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Relationships between vegetation and stomata, and between vegetation and pollen surface soil in Yunnan, Southwest China

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Surface pollen and stomata of 61 samples collected in a study area ranging from tropical seasonal rainforest to oak forest (*Quercus spinosa*) in the Yulong Snow Mountain region in Yunnan, China, are used to distinguish vegetation communities. The results show that tropical seasonal rainforest (and mountain rainforest), south subtropical evergreen broad-leaved forest, and *Quercus* shrub are distinguished effectively from other vegetation types by analysis of surface pollen. The south subtropical evergreen broad-leaved forest, *Pinus kesiya* forest and evergreen broadleaf forest are distinguished effectively from other types of vegetation by pollen analysis. However, *P. yunnanensis* forest is not distinguished from other vegetation types, and *P. armandii*, *P. densata* forest and temperate deciduous conifer mixed forest are not distinguished. The over-representation of *Pinus* pollen is the main reason that these vegetation communities are not distinguished from each other. Conifer stomata analysis is an effective tool for identifying and distinguishing different types of coniferous forest, and this method performs well even with a small number of sampling points.

Yunnan, pollen, stomata, topsoil, vegetation, palynology

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Pollen analysis provides important indicators which reconstruct palaeovegetation [1,2]. However, such results are often inaccurate because of uncertainty related to factors affecting pollen production, dissemination, deposition, and identification. Therefore, other assisting means of palaeovegetation reconstruction are needed. At present, there are two main auxiliary methods of reconstructing palaeovegetation. One method is based on examination of plant fossils, which identify species, and provides important information for accurate reconstruction of palaeovegetation [3]. However, adequate and reliable fossils are often unavailable. The other method relies on identification and quantification of stomata, and provides an accurate means of reconstructing palaeovegetation [4].

In the early 1950's, Trautmann [5] found that observations on conifer stomata served as a potential palaeoenvironmental analysis tool, and that some conifer stomata from vegetation in central Europe are morphologically distinctive. Parshall [6] found that conifer leaves could only transport a very short distance, scattering within approximately 20 m

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from the origin. The stomata found in closed lake sediments provide information on the plants which grew in the local area, serving as a substitute for observations of plant fossils [7,8]. Conifer stomata observations as an important supplement for pollen analysis have received extensive attention and research in Europe, North America, Siberia, and Latin America. In Europe, stomata analyses were applied to study timberline migration [9–13]; in North America, Hansen [14] first used the stomata to study vegetation changes, and further developed the identification standards of the stomata, on the basis of Trautmann's study. However, the scope of applications using stomata methodology has widened. Recently, stomata analysis has been applied to research conducted in alpine and arctic regions and Kennedy evaluated *Pinus* stomata to distinguish mountain forest from grassland in Dominica [15]. Stomata analysis has also been applied in the Yangtze River Delta and Liupangshan in China [16,17].

The study of vegetation changes in the Yunnan forest area in China, an ecological region strongly influenced by the Asian monsoon, is important for the understanding of monsoons and global climate change. Since the Quaternary, changes in the vertical belt of mountain forest in Northwest Yunnan may due to climate change [18–20]. Determination of the sequence and causes would be supported by accurate methods of reconstruction of the vegetation community. However, studies of surface pollen samples reveal that the accuracy of reconstruction of palaeovegetation by pollen is limited in such areas, because of the over-representation of *Pinus* pollen [21,22]. In addition, a wide range of gymnosperms are extensively distributed in Yunnan region, varying from cold temperate coniferous forest to subtropical *Pinus kesiya* forest, in which conifer species are dominant. These conifers have specific significance for ecological and palaeoecological research, if their stomata can be used as an auxiliary method to pollen analysis to reconstruct the ancient vegetation diversity, abundance and distribution. If validated, the method would provide a powerful means of reconstructing paleovegetation and paleoclimate.

The study of the relationship between current pollen observed in standard sampling to corresponding vegetation is a very important step toward the reconstruction of palaeovegetation [23], as a validation and calibration of the methods. Likewise, the study of the relationship of modern pollen stomata to corresponding vegetation can also provide important reference relationships for reconstruction of the ancient vegetation communities. Similar research has been conducted in North America, Central Asia, and South America [5,15,24–28]. This article reports the results of examinations of the relationships among vegetation, pollen and stomata, based on the study of pollen and stomata samples from different types of vegetation in Yunnan. The purpose of the research is to provide evidence and data as a basis for subsequent pollen-vegetation-stomata reconstructions.

1 Study area

Yunnan Province lies in the extreme southwest of China (21°9′–29°15′N, 97°30′–106°12′E). Complex mountains and valleys were formed during massive uplift and erosion during the end of the Tertiary and Quaternary. Elevation varies considerably, from less than 100 m on the Red River in southeast Yunnan, to more than 6000 m in the northwest mountains, within the range of foothills of the Himalayas. In eastern Yunnan, rivers generally flow eastward in relatively shallow valleys, but in the west, the riversf low southward or southeastward, deeply dissecting the plateau landscape by as much as 1000 m. Altitude drops from north to south [29].

The climate of Yunnan is influenced by the interaction of several circulation systems, typically sub-tropical monsoonal, affected by monsoons from the southwest, southeast and northeast, and extra-tropical westerlies. The high altitude strongly influences temperatures within the region. Yunnan has a relatively mild climate, with maximum and minimum annual temperatures of 22.1 and 9.1°C. Average annual precipitation is distinctly seasonal and varies from 800 mm in dry valleys to 1700 mm in the southern and western areas, with 80%–90% during May to October.

The topographical variety within the province has resulted in a rich diversity of vegetation, ranging from tropical rainforest to alpine exposed gravel, and cold desert from Xishuangbanna in southern Yunnan to Yulong Snow Mountain in northwestern Yunnan, within a straight-line distance of more than 600 km [29].

Within the study area, a total of 61 surface pollen and stomata samples were taken from Xishuangbanna in southern Yunnan to Yulong Snow Mountain region in northwestern Yunnan (Figure 1). The main vegetation types are shown in Figure 1 and Table 1.

2 Materials and methods

In 2005 and 2006, samples were collected from the southern tropical rainforest in Xishuangbanna to the northern cold coniferous forest of Yulong Snow Mountain in Yunnan (Figure 1). The main material collected in each sample is topsoil and moss. At each sampling point, 4–5 samples of moss or topsoil were randomly selected and collected within an area of approximately 100 m^2, then combined into one bulked sample and tagged with the location and altitude of each sampling point by GPS (Table 1). In order to study whether the stomata can accurately indicate the existence of cold temperate coniferous vegetation communities, samples were also collected in the forest sites where cold temperate tree species are not dominant to test the accuracy of analysis of stomata as indicators.

Standard hydrofluoric (HF) methods are used to prepare the samples treatment [30]. The number of pollen in each sample was counted, usually at least 500 grains. However,

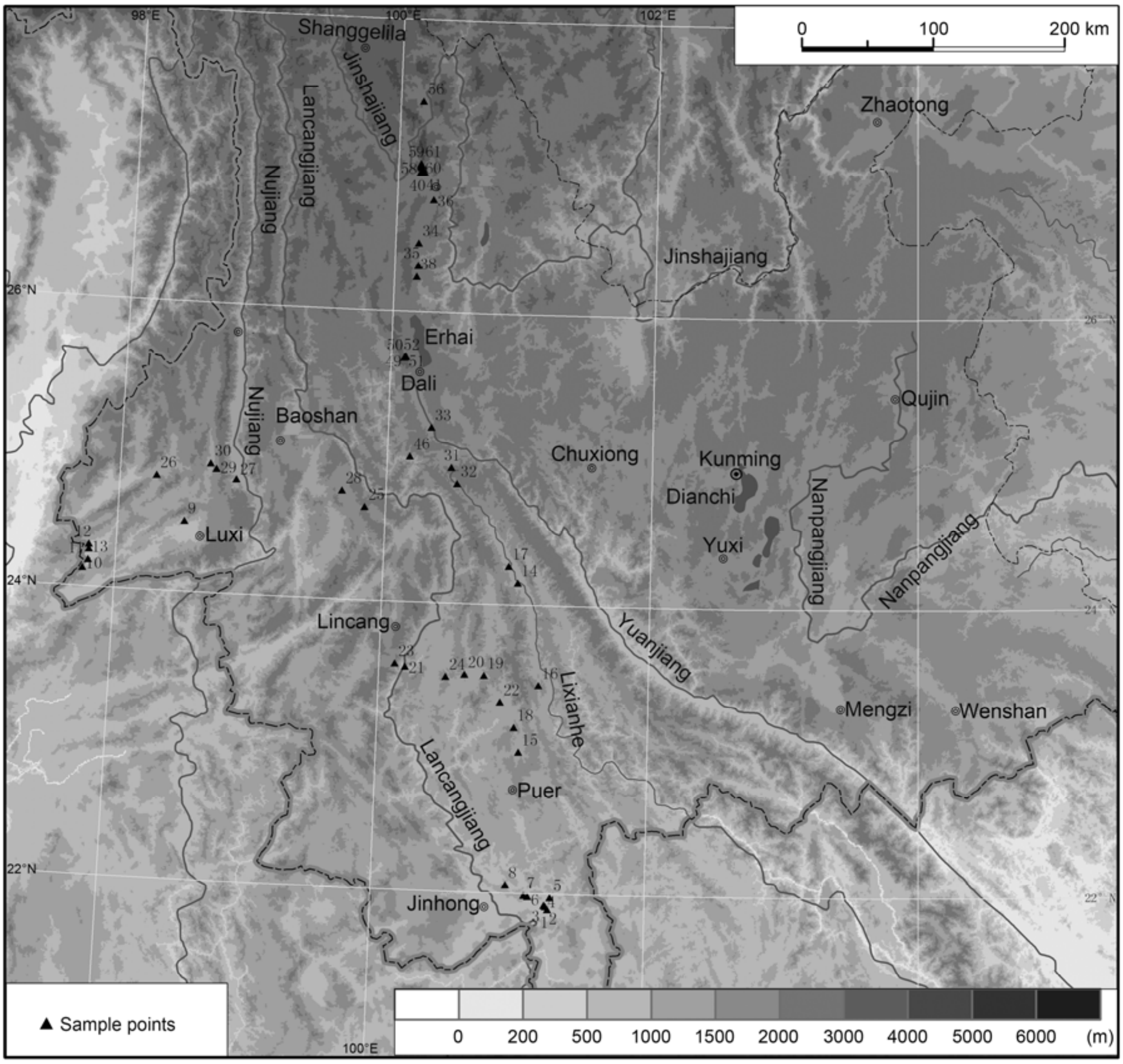

Figure 1 The locations of the sampling points.

samples with less than 500 grains of pollen were counted in a few samples because the content of the pollen is very low in those cases. At the same time, stomata were identified in the samples [24,31,32]. The percentages of pollen and stomata were calculated using the terrestrial plants as the base, and at the same time, the percentages of fern spores found in the samples were calculated using the sum of the total number of terrestrial plants and the total number of fern spores as the base. The calculation and mapping is based on software programs Tilia and TGView [33,34].

To study the relationship between pollen and modern vegetation, the pollen data is subjected to Detrended Correspondence Analysis (DCA) analysis. This method is used for pollen types for which the content of those pollen is more than 2% and those types appeared in more than 3 samples. CANOCO4.5 software is used to calculate results [35], and the square root transformation is used to convert the percentage data of the pollen counts.

3 Results

3.1 Surface pollen and stomata

More than 300 genera of pollen were identified in the 61 samples. The percentages of surface pollen and stomata are shown in Figure 2 according to the vegetation types, pollen and stomata (Figures 2 and 3).

(i) Tropical mountain rainforest and seasonal forest. Tropical and subtropical species (except *Pinus*) are dominant in the main woody pollen, including *Lithocarpus*/*Castanea*, *Castanopsis*, *Quercus* evergreen, *Mallotus*, *Macaranga*, *Moraceae*, *Eurya genus*, *Magnolia*, *Ficus* and *Theaceae*. Herbs are mainly *Gramineae*, *Artemisia* and *Euphorbiaceae*. The content of fern spores is high (25%–95%) and with multiple types. Samples 1 and 7 contained some stomata which cannot be identified.

(ii) South subtropical broadleaf forest. The relative amountof *Pinus* pollen in samples 9, 10 and 14 is dominant

Table 1 Elevation, latitude, longitude, vegetation types and the main vegetation of the sampling locations

Sampling points	Elevation (m)	Latitude (°)	Longitude (°)	Vegetation types	Main vegetation
1	557	21.891	101.296	seasonal rainforest	*Lagerstroemia*, *Yushania*, *Ficus*, *Ormosia*, *Duabanga grandiflora*, *Macaranga denticulata*, *Pinus tabulaeformis*, *Sterculia*, *Elaeocarpus*, *Malugay*, *Homalium cochinchinense*, *Lithocarpus*, *Dendrobenthamia angustata* (Chun) Fang
2	673	21.911	101.281	seasonal rainforest	*Caryota ochlandra* Hance, *Ficus*, *Lithocarpus*, *Macaranga denticulata*, *Acacia pennata*, *Phoebe*, *Ficus*
3	586	21.919	101.271	seasonal rainforest	*Tetrameles*, *Musa paradisiaca*, *Dimocarpus longgana* Lour., *Schefflera octophylla*(Lour.)Harms, *Erythrina indica* Lam, *Combretaceae* nom. conserv.
4	592	21.92	101.27	seasonal rainforest	*Carallia brachiata*, *mangrove*, *Ficusstricta*, *Melia azedarach*, *Semecarpus anacardius* L.f., *Macaranga tanarius*, *Baccaurea ramiflora* Lour
5	589	21.976	101.312	tropical mountain rainforest	same as above, *Rosa* spp., *Duabanga grandiflora*, *Ormosia*, *Yushania*, *Olea*, *Arecaceae*, *Magnolia henryi* Dunn
6	781	21.979	101.148	tropical mountain rainforest	Mainly: *Ficus*, *Clerodendrum bungei*, *Macaranga tanarius*, *Meliaceae*, *Schima superba* Gardn et Champ, Few: *Betula*, *Mussaenda pubescens*, *Rubia cordifolia*, *Bischofia polycarpa*
7	1074	21.986	101.12	tropical mountain rainforest	*Bauhinia purpurea* L., *Cinnamomum rigidissimum*, *M. basjoo*, *P. australis*, *Ficus*, *Duabanga grandiflora*, *Paramichelia baillonii* (Pierre) Hu
8	800	22.056	100.984	tropical mountain rainforest	*Schima superba* Gardn et Champ, *Castanopsis fargesii*, *Lauraceae*, *Duabanga grandiflora*, *Sapotaceae*, *Rhodoleia*, *Hamamelidaceae*, *Rhus chinensis*, *Ficus*
9	1506	24.815	98.833	south subtropical evergreen broad-leaved forest	*Castanopsis fargesii*, *Lauraceae*, *Theaceae*
10	1255	24.213	97.794	south subtropical evergreen broad-leaved forest	*Castanopsis fargesii*, *Lauraceae*, *Theaceae*
11	1559	24.378	97.823	south subtropical evergreen broad-leaved forest	*Castanopsis fargesii*, *Lauraceae*, *Theaceae*
12	1760	24.392	97.822	south subtropical evergreen broad-leaved forest	*Castanopsis fargesii*, *Lauraceae*, *Theaceae*
13	1550	24.392	97.822	south subtropical evergreen broad-leaved forest	*Castanopsis fargesii*, *Lauraceae*, *Theaceae*
14	1120	24.152	101.011	south subtropical evergreen broad-leaved forest	*B. ceiba*, *Ficus microcarpa*, *Aleurites moluccana*, *Mangifera indica* Linn, *Cyclobalanopsis*, *Quercus acutissima* Carruth, *Syzygium jambos* (L.) Alston.
15	1333	22.981	101.052	*Pinus kesiya* forest	*Phyllanthus emblica* Linn., *Betula*, *Pinus kesiya*, *Toxicodenddron sylvestre* (Sieb. et Zucc.) O.Kunrze., *Ternstroemia*, *Schima superba* Gardn et Champ, *Ficus altissima* Bl., *Amla*
16	1265	23.446	101.186	*Pinus kesiya* forest	*Pinus kesiya*, *Castanopsis* fargesii, *Ailanthus* altissima (Mill.) Swingle, *Ficus microcarpa*, *Schima superba* Gardn et Champ
17	1204	24.266	100.941	*Pinus kesiya* forest	*Pinus kesiya*, *Ficus*
18	1207	23.15	101.012	*Pinus kesiya* forest	*Pinus kesiya*, *Toxicodendron verniciflua*(Stokes) F.A.Barkl *Cinnamomum camphora* (L.) Presl, *Rutaceae*
19	1149	23.505	100.78	*Pinus kesiya* forest	*Pinus kesiya*, *Toxicodendron verniciflua*(Stokes)F.A.Barkl *Cinnamomum camphora* (L.) Presl, *Rutaceae*
20	1436	23.507	100.635	*Pinus kesiya* forest	*Pinus kesiya*, *Fagaceae*, *Amelanchier*, *C. bungei*, *Alsophila spinulosa* (Hook.) Tryon
21	920	23.55	100.193	*Pinus kesiya* forest	*Fagaceae*, *Pinus kesiya*, *Amelanchier*, *C. bungei*, *Alsophila spinulosa (Hook.)* Tryon,
22	932	23.324	100.905	*Pinus kesiya* forest	*Fagaceae*, *Pinus kesiya*, *Amelanchier*, *C. bungei*, *Alsophila spinulosa* (Hook.) Tryon,
23	1520	23.569	100.116	*Pinus kesiya* forest	*Fagaceae*, *Pinus kesiya*, *Amelanchier*, *C. bungei*, *Alsophila spinulosa* (Hook.) Tryon
24	1723	23.489	100.495	*Pinus kesiya* forest	*Fagaceae*, *Pinus kesiya*, *Amelanchier*, *C. bungei*, *Alsophila spinulosa* (Hook.) Tryon
25	2178	24.644	99.845	*Pinus kesiya* forest	*Fagaceae*, *Pinus kesiya*, *Amelanchier*, *C. bungei*, *Alsophila spinulosa* (Hook.) Tryon,
26	1159	24.793	98.273	evergreen broad-leaved forest	*Lithocarpus echinotholus,* *Cyclobalanopsis glaucoides* Schottky, *C. delavayi* Franch., *Castanopsis delavayi* Franch., *Quercus*

(*To be continued on the next page*)

(*Continued*)

Sampling points	Elevation (m)	Latitude (°)	Longitude (°)	Vegetation types	Main vegetation
27	1250	24.791	98.873	evergreen broad-leaved forest	*Lithocarpus echinotholus*
28	1563	24.75	99.67	evergreen broad-leaved forest	*Lithocarpus echinotholus*
29	1606	24.857	98.721	evergreen broad-leaved forest	*Lithocarpus echinotholus*
30	1679	24.892	98.676	evergreen broad-leaved forest	*Lithocarpus echinotholus*
31	2054	24.935	100.486	*P. yunnanensis* forest	*Eucalyptus*, secondary *P. yunnanensis* Franch., *Quercus*, *Schima superba* Gardn et Champ
32	1686	24.824	100.533	*P. yunnanensis* forest	secondary *P. yunnanensis* Franch.
33	1919	25.206	100.325	*P. yunnanensis* forest	*Rosaceae*, *P. yunnanensis* Franch., *Eucalyptus*, *Cyclobalanopsis*
34	2324	26.468	100.175	*P. yunnanensis* forest	*Eucalyptus*, *P. yunnanensis* Franch.
35	2255	26.314	100.178	*P. yunnanensis* forest	*P. yunnanensis* Franch., *Alnus nepalensis* D.Don, *Keteleeria fortunei* (Murr) Carr, *Quercus*, *Lithocarpus*
36	2425	26.771	100.278	*P. yunnanensis* forest	*P. yunnanensis* Franch., *Photinia prionophylla* (Franch.) Schneid., *P. armandii* Franch.
37	2700	26.955	100.202	*P. armandii* and *P. densata* forest	*P. densata* Mast., *P. yunnanensis* Franch., *P. armandii* Franch., *Rhododendron*
38	2371	26.24	100.169	*P. armandii* and *P. densata* forest	*Duercus spinosa* David ex Fr., *Rhododendron*, *Heaceae*, *Myrica rubra* (Lour.)Zucc., *Keteleeria fortunei* (Murr) Carr, *Rhododendron delavayi* Franch, *Caprifoliaceae*, *P. armandii* Franch.
39	2838	26.954	100.192	*P. armandii* and *P. densata* forest	*P. armandii* Franch., *Populus*, *Salix*
40	2948	26.952	100.17	*P. armandii* and *P. densata* forest	*Picea*, *P. armandii* Franch., *P. densata* Mast., *Viburnum dilatatum* Thunb, *Populus davidiana* Dode, *Duercus spinosa* David ex Fr.
41	3054	26.953	100.182	*P. armandii* and *P. densata* forest	*P. armandii* Franch., *P. densata* Mast., *Populus davidiana* Dode, *Picea, Abies*
42	3138	26.969	100.182	*P. armandii* and *P. densata* forest	*P. armandii* Franch., *P. densata* Mast., *Quercus spinosa* David ex Fr., *Populus*, *Rhododendron*
43	3262	26.988	100.174	*P. armandii* and *P. densata* forest	*P. densata* Mast., *Tsuga*, *P. armandii* Franch., *Paeonia delavayi* var. *lutea* (Franch.) Finet. Et Gagnep.
44	3305	26.995	100.176	*P. densata* forest	Horse-intoxicated *Pieris*, *P. densata* Mast., *Q. pannosa* Hand.-Mazz., *Rhododendron*, *Viburnum dilatatum* Thunb, *P. armandii* Franch.
45	3321	26.998	100.17	*P. densata* forest	*P. densata* Mast., *Rhododendron*
46	3369	27.002	100.17	*P. densata* forest	*P. densata* Mast., *Quercus*, *Rhododendron*
47	3152	25.689	100.111	secondary shrub after temperate broad-leaved mixed conifer forest	*Spiraea*, *Polygonaceae*
48	3220	25.684	100.1	secondary shrub after temperate broad-leaved mixed conifer forest	*Rubus*
49	3152	25.689	100.111	temperate broad-leaved mixed conifer forest	*Lithocarpus*, *Populus*, *Tsuga*, *Phyllostachys pubescens* Mazel ex H. de Lehaie
50	3287	25.684	100.1	temperate broad-leaved mixed conifer forest	*Quercus*, *Abies*, *Tsuga*, *Rhododendron*, bamboo
51	3307	25.685	100.104	temperate broad-leaved mixed conifer forest	*Quercus*, *Abies*, *Tsuga*, *Rhododendron*, bamboo
52	3307	25.685	100.104	temperate broad-leaved mixed conifer forest	*Quercus*, *Abies*, *Tsuga*, *Rhododendron*, bamboo
53	3392	27.003	100.171	temperate broad-leaved mixed conifer forest	*Rhododendron*, *Abies*
54	3440	27.004	100.173	*Quercus* shrub	*Q. spinosa Rhododendron*, *Abies*, *Picea*
55	3472	27.007	100.174	*Quercus* shrub	*Q. spinosa Rhododendron*, *Abies*, *Picea*
56	3495	27.441	100.174	*Quercus* shrub	*Q. spinosa Rhododendron*, *Abies*, *Picea*
57	3530	27.009	100.173	*Quercus* shrub	*Q. spinosa*, *Rhododendron*, *Abies*
58	3571	27.01	100.172	*Quercus* shrub	*Q. spinosa*, Rhododendron, Abies
59	3581	27.011	100.172	*Quercus* shrub	*Q. pannosa* Hand.-Mazz., *Populus davidiana* Dode, *Q. spinosa*, *Abies*
60	3625	27.013	100.171	*Quercus* shrub	*Q. spinosa*
61	3679	27.014	100.172	*Quercus* shrub	*Q. spinosa*

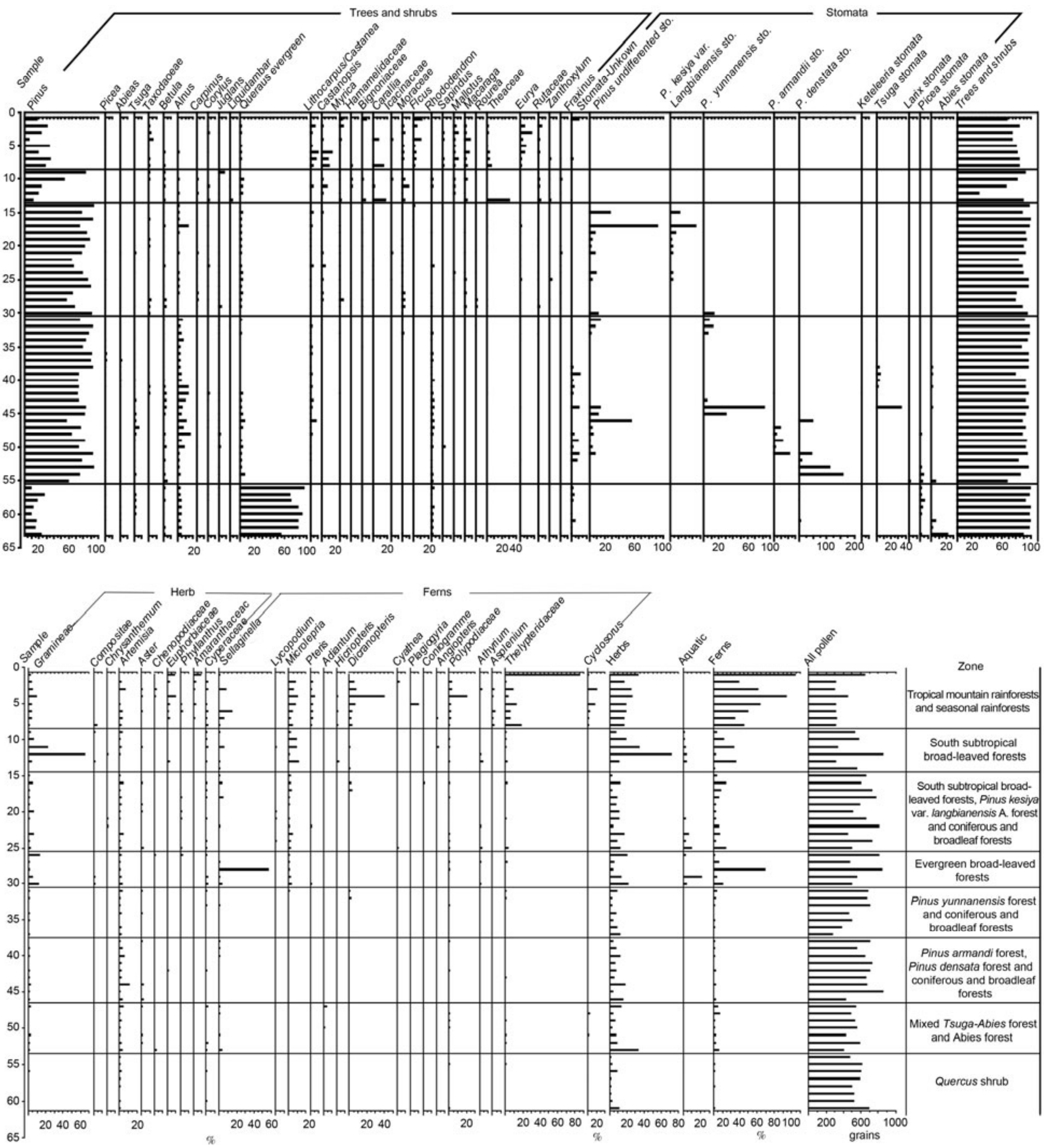

Figure 2 The percentages of surface pollen and stomata.

(maximum of 82.5%). The main woody pollen types are *Moraceae*, *Theaceae*, *Quercus* evergreen, *Castanopsis* and *Lithocarpus*/*Castanea*. The highest proportion of *Gramineae* in herb pollen is 65.5%, most of which is more than 38 μm (64.1%). Fern spore content is relatively high (5%–28.5%).

(iii) South subtropical *P. kesiya* forest. *Pinus* pollen accounted for 57.4%–93.8% and the content of broad-leaved tree pollen is relatively low, mainly including *Alnus*, *Lithocarpus*/*Castanea*, *Castanopsis* and *Quercus* evergreen; other pollen are mainly mainly *Gramineae* and *Artemisia*; ferns accounted for 3.5%–59.8% of the total pollen, which are the highest (50.5%). Conifer stomata are dominant, with mainly *P. kesiya* and *Pinus* sp. (*Pinus* which can not be identified, is recorded simply as *Pinus*).

(iv) Central subtropical evergreen broad-leaved forest. *Pinus* pollen is dominant (56.2%–90.9%) here, and other broad-leaved tree pollen types are mainly *Quercus* evergreen, *Lithocarpus*/*Castanea*, *Myrica* and *Moraceae*. Herb pollen are *Gramineae* and *Artemisia*, as is the case in other vegetation types, and fern spores are mainly *Selaginella*.

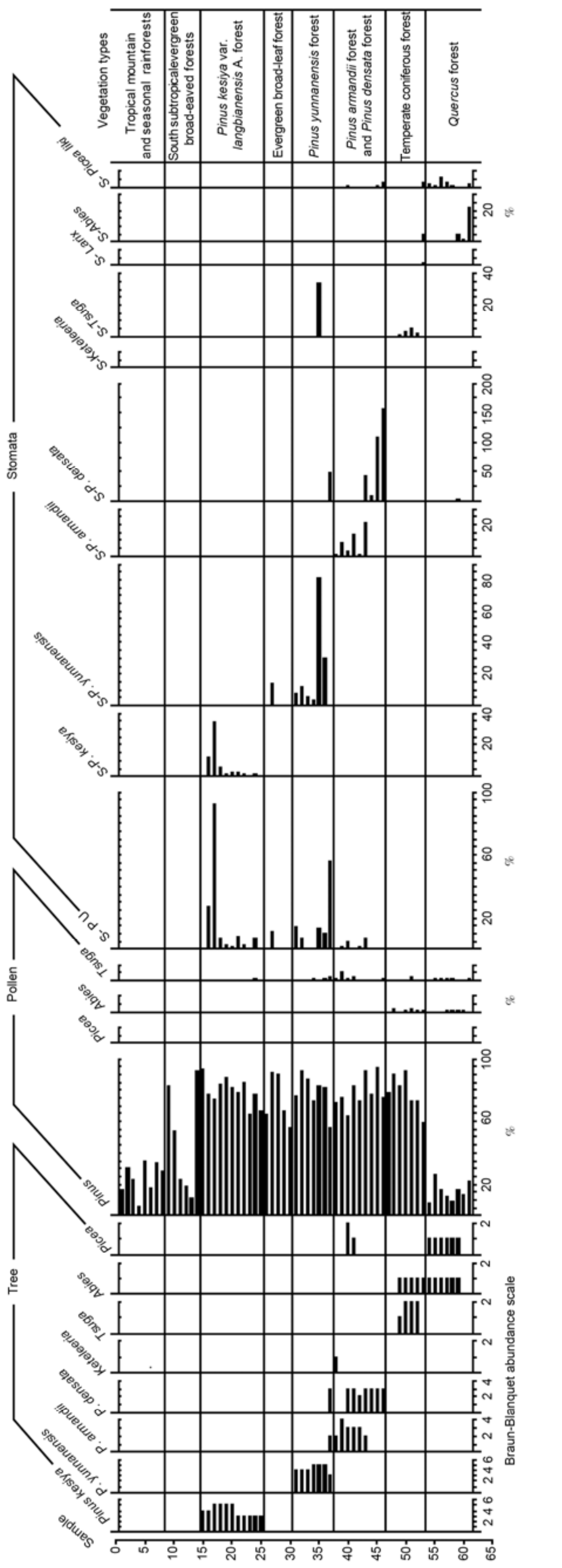

Figure 3 The percentages of surface pollen and stomata according to types of vegetation, pollen and stomata.

Sample 27 contained *Pinus* and *P. yunnanensis* stomata.

(v) *P. yunnanensis* forest. *Pinus* pollen accounted for 55.7%%–91.7% of the total. The maximum content of *Tsuga* is 1.9% and broad-leaved tree pollen is mainly from *Alnus* (1.7%–10.4%), *Lithocarpus/Castanea*, *Quercus* evergreen and *Betula*. Herb pollen is mainly *Artemisia*. *P. yunnanensis* Franch. is found in all samples except one (Sample 37). Stomata are found in all samples except Sample 34. The content of *Tsuga* stomata in Sample 36 is high and *P. densata* stomata is found in Sample 37.

(vi) *P. armandii* and *P. dansata* forest. *Pinus* pollen content is 63.4%–93.7% and *Tsuga* appeared in all samples. Broad-leaved tree pollen types are mainly *Alnus*, *Betula*, *Juglans*, *Quercus* evergreen and *Lithocarpus*/*Castanea*. Shrubs are mainly *Rhododendron*. Herb pollen types are mainly *Gramineae*, *Aster* and *Artemisia*. *P. armandii* stomata are found in samples 38–43 and Coniferous stomata from higher elevation area are found in samples 43–46.

(vii) Temperate broad-leaved mixed conifer forest. *Pinus* pollen is dominant here. *Tsuga* and *Abies* pollen are rare. Broad-leaved trees are mainly *Alnus* and *Lithocarpus*/*Castanea*. Samples 47 and 48 are the secondary shrub, with no stomata found in them. *Tsuga* and *Abies* stomata are found in the samples from sites where *Abies* and *Tsuga* appeared. Sample 53 contained larch, *Tsuga* and *Abies* stomata.

(viii) *Quercus* shrub. *Pinus* pollen content is lower, and samples contained mainly *Quercus* evergreen pollen, and typically lesser amounts of *Alnus*. These samples contain *Picea* and *Abies* stomatas, with *Picea* mainly found in low elevation samples and *Abies* mainly appearing in higher elevation samples.

3.2 DCA analysis

The results of the first and second axis coordinate chart of the DCA analysis are shown in Figures 4 and 5. The first Eigenvalue of 0.428 cumulatively explain 21.2% of the pollen changes. In the sample distribution diagram (Figure 4), tropical vegetation located in the negative terminal of the first axis while cold temperate vegetation distributed in the positive terminal of the first axis. In the species distribution diagram (Figure 5), fern spores and tropical species are distributed in the negative terminal of the first axis, while *Picea*, *Abies* and *Quercus* evergreen are distributed in the positive terminal. The first axis reflect the temperature gradient, and fully distinguish the tropical seasonal forest, montane rain forest and *Q. spinosa* shrubs from other vegetation types, and also distinguish subtropical evergreen broad-leaved forest, evergreen broad-leaved forest and *P. kesiya* forest from other vegetation types. However, it is difficult to draw distinctions among these three classes. *P. yunnanensis* forest are not reliably distinguished from other vegetation types. A portion of these samples overlap with the evergreen broadleaf forest and *P. kesiya* in the lower latitude. A portion of the samples overlap with *P. armandii* forest vegetation

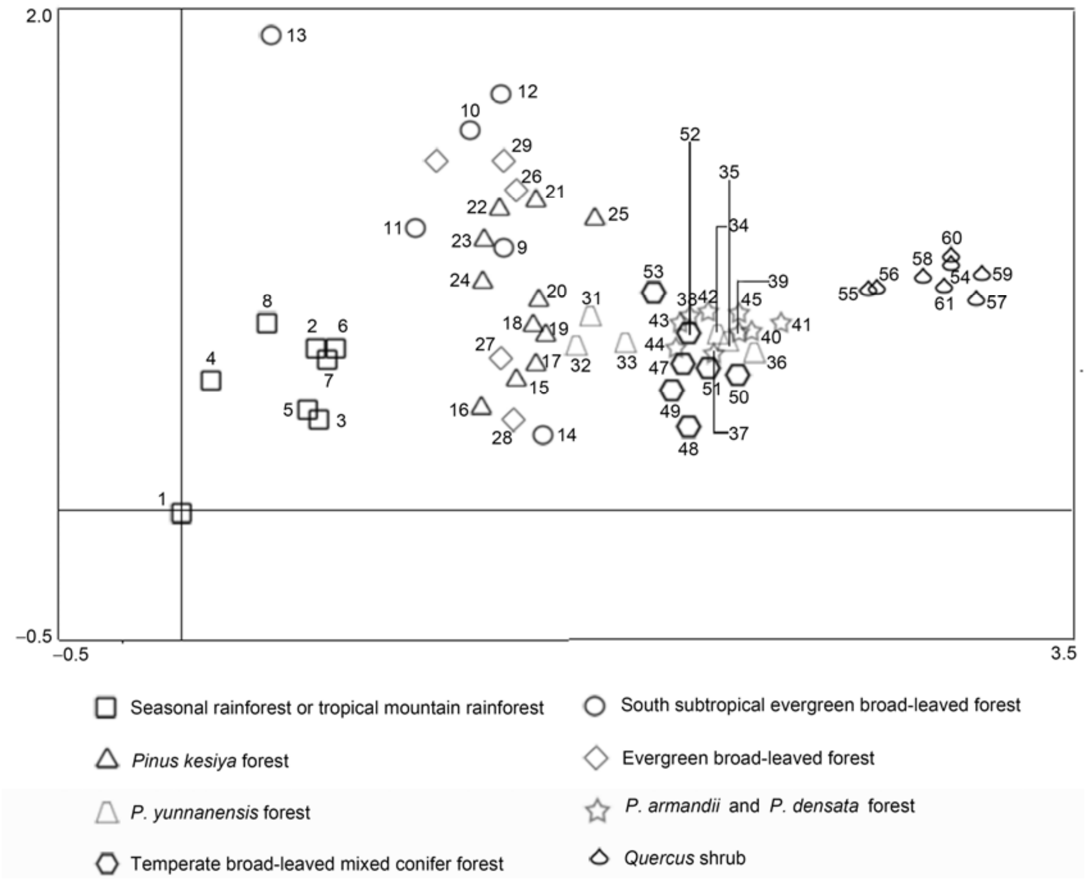

Figure 4 The sample distribution diagram.

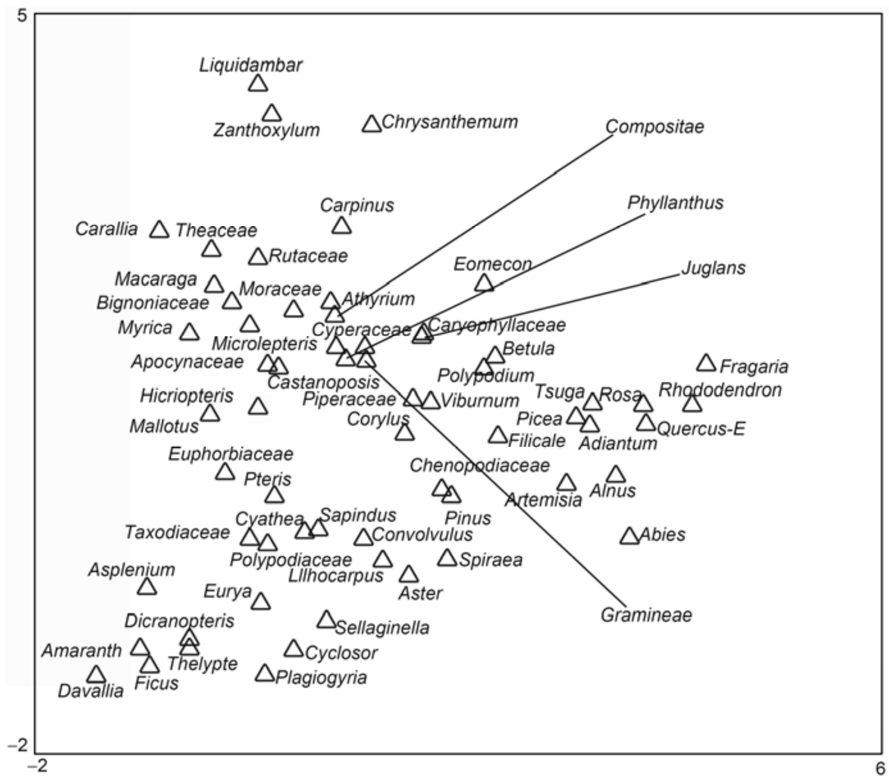

Figure 5 The species distribution diagram.

types in the high latitude or high elevation. *P. armandii* and mountain forest, temperate deciduous conifer mixed forest and *P. yunnanensis* remain difficult to distinguish.

4 Explanation and discussion

Variations in the content of pollen of tropical and subtropical evergreen broadleaf tree are relatively consistent with the vegetation distribution. *Ficus*, *Moraceae*, *Eurya*, *Lithocarpus*, and *Castanopsis* are common plants in tropical and subtropical sites. The relative content of pollen from these species is the highest in the tropical and subtropical soil samples. There is a good corresponding relationship between vegetation and pollen spectrum of tropical seasonal rainforest and mountain rainforest. The high number of species and high content of tropical and subtropical broad-leaved trees and ferns spore pollen reflect the diversity of the tropical vegetation, and also provide the key to distinguishing them from other vegetation types. The subtropical elements found in subtropical evergreen broad-leaved forest, *P. kesiya* forest and subtropical evergreen broad-leaved forest (such as the high content of *Castanopsis*, *Cyclobalanopsis* and *Moraceae* pollen) prove useful in distinguishing them from other types of vegetation. The dominant relative frequency of *Quercus* evergreen compared to *Pinus* pollen in the *Quercus* shrub pollen spectra is the key to distinguishing these communities from other vegetation types.

The results of determination of the pollen frequency spectrum and DCA analysis reveals the difficulty in making precise distinctions between different types of cool temperate vegetation communities based on pollen alone, in subtropical zones through to Yulong Snow Mountain. This uncertainty exists mainly when *Pinus* pollen is dominant and over-represented in the samples. Previous work in Yunnan and its adjacent areas showed that the content of *Pinus* pollen in the northern area of the south subtropical area is very high, while that of constructive species is low, however, we can distinguish different vegetation types based on high content of constructive species [21,22,36–40]. In this paper, all samples were taken from cold temperate deciduous conifer mixed forest. The content of *Picea*, *Abies* and *Tsuga* pollen is lower than 5%, which is less than that of pure forest, therefore, these communities can not be adequately differentiated.

4.1 *Pinus*

The over-representation effect in *Pinus* pollen is a feature of all of the 61 surface soil samples. The content of *Pinus* pollen from subtropical broad-leaved forest to cold-temperate coniferous and broad-leaved mixed forest is over 50%, with the highest reaching 93.7%. The over-representation effect of *Pinus* pollen is the main factor which precludes differentiation between broadleaf and coniferous forest. Based on the observations, we conclude that there are two reasons for the apparent lower content of *Pinus* pollen in secondary *Quercus* shrub: one is the representation of *Quercus*. Previous research found that the representation of *Quercus* pollen in the samples and sites is considerable, at least equivalent to the relative content of the vegetation [41,42]. The other factor is terrain, which can be an obstacle for *Pinus* pollen transport to the locations where mountain oak is a more common component of the forest stand. Collections of recent surface pollen in the Yulong Snow Mountain region show that the content of *Pinus* pollen located above 2000 m is over 60%, and the amount of *Pinus* pollen of surface pollen in modern lakes in Sichuan is more than 50% [22,39]. Topography may be the main factor leading to lower content of *Pinus* pollen in *Quercus* shrub.

Broad-leaved forest can be distinguished from coniferous forest by *Pinus* stomata. A sample of the evergreen broad-leaved forest contains *P. yunnanensis* stomata mainly because they grew at that sampling point. Since *Pinus* stomata can be identified to species, different *Pinus* are readily distinguished. *Sikaya*, *Matsu* and *P. yunnanensis* stomata are only found with *P. kesiya*, *P. yunnanensis* and *P. armandii*, while *P. densata* are distributed in high altitude and latitude. *Pinus* stomata are not found at sites where *Pinus* is absent. However, stomata do not appear in some of the samples which have *Pinus* pollen, such as the samples 15, 23 and 25, which containe *P. kesiya*, but no stomata. Sample 37 has signifcant growth of *P. yunnanensis* and *P. armandii,* but not their stomata, however a large number of *Pinus* stomata which cannot be identified to species are found. Samples 40 and 42 contain *Pinus* stomata, but *Pinus densata* Mast. stomata cannot be found, although it is clear that *Pinus densata* Mast. trees existed at the sites. These cases illustrate that identification of stomata species is the key to distinguishing different kinds of *Pinus*. Sample burial conditions and experimental processes may affect stomatal morphology thereby limiting the precise identification of the stomata.

The relative abundance of *Pinus* stomata is the highest of all conifer species in the study area. The samples with the highest proportion of *Pinus* stomata are found in sample points where the pine trees or pure forest are dominant.

4.2 *Picea*

As a result of the over-representation effect of *Pinus* pollen, the content of Yulong Snow Mountain's *Picea* pollen reached a maximum of 39.1%, although most samples do not exceed 20% [19]. In this study, the proportion of *Picea* forest is low and its pollen content is even lower, not exceeding 1%. *Picea* is found in or adjacent to the areas of samples 40, 53, and 55–58, but the content of their stomata is 0.3%–5.5%, much higher than the corresponding pollen percentage. However, *Picea* is not found adjacent to the locations of samples 45, 46, 54 and 61, and yet *Picea* stomata appeares in the samples. These samples are located at an altitude of over 3300 m where the *Picea-Abies* forest is distributed previously. After human-caused deforestation, secondary *P. densata* and *Q. spinosa* grew instead. *Picea* stomata may come from the surface soil. Another possible explanation for *Picea* and *Abies* stomata being found in Sample 61 assumes that leaves from the nearby *Picea-Abies* forest might have been blown to this sampling location by wind, since *Quercus* forest is located on the ridges and cold *Picea* forest is located on the northern slope.

4.3 *Abies*

Samples containing *Abies* are located in two places: one is the strip with an elevation of about 3100–3300 m in Cangshan, which supports *Abies*, *Tsuga*, *Quercus* evergreen and *Rhododendron* mixed forest. The proportion of coniferous forest is less than 10%. Primitive forest at the locations of samples 47 and 48 had been cut down, and thereafter generated mainly dominant *Spiraea* and *Rubus* shrubs. Another location is a narrow zone at elevation 3300–3600 m in Yulong Snow Mountain, where originally *Picea* and *Picea* forest dominated, but which is replaced by secondary forest cover after cutting, resulting in re-growth dominated by young *Q. spinosa*, *Picea* and *Abies*. *Abies* pollen had low representation, but its proportion in the *Abies* forest is still relatively high [43,44]. *Abies* are the dominant species in cold-temperate coniferous and broad-leaved mixed forest in southwest Sichuan, although its pollen content is not more than 10%, making it a "low" represented species [37,39]. The proportion of *Abies* is low in the sample points and its pollen content is even lower, with highest peak of 2.2%, but most of the samples have less than 1%, and mainly distributed around where the *Abies* grow. However, no *Abies* occurred at some of the sampling points, although *Abies* pollen could be found. *Abies* pollen could not be found in samples 49, 54–56. *Abies* stomatal content varied from 0.1% to 22% and no stomata have been found in samples 49, 51, 54–57. The *Abies* trees are scarce in the vicinity of the samples points and no or few pollen have been found, suggesting that the existence of *Abies* trees cannot be indicated by pollen and stomata in the cases with a very low proportion of *Abies* trees. Samples 59–61 belonged to secondary *Q. spinosa* shrubs. There is no *Abies* growing here, but stomata are present, for example, Sample 61 whose stomata content reached 22%. Possible causes are similar to those of *Picea* stomata.

4.4 *Tsuga*

Tsuga pollen distributed over a very wide range, from subtropical *Pinus kesiya* forest to *Q. spinosa* shrub, however, the content is generally below 1%. In the *Quercus* evergreen-*Tsuga-Abies* forest samples, the highest content of *Tsuga* is 1.7%, with the others making up less than 1%. Among all of the surface soil samples, the content of *Tsuga* in Sample 39 is the highest, reaching 5%, and at the same time, stomatal content is the highest, reaching 33.8%. However, *Tsuga* did not occur near the sampling point. The *Tsuga* pollen and stomata may have come from *Tsuga* forest, which is located in the upper mountain (the highest peak of the mountain has an elevation of 3942 m). The sampling point with significant *Tsuga* growth contains a certain amount of stomata, indicating that the observed presence of *Tsuga* stomatal can indicate the growth of *Tsuga*.

4.5 *Keteleeria* and *Larix*

Keteleeria grow mainly at the location of Sample 37. No *Keteleeria* pollen has been found but *Keteleeria* stomata appear in the sample, suggesting that the stomata may be a better indicator than pollen to identify the parent plants. Sample 53 is a *Rhododendron-Abies* forest. *Larch* is been found within 100 meters around the sampling point, but large *Sequoia* also appear. *Larch* pollen is absent, but *Larch* stomata (1.3%) are observed in the sample. Stomata may be transported by water or wind, although *Larch* trees occupy a small proportion of the forest. *Larch* drops the leaves annually, and therefore the stomatal content of the topsoil tends to be relatively high.

The analysis above shows that conifer stomata is a good indicator of the parent plants in most cases, even if the parent plants are rare in the forest. *Pinus* stomata can be identified to species, and used to reliably distinguish different types of pine forest. Hansen found that conifer stomata could only indicate the existence of the parent plants [14], however, in this paper, higher values of the stomata counts always appeared in the samples with a high value of abundance of the parent plants, demonstrating that high levels of stomata may indicate the abundance of the plants. This analysis of Yunnan surface pollen and stomata shows that evaluating both stomata and pollen can effectively assist in reconstructing the vegetation.

5 Conclusions

The results of sampling modern surface pollen in Yunnan show that tropical rainforest and mountain rainforest, subtropical vegetation and *Q. spinosa* shrub can be distinguished effectively from other vegetation types by pollen. However, the south Asian tropical broad-leaved forest, coniferous and broad-leaved mixed forest and *Pinus kesiya* forest cannot be distinguished from the others. Due to the over-representation effect of the *Pinus* pollen, subtropical broad-leaved forest, *P. yunnanensis* forest and its mixed forests, *P. armandii*, *P. densata* forests and its mixed forests, temperate coniferous and broad-leaf forests and *Abies* forests cannot be distinguished effectively from the others by pollen.

Conifer stomata can effectively add to the value of pollen analysis, to distinguish different types of coniferous forests and its mixed forests. Stomata data indicate the presence of the parent plants, even when *Tsuga*, *Abies* and *Picea* trees occupied such a small proportion of the forests that pollen of surface samples are not sufficient to indicate the existence of these trees.

Vertical vegetation zones existed in the mountainous because the stomata in the high altitude area may be transported to the low altitude areas by wind or water transportation.

The topsoil study in this paper shows that stomata could effectively assist with pollen analysis to reconstruct the vegetation.

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