Treeline migration and settlement recorded by Himalayan pencil cedar tree-rings in the highest alpine zone of western Himalaya, India

Himalayan pencil cedar (*Juniperus polycarpos*) is an evergreen tree distributed from Afghanistan, Baluchistan, Kagan valley, Kashmir, Lahaul-Spiti to upper reaches of western Tibet[1]. Naturally, the lower and upper limit of a tree species over a specific region varies due to the ecological settings of the area. Recently it has been noted that due to global/regional climatic changes there is marked change in distribution and composition of vegetation at its upper limit. Various studies from different parts of the globe indicate migration and shifting of treeline towards the higher altitudes[2–15]. Treeline migration/shifting have also been recorded from the Indian Himalaya by studying treeline dynamics over Uttarakhand-Himachal Pradesh[16,17] and Sikkim[18]. Himalayan pencil cedar is known to grow generally at high altitudes with the highest treeline recorded from the forest of *Juniperus tibetica* at southeast Tibet (~4900 masl)[19] and *Juniperus* sp. in Hunza-Karakorum (~3900 masl)[20]. Demarcation of the upper limit of treeline in complex mountainous terrains remains a challenging task.

In the western Himalaya treeline of the *Juniperus polycarpos* is not well defined because of the topographical barriers and variation in orography. Climatic variations and ecological conditions change rapidly from one valley to other due to the complex settings of mountain ranges in the Himalaya. Lahaul-Spiti is a cold desert, where major part of annual precipitation falls during winter to spring seasons by the western disturbances (WD). The region has very less influence of Indian summer monsoon (ISM) as it does not reach the Lahaul-Spiti due to high mountain peaks of Pir Panjal ranges. During winters precipitation occurs in forms of rain and snow and becomes the lifeline of socioeconomic activities for the region. During summers the valley gets heated by maximum temperature in July–August and temperature goes down to below zero in January. Himalayan pencil cedar has the ability to grow very old over such harsh and cold climatic conditions, which makes this species unique among the other conifers.

In the present study, the Yoche valley (32°38′11.3″, 77°15′31.7″) in Lahaul-Spiti, Himachal Pradesh was surveyed and Himalayan pencil cedar forests growing up to treeline were selected for the study (Figure 1). In the valley, Himalayan pencil cedar forest is very thin with trees growing disjunctly at altitudes ranging ~3500–4100 masl. Dry sandy soil covers the slope and it might be formed through the weathering of surrounding metamorphic rocks and glacial erosions. Peaks of the mountains surrounding the valley are covered by snow for maximum time of the year. In such harsh alpine climatic conditions trees are found growing healthy since the last millennium. During our field survey, we observed young saplings growing up to the height of 4122 masl (Figure 2), which makes the first-ever observation of treeline at such heights in the western Himalaya. Earlier it was believed that treeline limit of western Himalaya is around 3700 masl[1]. For trees growing in such high altitude, inaccessible pristine habitats are

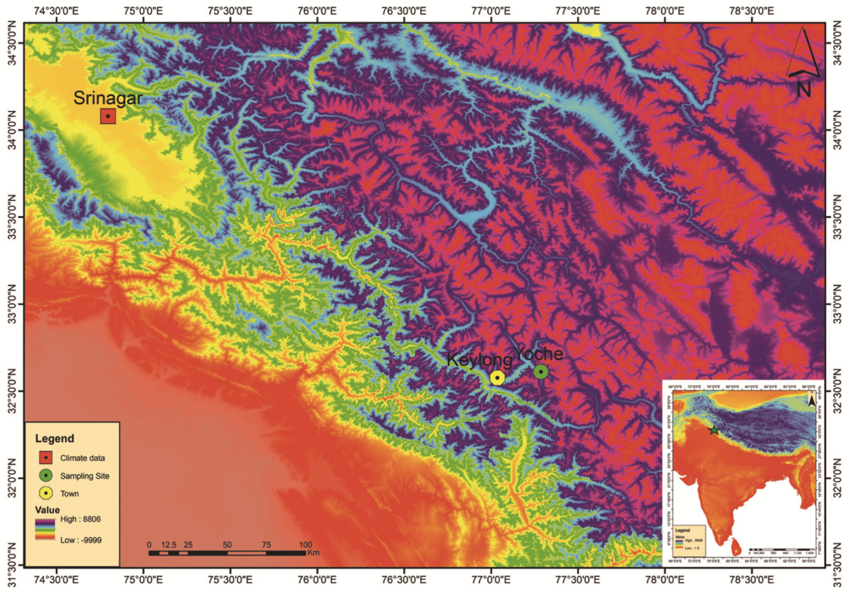

Figure 1. Map showing the location of the study area, tree ring sampling site and meteorological station used in the study.

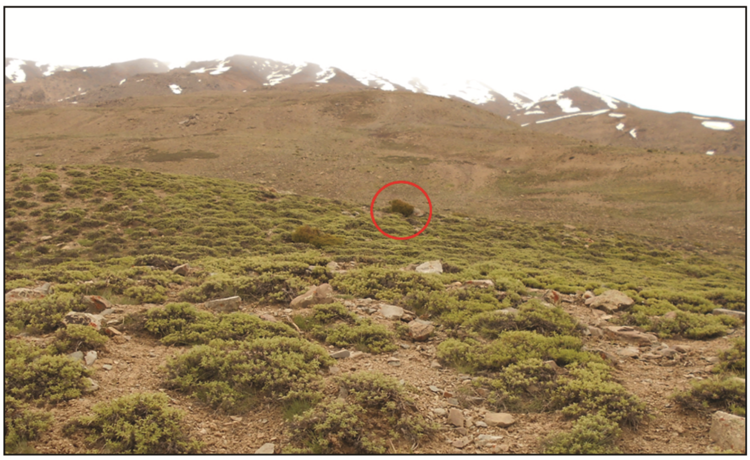

Figure 2. Young sapling of Himalayan pencil cedar (encircled) growing at the elevation of 4122 masl.

helpful for the understanding of climate in the long-term perspective. However, due to the increasing demand of fuel wood for the local population, such old trees are under potential anthropogenic pressure.

To investigate the tree establishment and climatic signal present in tree-ring series we collected 104 increment core samples from 73 trees of Himalayan pencil cedar. Generally, two increment cores from the opposite direction were collected from a tree growing on a steep slope at breast height (~1.4 m). After mounting of tree core samples in wooden frames, increment cores were polished with different grades of abrasives to make cross-surface clear under binocular microscope. Tree-ring sequences were precisely dated following standard dendrochronological techniques to assign exact calendar year to each ring. For cross-dating of the tree-ring samples, skeleton plots of each sample were prepared[21,22]. After cross-dating and assigning calendar years, the ring-widths in cores were measured up to 0.01 mm resolution using LINTAB measuring system (Rinntech, Germany) coupled with the personal computer. To check the measurement errors and ring-width pattern matching a dating quality control program COFECHA[23] and TSAP[24] were used. Samples showing measurement or dating errors were re-measured and errors, if any, corrected. Samples, dating of which could not be corrected were discarded for analyses. COFECHA analyses revealed that the mean correlation in the individual tree-ring-width series is 0.433. Inter-correlation is comparatively not much stronger as locality experiences frequent natural landslides/soil creep, due to which anomalous growth patterns were noticed in some portions of the tree-ring samples. The trees growing in harsh climatic conditions at high elevation were used to prepare chronologies from such remote areas to identify responsible climatic factors controlling the growth of such long-living trees.

Ring-width sequences of trees are influenced by climatic factors as well as internal/external factors such as biological growth, diseases and competition for the nutrient among the neighbouring trees, etc. Such biological growth trend from tree-ring series was removed for dendroclimatological studies by curve fitting techniques and detrending measures called ‘standardization’. Program ARSTAN was used for standardization of tree-ring series[25]. Before detrending the individual series data adaptive power transformation was applied to stabilize the variance[26]. Tree-ring-width measurement series were detrended using cubic smoothing spline with 50% amplitude over a two-third of the series length. By calculating biweight robust mean the individual tree-ring series were combined to mean chronology[25]. Using 104 tree-ring samples, 1226-year long chronology back to AD 789 was developed from the altitude of more than 4000 masl for the first time from the western Himalaya (Figure 3, Table 1). Oldest tree age over the site identified by cross-dating of the samples is ~1226 years. The threshold value of expressed population signal (EPS)[27] that is more than 0.85 was recorded from AD 1465. In some portions of the early part of the chronology, anomalous growth pattern is noticed which affected the EPS value. Residual chronology was used for establishing tree growth climate relationship (Figure 4). To identify dendroclimatic potential and climatic signal of the high-altitude tree-ring chronology of Himalayan pencil cedar, response function analyses was performed[22] using program DENDROCLIM2002 (ref. 28). Climate variables like monthly temperature and precipitation data of Srinagar (34°08′N, 74°48′E) were used in the response function analyses. Climatic window of the previous year October to current year September was used in the analyses to understand tree-growth and climate relationship. Bootstrap correlation analyses revealed that the correlation between the tree-ring chronology and previous year December to current year March except January precipitation was positive and significant, advocating that the tree-growth in the Yoche valley is influenced by winter precipitation. The precipitation of winter

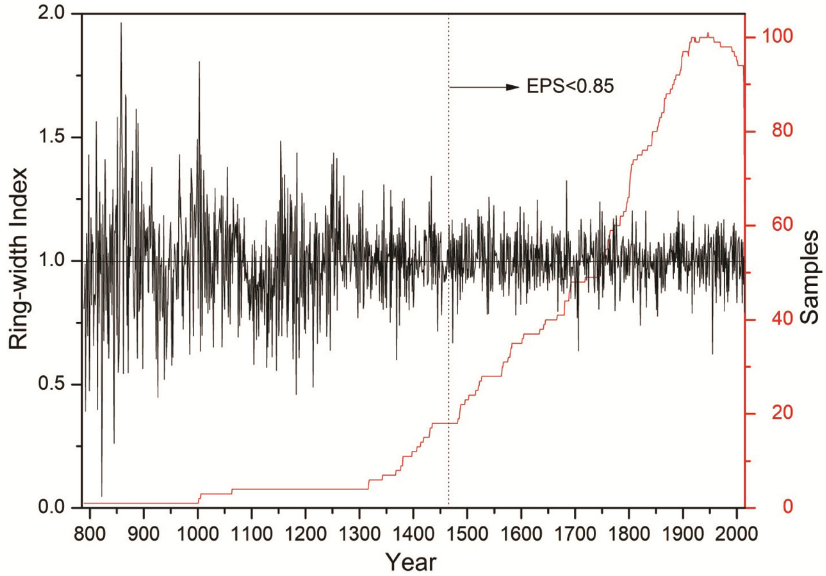

Figure 3. Himalayan pencil cedar chronology from Yoche, Lahaul-Spiti, Himachal Pradesh, India (AD 789–2014). Vertical line showing the EPS level (0.85) of the chronology at AD 1465, after which chronology exceeds threshold limit.

Table 1. Himalayan pencil cedar chronology statistics developed from Yoche, Lahaul-Spiti, Himachal Pradesh, India (AD 789–2014)

Latitude (N)	Longitude (E)	Elevation (m)	Cores/trees	Chronology span AD (years)	Chronology with EPS >0.85	MI	MS	SD
32°38′11.4″– 32°38′12.9″	77°15′19″– 77°15′31.4″	~ 4000 Max (4122 m)	104/73	789–2014 (1226)	1465–2014	1.00	0.18	0.17

EPS, Expressed population signal; MI, Mean index; MS, Mean sensitivity; SD, Standard deviation.

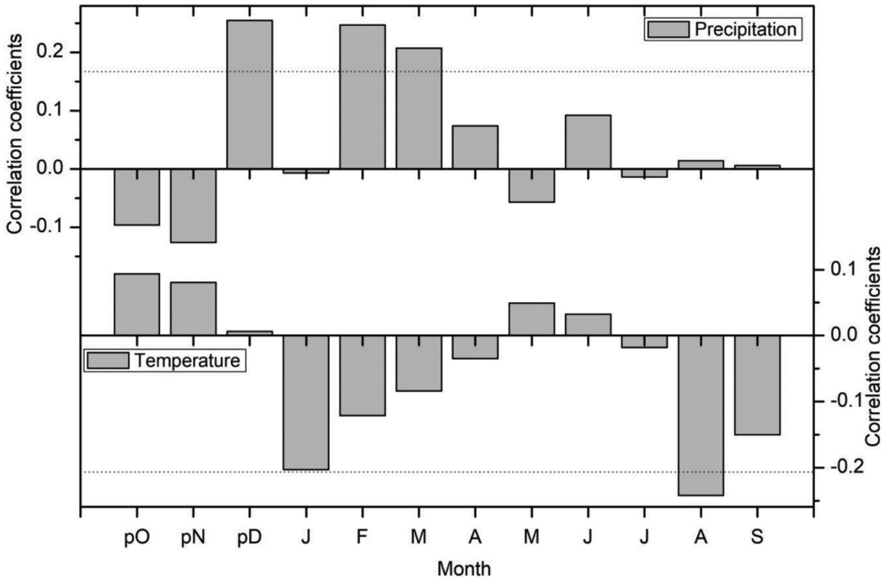

Figure 4. Bootstrap correlation of tree-ring chronology and climate variables such as monthly precipitation and temperature of Srinagar. The dotted line shows confidence level at 95%.

season recharges soil moisture in the growing period, as area is dry and sandy in nature. In general, response function analyses revealed that the cool and moist condition favour tree-growth and February–March precipitation is essential for the radial growth over high altitude tree-ring sites. However, tree-ring chronology showed significant negative correlation with August temperature data. It indicates that the increased temperature of August hampers the growth of alpine Himalayan pencil cedar trees in the valley. Dry and hot conditions during summer are inversely related to tree-growth.

Treeline limit over the altitude of 4100 masl in the Himalayan region has been recorded for the first time by us. Krumholz trees along with young trees/saplings growing up to the height of 4122 masl in the Yoche valley are demarcating the boundary of treeline and a new habitat of Himalayan pencil cedar. In this study, we have developed over millennium-long tree-ring chronology of Himalayan pencil cedar (extending back to AD 789; 1226 years) from the western Himalaya at an altitude >4000 masl. The study indicates dendroclimatic potential of such high altitude treeline species which are growing at climatic threshold limit in very harsh condition. Climatic variables like precipitation, temperature, etc. play significant role in the radial progression and vertical expansion of trees over the region. Tree-growth–climate relationship revealed that cool and moist climatic conditions favour tree-growth and increasing temperature during summer hamper tree-growth. Correlations between tree-ring and climatic variables established that such chronologies should be useful for long-term climatic reconstruction from treeline ecosystem. We are sanguine about the dendroclimatic utility of network of pristine high-altitude tree-ring chronologies from inaccessible areas with negligible anthropogenic impact on the natural vegetation. Such long-term climatic reconstructions should be of immense help to understand the climatic variability over the past millennium and treeline expansion history.

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In vitro rearing and gallery tunnelling pattern of Island pinhole borer, *Xyleborus perforans* (Wollaston), a scolytid associated with pomegranate wilt complex

Wilt, a devastating disease in pomegranate (*Punica granatum* L.) plantations causes complete death of young and old plants alike. This disease has become a threat to crop cultivation across the major pomegranate-growing countries like India, China, Iran and Greece, posing a potential crisis for farmers. Wilt-affected plants exhibit gradual yellowing, drying of leaves in a particular branch that spreads to others, leading to dieback and finally the infected plant dies within the next few weeks[1,2]. Pioneering studies have revealed that this disease shows symptoms caused by many contributing biotic and abiotic factors. Several biotic factors like fungal pathogens (viz. *Ceratocystis fimbriata*, *Fusarium* spp., *Macrophomina phaseolina*, *Phytophthora* spp., *Rhizoctonia bataticola*, *Rosellenia necatrix*, *Verticillium dahliae*), insects (scolytid beetle, *Xyleborus perforans* (Wollaston)) and nematodes (root-knot nematode, *Meloidogyne incognita*) were found to play a crucial role in disease progression[3].

The role of Island pinhole borer, *Xyleborus perforans* (Wollaston) (Coleoptera: Scolytidae) popularly known as pomegranate shothole borer (SHB), in causing pomegranate wilt is well established[4–7]. Besides their direct role in the mechanical transmission of wilt pathogens, these tiny beetles breed in the woody tissues of pomegranate plant by excavating galleries, particularly in the collar regions thereby damaging the plant vascular tissues. These scolytid beetles exclusively live in nutritional symbiosis with ambrosia fungi[8,9]. Therefore, the adult females (=foundresses) cultivate the ambrosia fungi in these galleries, as their exclusive source of nutrition for adults and young ones alike[6]. Studying the behaviour of these scolytid beetles, the nature of brood establishment, fungal cultivation and dispersal mechanism is extremely difficult in the field due to their invisible galleries hidden underneath the bark within the collar region of the plant. To overcome such difficulties associated with scolytids in general, which attack several tree species, many researchers have tried developing an artificial medium for rearing them in the laboratory[7–11]. A 'phloem sandwich' technique was explored, where a piece of phloem was sandwiched between acrylic or glass sheets sealed with parafilm to rear scolytid beetles within (that infest pine trees)[12].

In this study, the semi-synthetic medium established previously for other ambrosia beetles was customized to suit the nutrition requirements of *X. perforans* by addition of host wood sawdust (pomegranate), as the earlier medium did not support establishment of the beetles. The modified medium successfully supported the *in vitro* rearing of scolytid beetles that are associated with pomegranate wilt to facilitate the studies on their biology and etiology using *X. perforans* as a model species. The present study not only improves our understanding of tunnelling behaviour and biology of this scolytid beetle, but also demonstrates the feasibility of its *in vitro* rearing.