero temperatures. He also attributed the production of melanin by these fungi as a protective mechanism for survival under extreme temperatures.

Antarctica is a continent located at the South Pole. Barring 2% of the area, thick sheets of ice cover the remaining parts. Only a few species of fungi and bacteria have been described from the region in the recent past, most of them being from the marine environment, i.e. sea water and sea ice. Little investigation has been carried out on soil microorganisms of ice-free areas. Studies of Nichols *et al.*[5] resulted in the recovery of 769 strains of Actinobacteria from the Antarctica. They suggested that the terrestrial environments of the region are a rich source of novel and rare genera. They also studied at molecular level, the total microbial diversity of the polar and deep-sea environment.

India has established a permanent research station, Maitri at Schirmacher Oasis, East Antarctica and launched a series of scientific expeditions since 1981. Earlier studies suggested that life at Schirmacher Oasis is dominated by lichens[6], mosses[7] and algae[8,9]. Studies on bacteria and yeast were conducted by Shivaji[10]. Effect of temperature on bacterial populations was observed by Matondkar[11]. Microfaunal studies of the region were carried out by Ingole and Parulekar[12].

Sharma[13] reported nine species of fungi from the Antarctica region. These include *Arthrobotrys ferox* on moss, *Torulopsis psychrophila* and *Phoma herbarum* on bird excreta, *P. herbarum* on skeletal remains, *Acremonium antarcticum* and *A. psychrophilum* on lichens and species of *Torulopsis*, *Psychrophila* and *Cryptococcus* on ornithogenic soils. Besides, a few alien species of fungi, viz. *Hormoconis resinae* on oil spills and species of *Dacrymyces* and *Exidia* on wooden debris have also been reported by Sharma[13]. Some of the tropical saprophytic fungi, viz. *Chaetomium globosum*, *Stemphylium* sp., *Curvularia lunata*, *Memnoniella echinata*, *Aureobasidium pullulans*, *Aspergillus niger*, *Paecilomyces varioti*, *Penicillium funiculosum* and *Cladosporium* sp., were exposed to Antarctic environment for a period of 14 months by Dayal *et al.*[14], in order to study their viability, growth rate and virulence, but no major variation in activity was observed. In subsequent years, steady increase in summer temperatures and concomitant glaciological changes resulted in further exposure of soils in Antarctica and warranted continued studies on the life of the region.

The authors had an opportunity to examine the fungal diversity of soils of Schirmacher Oasis based on samples collected during a recent expedition by one of the authors.

*For correspondence. (e-mail: smsingh@ncaor.org)