Supplementary comments on 'endemic, rare and threatened flowering plants of South India'

This note is to supplement Meher Homji's comments (*Curr. Sci.*, 1995, **68**, 1083–1084), on the article by Ranjit Daniels *et al.* (*Curr. Sci.*, 1995, **68**, 493–495).

Coining a term 'pseudoendemic' for a plant species like *Piper barberi* occurring in the southernmost parts of the W. Ghats in Kanyakumari District and also in a very closely adjacent and contiguous area in Kulathupuzha is rather misleading. Plants can be either 'endemic' or wide species. There is no 'pseudo' category in the plant or animal world. There are well-defined categories of endemics such as palaeoendemic, neoendemic, patroendemic, schizoendemic and apoendemic. It is better not to add new phraseology like 'pseudoendemic'.

As mentioned by Meher Homji, conjectural propositions on extinction phenomena based on insufficient data, that too from a small politico-geographical area, (Tamil Nadu!) are not appropriate. Biological extinction and man-made extinction must be conceived in their true perspectives. Biological extinctions involve the processes of 'way out' of species through millions of years of speciation and extinction in time space continuum. Unfortunately the current crisis of extinctions of biota is steered by anthropogenic intervention and habitat loss.

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R. J. Ranjit Daniels et al.'s reply:

We thank both V. M. Meher-Homji and M. P. Nayar for their interesting comments on our paper (*Curr. Sci.*, 1995, **68**, 493–495). In fact, the primary purpose of the paper was to invoke some discussion.

Regarding the issues raised by Meher-Homji (*Curr. Sci.*, 1995, **68**, 1083–1084) and that subsequently quoted by Nayar, we admit that Tamil Nadu is not 'south India'. However, we have just chosen to treat the plants of Tamil Nadu listed in the *Red Data Books* as a subset of those reported from the 'Madras Presidency' by Gamble. Madras Presidency, which included, at least in part, the four south Indian states, viz. Kerala, Tamil Nadu, Karnataka and Andhra Pradesh is in our opinion and geographically a fairly good representation of south India. We therefore feel that it is statistically appropriate to work with the available data on small subsets for drawing more general inferences.

On the issue of 'biological extinction and human-induced extinction', it is not clear to us how the distinction can be easily made. We are only highlighting the fact that certain species are 'more extinction-prone' than the others. We have also illustrated some of the possible reasons that make such species extinction-prone. Species on their natural 'way out' are as vulnerable to extinction as those which are being 'led' by humans. Whether we pay more importance to one at the expense of the other becomes more of a philosophical issue.

Finally, regarding the 'pseudoendemics' and the contention that there is nothing 'pseudo' in the natural world, we have an explanation as well. One has to admit the fact that all the status that we humans attribute to living organisms are 'relative' and at any stage be claimed as false. While it cannot be rationally argued as to why we should not use such terms as pseudo, it is easier to bring to the attention of the contender the fact that this term was not 'coined' by us. Although while writing this paper we thought we were introducing a new term to describe 'endemic' species, we discovered later that this term has been in use since 1990 after its first introduction by Nelson *et al.* (*Nature*, **345**, 714–716). The term was, as much as we have done, used to recognize 'endemic' species whose full range has not been sampled due to inadequate or uneven surveys.

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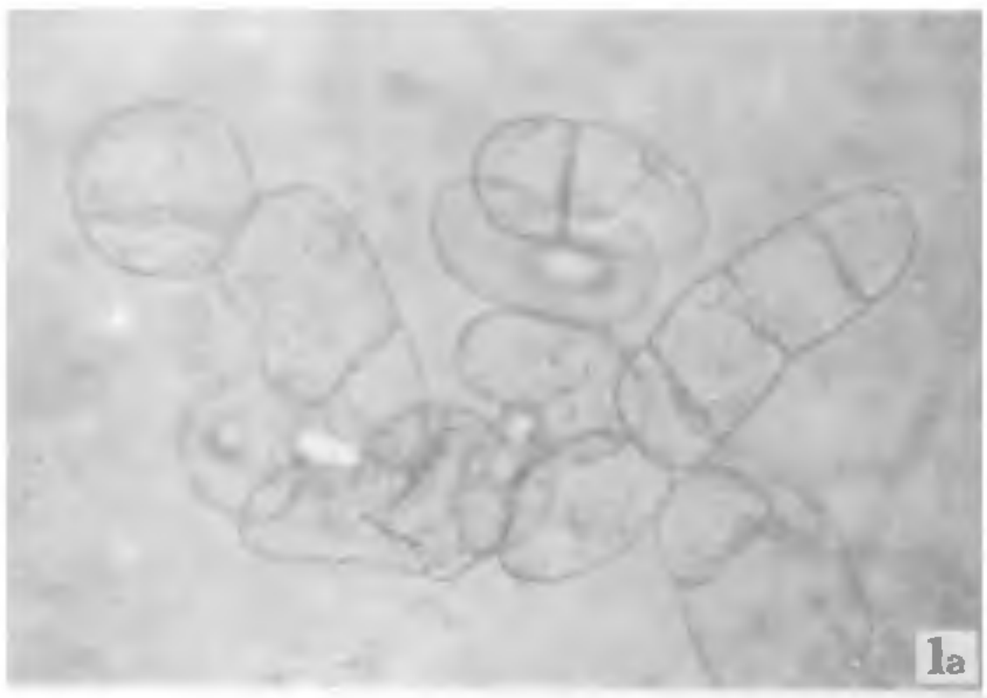
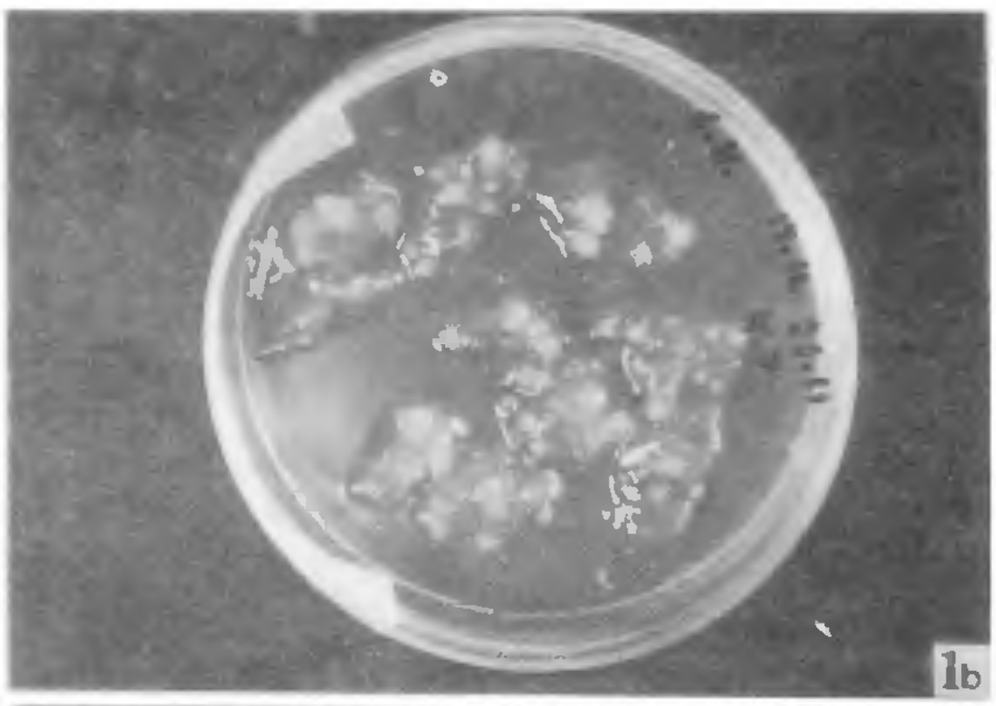
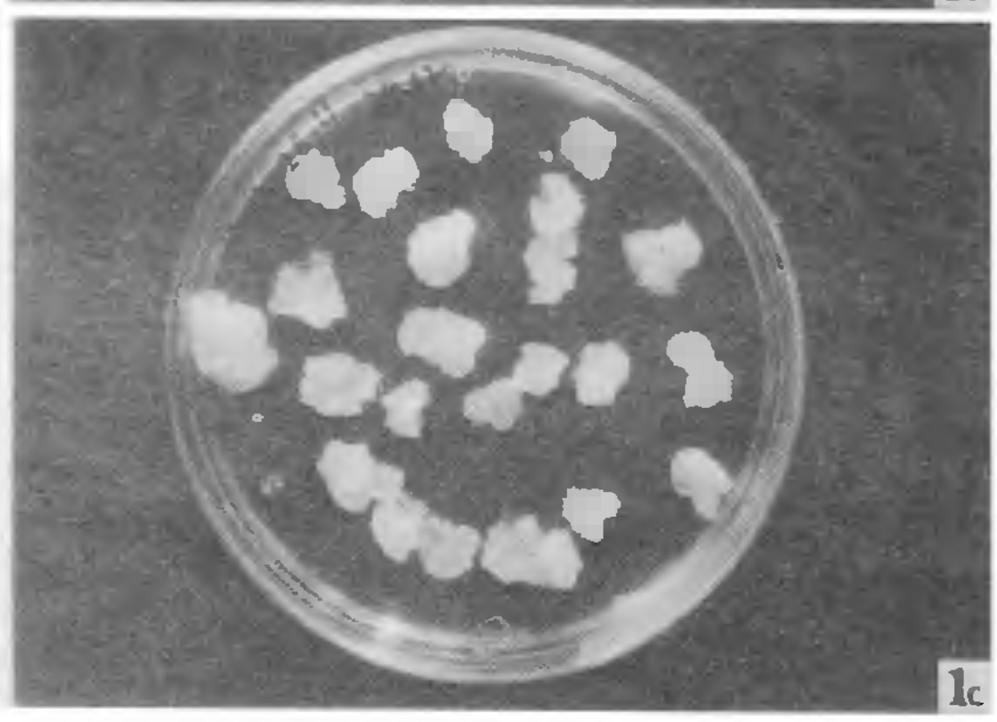
A fast-growing cell line of *Taxus baccata* L. (Himalayan yew) as a potential source of taxol precursor

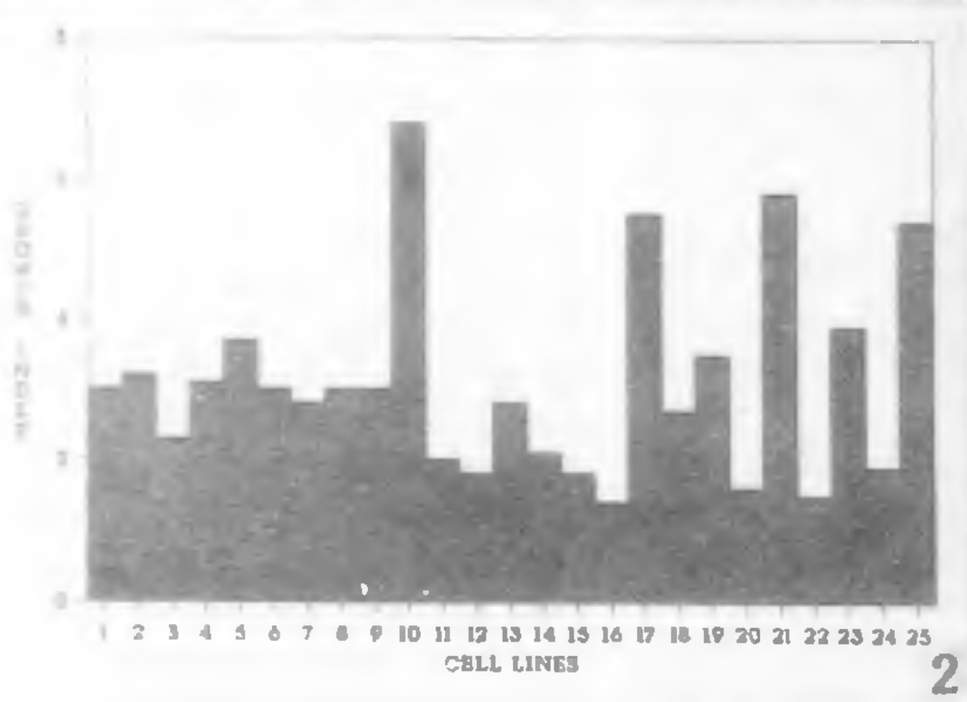
Taxol, a diterpene with exceptional anticancerous activity, is presently isolated from bark of Pacific yew, *Taxus brevifolia*[1]. It occurs as a very minor component in several species of *Taxus*[2]. Continued bark harvesting for taxol does not appear to be an adequate source to meet its demand[3]. There is a growing interest in alternative methods for taxol production such as from tissue culture of *Taxus* sp.[4–6]. The Himalayan yew, *T. baccata*, has been reported[7] to contain taxol and 10-deacetyl baccatin III, a precursor for synthesis of taxol. Here we report selection and cloning of fast-growing cell lines of Himalayan *T. baccata* for the production of taxanes.

Callus cultures were initiated from needle leaf of mature trees of *T. baccata* (Linn.) collected from Darjeeling area during July–October 1993. Callus was induced on explants on B_5 medium[8] supplemented with 2 mg/l 2,4-D, 0.1 mg/l kinetin and 0.1% casein hydrolysate, as reported for other species of *Taxus*[4–6]. Culture growth was main-









Figures 1 and 2. **1***a*, Early exponential phase cells of *T. baccata* suspension, *b*, visible colonies following plating after 21 days and *c*, variability in growth rates of cell colonies after 8 weeks of plating. **2.** Comparison of growth index of 25 established cell lines of *T baccata*.

Table 1. Taxol and 10-deacetyl baccatin III content in four fast growing cell lines of Himalayan *T. baccata* after 6 months of culture

		Taxol		10-deacetyl baccatin III	
Cell line	GI	*Cell suspension (mg/100 g)	Media (µg/l)	*Cell suspension (mg/100 g)	Media (µg/l)
Kp 510	6 8	3 ± 0.5	10	242 ± 15	620
Kp 517	5 5	10 ± 1.5	33	177 ± 21	370
Kp 535	5.8	6 ± 0.5	15	160 ± 11	310
Kp 551	5.4	7 ± 1 2	Not detectable	168 ± 10	112

*Values represent mean ± standard error expressed on a dry matter basis.

tained on the same medium and was subcultured at 4-week intervals for six months. Suspension cultures were initiated by transferring callus tissues (2 g fresh wt) into 50 ml B_5 medium containing 2,4-D (0.2 mg/l) and kinetin (0.1 mg/l) in 250 ml Erlenmeyer flasks. The cultures were incubated on a rotary shaker (125 rpm) at 25 ± 2°C under continuous dark and were transferred every 15 days. Cell suspension cultures were maintained by inoculating aliquotes of filtered cultures (nylon mesh 0.5 mm pore size) equivalent to 10 mg dry wt (100°C/5 h) per ml of fresh medium containing 1 mg/l 2,4-D and 0.05 mg/l kinetin. Cultures were regularly passed through nylon sieve (0.5 mm) up to 6 months to obtain a fine cell suspension culture.

For selection of fast-growing cell lines, 3-week-old cell suspension cultures (GI 1.8 to 2.5) were centrifuged at 2000 rpm for 5 min, the liquid medium discarded and fresh medium added. Appropriate aliquotes were mixed in petri dishes with MS agar medium, held liquid at 40°C to give a final cell density of 5×10^4 cells/ml. The final agar concentration was 0.6%. The petri plates were incubated under continuous dark at 25 ± 2°C for 45 days. Cell division started within 7 days (Figure 1 *a*, *b*) and visible colonies (Figure 1 *c*) appeared on agar plates within 21 days of culture. Variability in cell growth rate was observed in different cell lines (Figure 1 *d*) initiated from the same explant. Growth index was calculated as (final wt – initial wt)/initial wt. Using this procedure, it was possible to select several cell lines showing faster growth. One major problem associated with *Taxus* cell suspension culture is the production of red coloured exudates (presumably phenolics) which effected cell growth. By following selection procedure as

described above, creamish-white cell suspension cultures could be established with minimum exudation of phenolics. These cell lines have been maintained for over 12 months in culture.

Taxanes in cell suspension cultures were measured using previously described methods[6]. For analysis of taxanes in cell suspensions and cultured media, cultures were harvested after 3 weeks of each passage, fresh weight of cell cultures recorded and the material lyophilized and extracted with chloroform (20 ml/g cells). The liquid medium was shaken with chloroform (100 ml/l). The chloroform extracts were evaporated to dryness, suspended in 5 ml methanol (HPLC grade) and filtered (0.2 µm) prior to HPLC analysis. HPLC analysis of taxol and 10-deacetyl baccatin III was performed in a Shimadzu liquid chromatograph (LC 10 AD) employing a reverse phase ODS–Supelcosil C-18 column (25 cm × 4.6 mm id), a Supelco guard column (Pelliguard™ LC-18 kit with 2 cm × 4.6 mm cartridge) and isocratic elution with acetonitrile:water:methanol (30:30:40). The flow rate was 1 ml/min and all chromatographs were plotted at 227 nm, using a UV detector (SPD 10A). A 0.25 mg/ml solution of standard samples of taxanes in MeOH was prepared and filtered through 0.2 µm filter prior to HPLC analysis. Identity of taxol and 10-deacetyl baccatin III was confirmed by retention time, spiking with standard and on the basis of spectral properties[9,10].

Extreme variability in cell growth rate was observed (Figure 2) in cell lines established by plating. Selection and recloning of faster-growing colonies led to establishment of four cell lines with rapid doubling time. Such variability in growth rates of cell lines has been reported in cell cultures of *T. brevifolia*[7,11].

Suspension cultures were established from the four cell lines (GI 5.5–6.8) and taxol and 10-deacetyl baccatin III were detected in cell suspension and culture media. However, taxol content was low and ranged from 0.003% to 0.007% in the four cell lines. The content of 10-deacetyl baccatin III varied from 0.162% to 0.212% (Table 1). Low levels of taxanes were also exuded in the liquid culture medium. The results are particularly significant since total chemical synthesis of taxol has not yet been achieved and an efficient semi-synthetic method starting from 10-deacetyl baccatin III has been reported[12]. Thus cell suspension culture of Himalayan *T. baccata* may be used as a source of precursor for synthesis of taxol.

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