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## Millennium-long ring-width chronology of Himalayan cedar from Garhwal Himalaya and its potential in climate change studies

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We report here a 1198-year long (AD 805–2002) ring-width chronology of Himalayan cedar (*Cedrus deodara*) from a site in Bhaironghati, Garhwal, Uttaranchal. This provides the longest record of ring-width chronology prepared so far using living tree samples from the Himalayan region. The forest from which the constituent samples were derived is a natural stand of mixed age. Many of the trees are several centuries old, with average age reaching 532 years. The ring-width chronology shows strong indirect relationship with mean monthly temperature from February to May. Strong temperature signal present in the series shows the potential of such long-term chronologies in developing climatic reconstructions useful for evaluating the recent climatic changes under the background influence of increasing concentration of greenhouse gases.

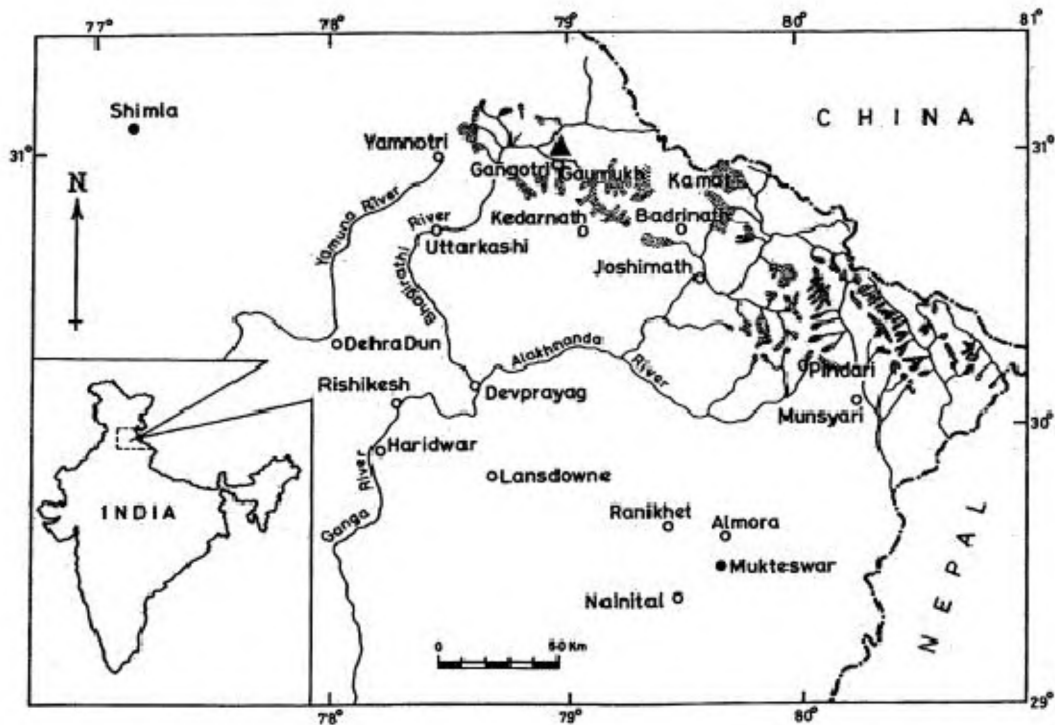
UNDERSTANDING the causes and mechanisms of the earth's climate variability requires developing high-resolution proxy records of climate spanning several centuries and millennia<sup>1</sup>. The proxy records spanning millennia and beyond, available thus far, are largely restricted to high-latitude Northern and a few from the Southern Hemisphere<sup>1,2</sup>. However, for the Himalayan region, which is climatically important, such long-term records are not yet available, except for the lone tree-ring-width chronology of Himalayan cedar spanning from AD 1168 to 1988 (821 years) prepared using living tree samples from Harshil, Uttarkashi, Uttaranchal<sup>3</sup>. To fulfill the need of millennium long

chronology we describe here a 1198-year long ring-width chronology of Himalayan cedar from a site featured by rocky slopes with thin soil cover in Bhaironghati, Uttarkashi, Uttaranchal. The chronology provides valuable input to the global data network required to infer the hemispheric-scale changes in climate.

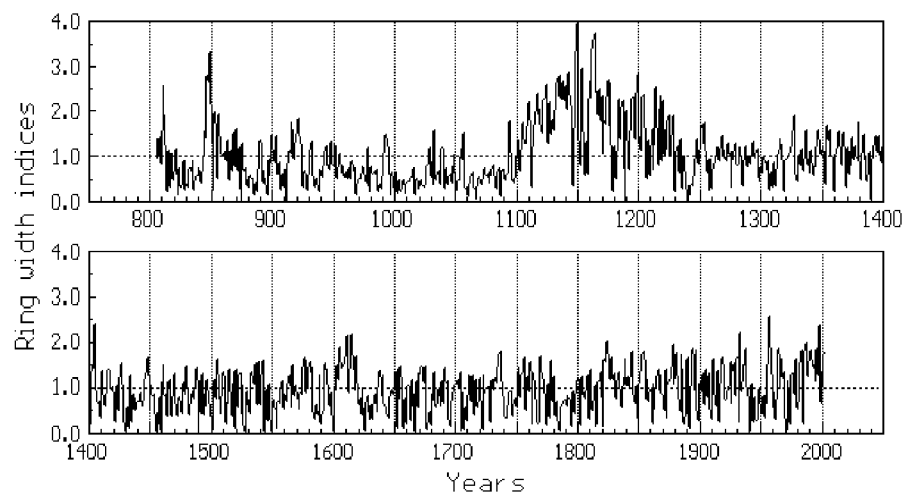
The tree-ring samples in the form of increment cores (usually two from each tree) were collected from 30 living trees during two field trips undertaken in September 2000 and June 2003 from a site near Bhaironghati (Figure 1). The krummholz nature and crown dieback with umbrella-shaped canopy of the trees indicate old age and climatic stress. Several trees at this site were found to be 700–800 years old, except one tree where the number of rings in the extracted core was around 1200. Complete age of this tree could not be determined, as the core length retrieved was limited due to heart rot. However, in order to obtain longer series, we attempted to take six cores from different directions of the main stem at variable heights. Due to the presence of narrow rings towards the inner end of the cores, we expect that the age of the tree could extend beyond 1500 years.

The increment cores collected were air-dried and glued on grooved wooden supports for safe handling in the laboratory. The growth ring sequences were cross-dated using skeleton plotting<sup>4</sup>. In this procedure, the relative narrowness of rings in comparison with the adjacent ones was plotted on graph-paper strips and then ring-width sequences among the samples crossmatched. About 2.3% rings were recorded missing in samples, indicating climatic stress experienced by the trees. The nature and location of the site also indicate that the trees might be subjected to frequent stone-fall injuries. The falling stones damage the trees coming in their way. The damages due to stone fall are healed in few years, leaving no visible scar on the outer surface. If the increment corer passes through such old injuries, the core extracted has several missing rings depending on the magnitude of the damage. Cores with a number of missing rings were noted in many samples in our collection. When dating in such cases was not possible, the sample in question was rejected for further study. We have been able to successfully cross-date 43 core samples derived from 24 trees. The ring-widths of dated growth-ring sequences were measured to 0.01 mm accuracy using linear encoder coupled with a personal computer. The accuracy of cross-dating among the samples was cross-checked using a dating quality control program, COFECHA<sup>5</sup>. This helps in identifying segments of a core or group of cores where dating or measurement errors may have occurred. Samples that showed ambiguity were rechecked, and where problems could not be corrected, the material in question was excluded from further analysis. The tree-ring-width patterns within the samples showed good cross-dating. COFECHA showed a mean correlation of all 43 radii from 24 trees with the master series of 0.83. Such a high correlation and good

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**Figure 1.** Location of tree-ring site (filled triangle) and meteorological stations (filled circles).



**Figure 2.** Himalayan cedar ring-width chronology (AD 805–2002).

cross-dating strongly suggest the presence of a dominant climatic signal in the tree-ring data.

The individual ring-width measurement series were standardized (i.e. detrended) to remove the long-term growth trends largely attributed to increasing age and tree size<sup>6</sup>. In order to minimize the removal of any long-term variance in the process, we used negative exponential curves or straight lines fitted to each ring-width measurement series of each individual tree core. The indices for each series were derived by taking the ratio of the meas-

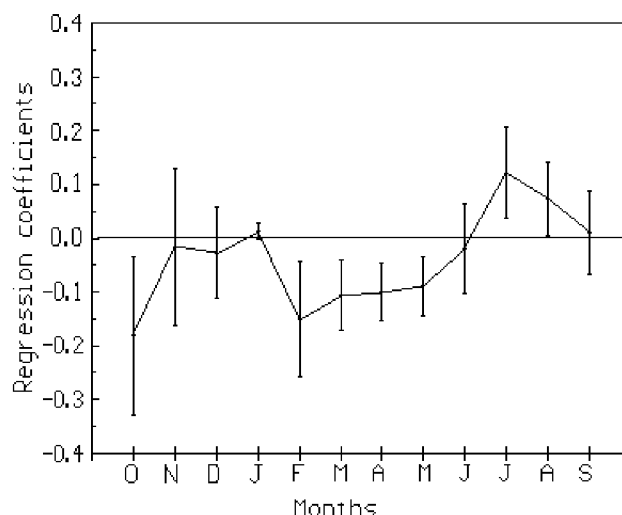
urement to the fitted value in each year. These indices, after removing the autocorrelation functions using an autoregressive model selected on the basis of the minimum Akaike criterion, were combined across all series in each year using biweight robust estimation of the mean to discount the influence of outliers. A set of three chronologies was developed; a standard chronology, a residual containing only the high-frequency variations and an arstan composed of the residual chronology with the pooled autoregression reincorporated<sup>7</sup>. The standard chronology

**Table 1.** Chronology statistics of Himalayan cedar

Chronology span (years)	AD 805–2002 (1198)
Number of trees (cores)	24 (43)
Mean sensitivity	0.51
Standard deviation	0.58
Autocorrelation	
Order 1	0.53
Order 2	0.19
Order 3	0.15
Common interval analysis period (years)	AD 1561–2000 (440)
Number of trees (cores)	18 (29)
Mean correlation	
Among all radii	0.50
Between trees (Y variance)	0.49
Within trees	0.76
Signal-to-noise ratio	17.31
Expressed population signal	0.95
Variance in first eigenvector	52.93%

(AD 805–2002) is shown in Figure 2. The statistical features of the chronology (Table 1) comparable to other ring-width chronologies of Himalayan cedar prepared from different sites in Garhwal Himalaya<sup>8</sup> show its potential in climatic studies. Though the chronology spans the past 1198 years, acceptable chronology confidence gauged using the subsample signal strength (a combination of mean interseries correlation and the number of tree-core samples represented in the chronology)<sup>9</sup> is achieved with a minimum sample replication of four tree samples, i.e. from AD 1227 onwards. The expressed population signal declined gradually towards the early part of chronology, reaching up to 75% back to AD 1223. The chronology back to AD 1223 is represented by six series from only one tree sample and among these only one core from AD 805 to 974. As one tree sample statistically represents 61% of the population signal, the climatic interpretation of the early part of the chronology needs to be done cautiously.

Site features such as steep rocky slopes and thin soil cover show that the sampled trees are subjected to moisture stress and therefore could be a valuable proxy of precipitation. But the precipitation records near the sampling site, best suited to calibrate ring-width data, are not available. Topography-forced high spatial variability in precipitation limits the applicability of data from distant stations, which could not be taken as representative for the site in question. However, contrary to this in our earlier detailed study<sup>10</sup>, we have found that the temperature records from distant regions having strong coherence represent the regional features and therefore, provide valuable data to calibrate the tree-ring data even from far-off distances. For tree-ring sites like the present one, high temperature could exacerbate the soil moisture deficit for the trees by increasing the evapotranspiration rate. Thereby, the tree-ring data from such sites also serve as a valuable proxy of temperature. Considering this, we have taken the mean temperature data of Shimla (31°10'N, 77°17'E; 2205 masl) and Mukteswar (29°28'N, 79°39'E;



**Figure 3.** Response function of residual chronology with mean monthly temperature (1901–98). Vertical bars are the 95% confidence limits.

2311 masl) for calibration with tree-ring-width data. The mean temperature of the above two stations has earlier been found to be useful in developing strong climatic reconstruction using tree-ring-data network of Himalayan cedar for western Himalaya<sup>11</sup>.

We used response function analysis<sup>6</sup> to identify the influence of temperature on tree growth. This is a multiple regression analysis in which monthly climate variables are taken as predictors and tree-ring data as predictand. The residual chronology and the mean temperature beginning in October of the previous growth year and ending in September of the current growth year over the period 1901–98 were used for this purpose (Figure 3). The temperature data during months prior to the growing season were included in the response function study, because growing-season ring-widths can integrate climate over a longer period<sup>6</sup>. The study shows that the tree growth has negative relationship with the mean monthly temperature ranging from February to May of the growth year. The significant months (February–May) correspond to the onset of bud break, tree growth and the formation of early wood. The above warm months cause intense evapotranspiration, which reduces the soil-moisture budget thus limiting the tree growth.

Establishment of successful cross-dating among ring-width sequences in the samples from a climatically stressed site has enabled us to develop a 1198-year (AD 805–2002)-long ring-width chronology of Himalayan cedar. This provides the longest record of ring-width chronology developed so far from the Himalayan region using living tree samples. Old natural forests, still available in interior regions, where human disturbances are least could provide valuable data to prepare long-term tree-ring chronologies.

Chronology statistics such as mean sensitivity (0.51) and standard deviation (0.58) indicate high variability in ring-width patterns, largely due to high sensitivity of trees to climatic fluctuations. Existence of strong correlation between trees ( $r=0.49$ ) shows the presence of coherent growth pattern among trees, which is largely controlled by climate. The tree-ring chronology shows a surge in ring-width indices from AD 1100 to 1228 (Figure 2). Whether the high index values for such a century-long period are tree-specific or climate-forced is difficult to conclude at present until this part of the chronology is replicated with more tree samples.

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## Isolation, characterization and antimicrobial activity of marine halophilic *Actinopolyspora* species AH1 from the west coast of India

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A halophilic *Actinopolyspora* species AH1 was isolated from a marine sediment sample obtained from Alibag coast, Maharashtra. The strain showed good growth in medium containing 10 to 15% (w/v) NaCl and with 30 to 36°C temperature. Cultural properties of *Actinopolyspora* were studied extensively on starch casein agar and other media. Strain AH1 showed an elon-

gated and circular shape with 20 to 30 spore-chain structures observed by slide culture technique and scanning electron microscopy. The morphological, biochemical and physiological characters of the isolate conformed to the characteristics of the genus *Actinopolyspora*, which contained only three species, viz. *A. halophila*, *A. mortivallis* and *A. iraqiensis*. However, the *Actinopolyspora* species AH1 isolated and characterized by us showed different properties compared to the known species. It showed resistance to clindamycin, vancomycin, nalixidic acid and streptomycin antibiotics. Interestingly, it showed good antibacterial activity against *Staphylococcus aureus*, *S. epidermidis*, *Bacillus subtilis* and antifungal activities against *Aspergillus niger*, *A. fumigatus*, *A. flavus*, *Fusarium oxysporum*, *Penicillium* species and *Trichoderma* species. This is a report of the antimicrobial activity exhibited by *Actinopolyspora* species AH1 isolated from the marine sediment.

IN recent years marine microorganisms have become important in the study of novel microbial products exhibiting antimicrobial, antiviral, antitumor as well as anticoagulant and cardioactive properties<sup>1–3</sup>. These active compounds may serve as model systems in the discovery of new drugs<sup>4,5</sup>. Halophilic species have been described as *Actinopolyspora halophila*, *Actinopolyspora mortivallis* and *Actinopolyspora iraqiensis*<sup>6–9</sup>. During the course of screening for antibiotics from marine actinomycetes, a halophilic *Actinopolyspora* species was isolated from Alibag, a coastal region in the west coast of India. Species AH1 isolated in the present study required 8 to 15% (w/v) NaCl for growth. This communication deals with isolation, characterization and antimicrobial activities of marine halophilic *Actinopolyspora* species AH1.

Strain AH1 showed good growth on starch casein agar (SCA) after 3 days. The medium consisted of 1% soluble starch, 0.1% casein, 0.05% KH<sub>2</sub>PO<sub>4</sub>, 0.05% MgSO<sub>4</sub>, 3% NaCl, 50% natural sea water collected from Alibag sea coast and 50% distilled water as a base. The final pH of the medium was adjusted to 7.6 with 0.1 N NaOH before sterilization<sup>10–13</sup>. The inoculated plates were incubated at 28°C for 7 days. After isolation, strain AH1 was purified by streak plate method and stock culture was maintained on SCA slant at 4°C in a refrigerator<sup>14,15</sup>. Growth characteristics were observed using different types of media, such as modified SCA, glucose asparagine agar, glycerol asparagine agar, tyrosine agar, yeast extract–malt extract agar, nutrient agar, maltose yeast extract agar and glycerol glycine agar<sup>16,17</sup>. Colonies showed wrinkled appearance on the surface of SCA containing 8 to 15% (w/v) NaCl, and hence the strain was considered as a halophilic species of the genus *Actinopolyspora*. The growth was found to take place in the temperature range of 10–45°C and 28°C was found to be optimum for growth. The strain was grown on SCA at different pH values such as 3.5, 5, 7 and 9 for 8 to 10 days, and pH 7.6 was found to be optimum for

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