Plagiochila porelloides (Plagiochilaceae, Hepaticae) from Changbai Mountain, new to China, with chemical characterization and chromosome measurements†

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The hepatic Plagiochila porelloides (Nees) Lindenb. is reported for the first time from China. It was collected on Changbai Mt., Jilin Province, at four localities from 1600–2200 m a.s.l. on boulders, alpine heathland and decaying logs. Chromosome counts shows $n = 9 (8+m)$. It has been characterized chemically using NMR fingerprinting and GC-MS. The lipophilic chemical profile is dominated by $\beta$-barbatene, but 2,3-secoaromadendrane are also present. The Chinese plants therefore belong to a similar chemotype as P. porelloides from Europe. The main 2,3-secoaromadendrane has been isolated, characterized and found to be new; a structure for this compound, plagiochiline V, is proposed on the basis of its NMR parameters.

Key words: chemistry, China, chromosomes, Plagiochila porelloides, taxonomy

INTRODUCTION

During the excursion to Changbai Mountain, Jilin Province, north-east China, in connection with the IAB Conference in Beijing, 1997, a Plagiochila species of the P. asplenoides group was found with perianths and antheridia. This turned out to be P. porelloides (Nees) Lindenb., a species not reported previously from China. In this paper the relation to other regional species, distribution, chemical characterization and chromosomes, are described. Distribution and relationships were studied mainly by Söderström, chemical characterization by Rycroft and Cole, and chromosome measurements by Sha Wei.

Plagiochila porelloides was collected at four different localities.
1. Ca. 5 km N of Tianchi (Sky Lake; 41°30’N 128°10’E), at the road junction, upper oro-
real Betula ermanii forest, alt. ca. 1800 m, on

† Part 6 in the series “NMR Fingerprinting of Liverworts”. For part 5, see Rycroft et al. (1999)
the side of a big boulder. 3. VI. 1997, Söderström no. 97/69
2. Ca. 2 km N of Tianchi (Sky Lake; 41°30'N 128°10'E), alpine heath, alt. ca. 2 200 m, 3. VI. 1997, Söderström no. 97/70, on a N-facing cliff, 3. VI. 1997, Söderström no. 97/80.
3. Yin Huan Hu ("Silver Ring Lake", formerly Xiao Tian Chi "Small Sky Lake"; 41°30'N 128°10'E), upper oreoboreal Betula ermanii forest, alt. ca. 1 700 m, on a boulder, 4. VI. 1997, Söderström no. 97/89.
4. Gu Di Sen Lin (41°30'N 128°10'E), middle oreoboreal Abies nephrolepis-Picea jezoensis forest, alt. ca 1 600 m, along the stream margin, 4. VI. 1997, Söderström & Rycroft LS no. 97/116, ca. fr. and antheridia; Rycroft no. 97059a, c. fr.; 5. VI. 1997, Rycroft no. 97066, with antheridia.

DISTRIBUTION AND RELATION TO OTHER REGIONAL SPECIES

The Plagiochila asplenioides complex includes four taxa in northeastern Asia: P. porrelloides, P. asplenioides (L.) Dum., P. satoi Hatt. and P. ovalifolia Mitt.

Plagiochila porrelloides is a boreal and arctic circumpolar species and is common in Europe, North America and arctic and boreal Siberia. In Asia it has been reported from northern Siberia (Konstantinova & Potemkin 1996), the Khanbarovsk region (Koponen et al. 1978), the Sayan Mts. (Konstantinova & Vasiljev 1994) and the Altai Mountains (Váňa & Ignatov 1995).

Plagiochila asplenioides is also common in northern Europe. Additionally it occurs in Turkey (Göklér & Öztürk 1991) and the Caucasus (Manakyan 1995). It is lacking from eastern North America but occurs in the west from Colorado and Washington to Alaska (Schuster 1980) and is found also in the nearby Chukotka Peninsula (Abramova et al. 1987). In China it has been reported from Hebei and Zhejiang provinces (Pippo 1990). However, all specimens from China should be re-examined to determine whether they are true P. asplenioides or if they belong to P. porrelloides or any other related taxon.

Plagiochila satoi has a circumpacific distribution. In North America it occurs from Oregon to Alaska (Hong 1987). It is known in Asia from the Russian Far East (Konstantinova et al. 1992), Japan (H. Inoue 1958), Korea (Yamada & Choe 1997) and in China from Heilongjiang, Jilin and Hebei provinces (Pippo 1990).

Plagiochila ovalifolia is an Asiatic endemic known from China (Tibet and Fujian provinces; Pippo 1990), Taiwan (Pippo 1990), Japan (Mizutani 1984) and Korea (Yamada & Choe 1997).

Plagiochila porrelloides is closely related to P. asplenioides and often regarded as a subspecies of it (e.g. Schuster 1980). It differs in the smaller size (3–6 vs. 6–10 cm long and 3–6 vs. 6–8 mm wide) and cells (median cells 25–32 μm vs. 30–36 μm). Plagiochila satoi has even smaller cells (21–30 × 25–34 μm) and more dentate leaves (P. porrelloides is sometimes nearly edentate, as in the specimens from Changbai Mt.). P. ovalifolia has larger cells and the perianth mouth is oblique versus straight in P. porrelloides.

The following key is provided to distinguish the regional taxa.

1. Plants with large cells (median cells 32–36 × 35–40 μm, marginal cells 25–32 μm wide) ........................................ 2
2. Plants with smaller cells (median cells 21–32 μm or smaller, marginal cells 17–25 μm wide) ............................. 3
3. Plants large (6–10 cm long and 3–6 mm wide). Perianth mouth straight. Leaves edentate to dentate with small teeth ........................................  P. asplenioides
4. Plants smaller (to 6 cm long and 4.5 mm wide). Perianth mouth oblique, dentate. Leaves coarsely dentate .............................  P. ovalifolia
5. Median cells 21–25 × 22–26 μm, marginal cells 10–20 μm wide, leaves coarsely dentate (very rarely with reduced teeth) ........................ P. satoi

CHEMICAL CHARACTERIZATION

Members of the liverwort genus Plagiochila have been classified into eight chemotypes (Asakawa 1995); a ninth chemotype, to which P. spinulosa (Dicks.) Dum., P. punctata Tayl., P. killarniensis and some Neotropical Plagiochilae belong, is characterized by the presence of 9,10-
dihydrophananthrenes (Anton et al. 1997, Rycroft 1998a, Rycroft et al. 1999). The largest chemotype group is characterized by the presence of 2,3-secoaromadendranes; plagiochilines A–S are among the known members of this class of compound (Asakawa 1995, Valcic et al. 1997). Plagiochila porelloides from Europe belongs to this chemotype as previous studies have reported the presence of plagiochilines A, C, D and H (Asakawa et al. 1980) as well as 2,3-secoaromadendrane esters of fatty acids (Toyota et al. 1994).

Two specimens, one female and one male, of Plagiochila porelloides from China have been studied using the complementary techniques of NMR fingerprinting (Rycroft 1996, 1998) and GC-MS. GC, GC-MS and $^1$H NMR (360 or 400 MHz, CDCl$_3$) were performed as described previously (Rycroft et al. 1998b). Extracts were prepared by triturating dried plant material with sufficient CDCl$_3$ to produce 0.6–0.7 mL of a filtered solution.

The results (Table 1) show that the lipophilic chemical profiles are dominated by β-barbatene (peak 4, concentration ca. 7 mM in extract 96059a from comparison with the intensity of the 0.2% residual CHCl$_3$ signal in the $^1$H NMR spectrum). β-barbatene (Fig. 1) is a common liverwort constituent (Toyota et al. 1996) along with bicyclogermacrene, octenyl acetate, α- and β-chamigrene. Several plagiochiline-type compounds are also present (the proton NMR signals of H-2 and H-3 of many 2,3-secoaromadendranes are readily visible in a characteristic region of the spectrum). The only positive iden-

![Fig. 1. Chemical structures.](image-url)
Table 1. GC-MS analysis of CDCl₃ extracts of two specimens of *Plagiochila porelloides* from China.

<table>
<thead>
<tr>
<th>Peak No.</th>
<th>Assignment</th>
<th>Rᵣ</th>
<th>M⁺</th>
<th>Base peak</th>
<th>Relative abundance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>octenyl acetate</td>
<td>1091</td>
<td>[128] (9)</td>
<td>48</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>(α-barbatene)</td>
<td>1387</td>
<td>204 (22)</td>
<td>93</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>1406</td>
<td>[179] (11)</td>
<td>135</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>β-barbatene</td>
<td>1416</td>
<td>204 (4)</td>
<td>93</td>
<td>100</td>
</tr>
<tr>
<td>5</td>
<td>β-chamigrene</td>
<td>1453</td>
<td>204 (38)</td>
<td>189</td>
<td>2</td>
</tr>
<tr>
<td>6</td>
<td>bicyclogermacrene</td>
<td>1475</td>
<td>204 (21)</td>
<td>121</td>
<td>9</td>
</tr>
<tr>
<td>7</td>
<td>α-chamigrene</td>
<td>1480</td>
<td>204 (21)</td>
<td>136</td>
<td>1</td>
</tr>
<tr>
<td>8</td>
<td></td>
<td>1507</td>
<td>204 (16)</td>
<td>69</td>
<td>3</td>
</tr>
<tr>
<td>9</td>
<td></td>
<td>1541</td>
<td>[205] (51)</td>
<td>43</td>
<td>5</td>
</tr>
<tr>
<td>10</td>
<td></td>
<td>1782</td>
<td>234 (1)</td>
<td>95</td>
<td>2</td>
</tr>
<tr>
<td>11</td>
<td></td>
<td>1837</td>
<td>270 (1)</td>
<td>68</td>
<td>4</td>
</tr>
<tr>
<td>12</td>
<td></td>
<td>1879</td>
<td>278 (16)</td>
<td>43</td>
<td>2</td>
</tr>
<tr>
<td>13</td>
<td></td>
<td>1913</td>
<td>260 (4)</td>
<td>79</td>
<td>2</td>
</tr>
<tr>
<td>14</td>
<td>fusicoecadiene</td>
<td>1962</td>
<td>272 (21)</td>
<td>135</td>
<td>3</td>
</tr>
<tr>
<td>15</td>
<td>plagiochiline C</td>
<td>2085</td>
<td>[274] (1)</td>
<td>43</td>
<td>2</td>
</tr>
<tr>
<td>16</td>
<td>plagiochiline V</td>
<td>2183</td>
<td>[292] (2)</td>
<td>43</td>
<td>2</td>
</tr>
<tr>
<td>17</td>
<td></td>
<td>2196</td>
<td>304 (4)</td>
<td>43</td>
<td>2</td>
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<tr>
<td>18</td>
<td></td>
<td>2380</td>
<td>[336] (19)</td>
<td>43</td>
<td>1</td>
</tr>
<tr>
<td>19</td>
<td>plagiochiline-type</td>
<td>2557</td>
<td>[335] (1)</td>
<td>43</td>
<td>1</td>
</tr>
</tbody>
</table>

1) Collection *Rycraft 97059a*, at the stream crossing the path to the Underground Forest (Gu di sen lin), on a rotten log in the middle of the stream, 4.IV.1997, with sporophytes, 277 mg extracted 5.V.1998.


3) All components with an abundance in either extract greater than 1% of the most abundant component (β-barbatene) are included.

4) Based on comparison with authentic materials or literature retention indices and MS (Adams, 1995); assignments in parentheses are tentative.

5) Figures in square brackets indicate m/z of the heaviest ion observed rather than M⁺ (figures in parentheses show the % abundance relative to the base peak).

6) From GC-MS TIC integration (these figures are affected by different detection sensitivities and, in the absence of standards, do not quantitatively reflect the amounts present); + indicates that the compound was detectable in low amount.

been observed previously in plagiochilines, but can arise from cyclization of a β-hydroxyl substituent at C-8 (as occurs in plagiochilines O and P; Valcic *et al.* 1997), to an aldehyde function at C-14 (as occurs in other 2,3-secoaromadendranes; Asakawa 1995).

Although the new 2,3-secoaromadendrane, plagiochiline V, has been isolated and characterized, further work is in progress to obtain supporting evidence for the proposed structure and, in particular, to confirm the relative stereochemistry. Full details will be reported elsewhere.

CHROMOSOME MEASUREMENTS

For the chromosome number and karyological observations, stem tips of the male gametophytes were pre-treated in 1:1 mixture of saturated of p-dichlorobenzene and 1 % α-brohrnonaphtalene for 3-6 hours at 10⁰C before they were fixed in a 3:1 mixture of absolute alcohol and glacial acetic acid. They were then hydrolysed in a 1:1 mixture of hydrochloric acid and 95% alcohol for 15-20 minutes. After being washed with water, they were stained with car-
bol fuchsin before slide preparations were made. Many cells with well-dispersed chromosomes were observed, from which 5 cells were chosen for karyological analysis.

The chromosome number of the male gametophytes was nine. This number agrees with other species of the genus and the number given by other authors for *Plagiochila pellelloides* (H. Inoue 1967, 1968, 1975, 1977, S. Inoue et al. 1985, Segawa 1965a, b).

The karyotype of *Plagiochila pellelloides* is k(n) = 9 = 5V+3I+v. At the metaphase each chromosome in the complement was distinguishable in size and shape (Fig. 3d, f). Measurement of the length of the chromosomes (Table 2) shows that at the metaphase the constriction in chromosomes 3, 4, 7, 8 and 9 is submedian and in 2, 5 and 6 is subterminal. At the prometaphase the chromosomes are longer than

**Fig. 2.** NMR data of plagiochiline V.

**Fig. 3.** Chromosomes of *Plagiochila pellelloides*. - 1, 2: Resting nuclei. - 3, 4: Prometaphase. - 5, 6: Metaphase (x 2300).
Table 2. Measurements of somatic chromosomes of *Plagiochila pelloroides* from Changbai Mountain, Jilin Province, China.

<table>
<thead>
<tr>
<th>Chromosome No.</th>
<th>Length in µm</th>
<th>Relative length</th>
<th>Arm ratio</th>
<th>F%</th>
<th>Type</th>
<th>Length in µm</th>
<th>Relative length</th>
<th>Arm ratio</th>
<th>F%</th>
<th>Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.02±5.04=10.06</td>
<td>25.14</td>
<td>1.00</td>
<td>49.90</td>
<td>V</td>
<td>1.99±1.99=3.98</td>
<td>13.57</td>
<td>1.00</td>
<td>50.00</td>
<td>V</td>
</tr>
<tr>
<td>2</td>
<td>1.67±2.96=4.63</td>
<td>11.57</td>
<td>1.77</td>
<td>36.07</td>
<td>J</td>
<td>1.21±2.74=3.95</td>
<td>13.47</td>
<td>2.26</td>
<td>30.63</td>
<td>J</td>
</tr>
<tr>
<td>3</td>
<td>1.77±2.76=4.53</td>
<td>11.32</td>
<td>1.56</td>
<td>39.07</td>
<td>V</td>
<td>1.81±1.89=3.70</td>
<td>12.62</td>
<td>1.04</td>
<td>48.92</td>
<td>V</td>
</tr>
<tr>
<td>4</td>
<td>1.99±2.39=4.38</td>
<td>10.95</td>
<td>1.20</td>
<td>45.43</td>
<td>V</td>
<td>1.75±1.93=3.68</td>
<td>12.55</td>
<td>1.10</td>
<td>47.55</td>
<td>V</td>
</tr>
<tr>
<td>5</td>
<td>1.47±2.85=4.32</td>
<td>10.80</td>
<td>1.94</td>
<td>34.03</td>
<td>J</td>
<td>1.29±2.30=3.59</td>
<td>12.24</td>
<td>1.78</td>
<td>35.93</td>
<td>J</td>
</tr>
<tr>
<td>6</td>
<td>1.30±2.48=3.78</td>
<td>9.45</td>
<td>1.91</td>
<td>34.39</td>
<td>J</td>
<td>1.16±2.16=3.32</td>
<td>11.32</td>
<td>1.86</td>
<td>34.94</td>
<td>J</td>
</tr>
<tr>
<td>7</td>
<td>1.53±1.93=3.46</td>
<td>8.65</td>
<td>1.26</td>
<td>44.22</td>
<td>V</td>
<td>1.25±1.71=2.96</td>
<td>10.10</td>
<td>1.37</td>
<td>42.23</td>
<td>V</td>
</tr>
<tr>
<td>8</td>
<td>1.54±1.86=3.40</td>
<td>8.50</td>
<td>1.21</td>
<td>45.29</td>
<td>V</td>
<td>1.25±1.69=2.94</td>
<td>10.03</td>
<td>1.35</td>
<td>42.52</td>
<td>V</td>
</tr>
<tr>
<td>9</td>
<td>0.69±0.76=1.45</td>
<td>3.62</td>
<td>1.10</td>
<td>47.59</td>
<td>v</td>
<td>0.56±0.64=1.20</td>
<td>4.09</td>
<td>1.14</td>
<td>46.67</td>
<td>v</td>
</tr>
</tbody>
</table>

those at the metaphase, and the deeply stained regions (heterochromatic segments) could be distinguished from the lightly stained regions (euchromatic segments; Fig. 3c, e, Table 2). The longest chromosome is the sex chromosome (Y chromosome) with a large constriction in the heterochromatic segment in both arms. In the resting stage of the nuclei of the male gametophytes, two large and some small bodies were observed (Fig. 3a, b), with the largest one the Y chromosome.

CONCLUSION

The identification of *Plagiochila pelloroides* from China is perhaps not surprising when viewed in the context of the known flora of the Changbai Mountain ecosystem. Nevertheless the result is an extension of the boundaries of the known range of the species. The chromosome measurements confirm that we are dealing with a normal haploid species rather than a putative diploid derivative such as *P. britannica* Paton. Although we have not yet completed chemical investigations of European *P. pelloroides* using the same methodology as employed here, it is clear that European and Chinese *P. pelloroides* are closely related chemically.

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REFERENCES


Stream.
Rycroft, D. S. 1996: Fingerprinting of plant extracts using NMR spectroscopy: application to small samples of liverworts. - Chemical Communications: 2187-2188.
Toyota, M., Koyama, H., Mizutani, M. & Asakawa, Y. 1996: (-)-Ent-Spathulenol isolated from liverworts is an artifact. - Phytochemistry 41: 1347-1350.