Syo KUROKAWA* and Heinai SHIBUICHI**: Notes on Japanese species of Pilophoron

黒川 道* • 四分一平内**：日本産カムリゴケ属について

(Pl. III)

Pilophoron, a genus of the Stereocaulaceae, comprises about 10 species, which are mostly distributed in alpine or subalpine regions in the world. In 1941, when Sato summarized Japanese Pilophoron, he reported the occurrence of three species of the genus in Japan; P. aciculare (Ach.) Nyl., P. japonicum Zahlbr. (=P. hallii (Tuck.) Vain.), and P. nigricaule Sato. In 1940, he published another paper on the genus, in which he proposed a new infrageneric classification for the genus, establishing the section Nigricaulia.

We have studied chemical substances of Japanese species of Pilophoron both with the microchemical and thin layer chromatographic methods. The results of chemical testings will be reported in this paper. A new species, P. curtulum, will be described, with special reference to infrageneric classification of the genus. Specimens cited in this paper are preserved at TNS, unless otherwise indicated. Our sincere thanks are expressed to Dr. H. Krog, University of Oslo, for the loan of valuable specimens of P. cereolus (Ach.) Nyl.

Methods of chemical testing Specimens of Pilophoron preserved at TNS were tested both with the microchemical and thin layer chromatographic methods. Atranorin, stictic acid, caperatic acid, and zeorin were crystallized on slide glasses following the ordinary microcrystal methods, but these substances, especially zeorin, were not always demonstrated in some specimens, probably because of the low concentration. Thin layer chromatographic tests were also made for these substances, for they seem to be more sensitive to various substances. The acetone extract was spotted on Merck's silica gel coated slide glasses (5×10 cm). The chromatograms were developed

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with a mixture of \( n \)-hexane, ethyl ether, and formic acid (5:4:0.5) for about 6 minutes. After evaporation of the solvent, the chromatograms were sprayed with 10% sulphuric acid and heated over an alcohol flame. Substances detected with this method are shown on the Table 1.

Stictic acid, constictic acid, caperatic acid, zeorin, and atranorin were identified by comparison with acetone extracts of other lichens which contain these substances. An orange yellow spot at Rf 0.08 and a dark grey spot at Rf 0.10 are identified with stictic and constictic acids respectively, because these spots coincide with spots of the substances contained in _Menegazzia pertusa_ (Shrank) Stein., _Stereocaulon japonicum_ Th. Fr., and _Parmelia crinita_ Ach. A grey spot at Rf 0.24 agrees with that of caperatic acid demonstrated on chromatograms of acetone extracts of _Parmelia caperata_ (L.) Ach., _P. koyaensis_ Asah., and _Platismatia interrupta_ Culb. et Culb. Pure samples of zeorin and atranorin appear as a pinkish violet to purple spot at Rf 0.34 and an orange yellow spot at Rf 0.69 respectively. The other six spots have not yet been positively identified with any known substances; pinkish violet to purple spots at Rf 0.30, Rf 0.32 and Rf. 0.36, pinkish violet spots at Rf. 0.69 and 0.72 and pale grey spot at Rf. 0.85. These substances will be called Pil-1 (Rf 0.30), Pil-2 (Rf 0.32), Pil-3 (Rf 0.36), Pil-4 (Rf 0.69), Pil-5 (Rf 0.72) and Pil-6 (Rf 0.85) in this paper. Judging from color of spots, they appear

### Table 1. Chemical ingredients of Japanese _Pilophoron_ demonstrated with thin layer chromatography.

<table>
<thead>
<tr>
<th>chemical substance</th>
<th>Rf value</th>
<th>color on chromatograms</th>
</tr>
</thead>
<tbody>
<tr>
<td>stictic acid</td>
<td>0.08</td>
<td>orange yellow</td>
</tr>
<tr>
<td>constictic acid</td>
<td>0.10</td>
<td>dark grey</td>
</tr>
<tr>
<td>caperatic acid</td>
<td>0.24</td>
<td>grey</td>
</tr>
<tr>
<td>Pil-1</td>
<td>0.30</td>
<td>pinkish violet to purple</td>
</tr>
<tr>
<td>Pil-2</td>
<td>0.32</td>
<td>pinkish violet to purple</td>
</tr>
<tr>
<td>zeorin</td>
<td>0.34</td>
<td>pinkish violet to purple</td>
</tr>
<tr>
<td>Pil-3</td>
<td>0.36</td>
<td>pinkish violet to purple</td>
</tr>
<tr>
<td>atranorin</td>
<td>0.69</td>
<td>orange yellow</td>
</tr>
<tr>
<td>Pil-4</td>
<td>0.69</td>
<td>pinkish violet</td>
</tr>
<tr>
<td>Pil-5</td>
<td>0.72</td>
<td>pinkish violet</td>
</tr>
<tr>
<td>Pil-6</td>
<td>0.85</td>
<td>pale grey</td>
</tr>
</tbody>
</table>
to belong to triterpenes. Pil-2 and Pil-3 give the same Rf values and color as those of pure samples of N-3 (6α, 16β-di-O-acetyl-leucotyline) and N-2 (6-deoxy-16β-O-acetyl-leucotyline), when chromatograms are developed with a mixture of n-hexane, ethyl ether, and formic acid, which has been used in this study. However, these spots cannot be identified with N-3 or N-2, because no spot agrees with those of N-3 and N-2 on chromatograms developed with mixtures of benzene, dioxane, and acetic acid (25:10:1) or benzene, ethyl ether, and formic acid (50:20:1). The table 2 shows substances demonstrated in each species of Pilophoron with the chromatographic methods.

**Infrageneric classification**  Sato (1940) divided Pilophoron into two sections, section Eupilophoron and section Nigricaulia. According to him, section Eupilophoron is characterized by colorless hyaline axis, whereas section Nigricaulia by black carbonized axis. As pointed out by Sato, *P. nigricaule*, the type species of section Nigricaulia, has black carbonized axis, which is connected with black carbonized excipulum at the uppermost portion. In contrast, the axis is colorless and hyaline in most specimens of both *P. aciculare* and *P. hallii*, which were considered as members of section

<table>
<thead>
<tr>
<th>species</th>
<th><em>P. aciculare</em> (Japan)</th>
<th><em>P. aciculare</em> (North America)</th>
<th><em>P. curtulum</em></th>
<th><em>P. hallii</em></th>
<th><em>P. nigricaule</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>stictic acid</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>constictic acid</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>caperatic acid</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Pil-1</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Pil-2</td>
<td>trace or -</td>
<td>-</td>
<td>+ or -</td>
<td>trace or -</td>
<td></td>
</tr>
<tr>
<td>zeorin</td>
<td>+</td>
<td>+</td>
<td>+ or -</td>
<td>+</td>
<td>+ or trace</td>
</tr>
<tr>
<td>Pil-3</td>
<td>-</td>
<td>-</td>
<td>trace or -</td>
<td>+</td>
<td>trace or -</td>
</tr>
<tr>
<td>atranorin</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Pil-4</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Pil-5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Pil-6</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>
Eupilophoron by Sato. The excipulum, however, is dark brown or black in all specimens of these two species. In one specimen of *P. hallii* collected at open alpine area on Mt. Eboshi, central Japan, in addition, the axis is apparently dark brown and is connected with dark brown excipulum, just as in the case of *P. nigricaule*. In *P. curtulum*, a new species, the axis is usually dark brown or blackish brown, especially when the axis is exposed. However, it is colorless and hyaline in some specimens of *P. curtulum*. Therefore, the color of axis seems to vary with environmental factors or exposure to sun-light. As a result, blackened axis appears to have no consistent value as an infrageneric character. Thus we do not feel it is necessary to devide the genus *Pilophoron* into sections, especially based on the color of axes.

**Pilophoron** (Tuck.) Th. Fr., Stereoc. et Pilophor. Comment. 40. 1857.  

Type species: *Pilophoron aciculare* (Ach.) Nyl.  

Type species: *Pilophoron nigricaule* Sato.

**Key to the species of Japanese Pilophoron**

1. Fertile podetia more than 2 cm high; sterile ones sometimes less than 2 cm high and often sorediate; axis fistular at least near the base. .................
   1. *P. aciculare* (Ach.) Nyl.
2. Thallus thick (0.8-1.5 mm), irregularly cracked and distinctly areolate; surface of podetia also cracked and areolate; podetia P+orange red, containing stictic acid. .................. (4) *P. nigricaule* Sato
2. Thallus thin (less than 1 mm), scabrous or granular; surface of podetia smooth but sometimes decorticate in part; podetia P-, not containing stictic acid.
3. Apothecia ellipsoidal; caperatic acid present  ................... (3) *P. hallii* (Tuck.) Vain.
3. Apothecia globose; caperatic acid absent  ...................... (2) *P. curtulum* Kurok. et Shibuichi

This species is characterized by having very tall podetia and fistular axis. While fertile podetia are tall (usually more than 2 cm high) and esorediate, sterile ones are often sorediate and much shorter (less than 2 cm high). Specimens with sterile sorediate podetia may be confused with *P. cereolus*. However, podetia of *P. cereolus* are more densely sorediate and have almost solid axis.

In *P. aciculare*, cephalodia are scattered on the thallus as well as on podetia and are more or less densely distributed around the base of podetia. They are dark brown and 1.5–2 mm in diameter. Cortex of the cephalodia is more or less distinct and 15–25 μ thick. Blue green algae in the cephalodia belong to *Stigonema*.

Atranorin and zeorin were demonstrated in all specimens examined with thin layer chromatography. Pil-2, however, was demonstrated in small amount in only four specimens of 21 collections. It seems, therefore, to be an accessory component in this species. Although North American and Japanese specimens are identical in morphology, Pil-1 has never been demonstrated in Japanese specimens, whereas it seems to be a constant component in North American ones (Table 2).

**Pilophoron aciculare** is apparently related to *P. cereolus*, because they both have similar tall podetia and produce similar substances. However, the axis is fistular in *P. aciculare*, while it is composed of loosely interwoven hyphae even in the center in *P. cereolus*. Podetia of *P. cereolus*, in addition, are densely sorediate even when fertile, whereas only sterile podetia are often sorediate in *P. aciculare*. Chemical products of *P. cereolus* are identical with those of Japanese specimens of *P. aciculare*. However, Pil-2, an accessory component in *P. aciculare*, has never been demonstrated in *P. cereolus*.

**Pilophoron aciculare** is distributed mainly in subalpine areas in Japan, growing on non-calcareous rocks. It has been also reported from North America, southern Africa, and Australia.


(2) Pilophoron curtulum Kurok. et Shibuichi, sp. nov.

Thallus primarius crustaceus, substrato arcte adhaerens, glaucus, minutissime rimosus granulatusque, sorediis et isidiis destitutus, cephalodiis fusco-brunnescentibus et ex granulis minutissimis formatis praeditus. Podetia plus minusve dispersa, cylindrica, erecta vel suberecta, simplicia, curtula, 3-5 mm alta, 0.5-1.0 mm crassa; superficies podetii corticata sed raro decorticata, plus minusve granulata; axis fusco-brunnescens sed raro decoloratus, ex hyphis longitudinalibus conglutinatibusque formatus, sed in centro axis hyphis plus minusve laxe intricatis. Apothecia terminalia, simplicia, plerumque globosa, nigra, 0.8-1.2 mm diametro; epithecium indico coloratum; hymenium hyalinum, ca. 100 µ altum, J+coerulescens; hypothecium una cum excipulo fuscescens; asci elongato-clavati; sporeae 8-nae, simplices decolorataeque, fuciformes vel ellipsoideae, 5-7 x 20-25 µ.

Reactions: thallus and podetia K+pale yellow, C−, P−.

Chemical ingredients: atranorin as a constant component and zeorin and Pil-3 present or absent.

Type collection: Mt. Ontake, Prov. Hida, Japan. On rocks, elevation 1900-2800 m. S. Kurokawa 64180—holotype in TNS and isotypes in FH and O.

This new species has been long confused with P. hallii by Japanese lichenologists, because they both occur mainly in subalpine areas in Japan and have similar thalli and podetia. However, it is clearly distinguished from P. hallii by having more rounded apothecia and by lacking caperatic acid. The axis of this species is more often dark brown than in P. hallii. Thus, it also may be confused with P. nigricaule, from which it is easily separated by the thinner thalli and a negative reaction with P. While atranorin is a constant component in this species, zeorin seems to be an accessory, being demonstrated in only three of 15 specimens. This species is rather common
in subalpine areas in Japan, often growing together with _P. hallii_.


(3) **Pilophoron hallii** (Tuck.) Vain., Bot. Mag. Tokyo 35; 59. 1921.


Atranorin and zeorin were demonstrated in all specimens of _P. hallii_ with both microchemical and thin layer chromatographic methods. The presence of caperatic acid, in addition, was proved in all specimens of this species with thin layer chromatographic methods, though the acid was demonstrated in most specimens with crystal methods with some difficulty. This is the first record of the occurrence of a fatty acid in the genus _Pilophoron._ Some other substances were also demonstrated in this species with the chromatographic methods. Pil-3, Pil-4, and Pil-5 were demonstrated in all specimens, but Pil-2 in 44 of 58 specimens.

This species was known as _P. japonicum_ Zahlbr. in Japan. Recently, however, Sato (1962) pointed out that the name _P. hallii_ should be used for this species. As mentioned above, _P. hallii_ is related to _P. curtulum_ and the difference between these two species was discussed under _P. curtulum._

_Pilophoron hallii_ is widely distributed in temperate and subalpine areas in Japan. It also has been reported from Korea and North America. In addition, two specimens collected in Taiwan are identical with this species. Thus, _P. hallii_ shows a disjunctive distribution between eastern Asia and North America.


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This species resembles P. curtulum, because they both have rather short podetia, globose apothecia, and dark brown axis. However, it is easily separated from P. curtulum by thicker thalli and orange color reaction with P. While the cortex of cephalodia is thin (15-20 μ) and indistinct in P. curtulum, it is rather thick (20-25 μ) and distinct in P. nigricaule.

Even though Sato reported negative reaction with P, the thallus and podetia are apparently P+orange red in the holotype of P. nigricaule, containing stictic acid. Krog (1968) recently reported the presence of atranorin, stictic acid and zeorin in an Alaskan specimen of this species. The results—16—
of thin layer chromatographic tests show the presence of constictic acid and Pil-6 in this species along with atranorin, stictic acid, and zeorin. In addition, Pil-2 was demonstrated in seven of 17 specimens and Pil-3 in ten of 17 specimens.

Pilophoron nigricaule is distributed in open alpine areas in central and northern Japan. Although it had been long considered to be endemic to Japan, Krog (1968) reported the occurrence of this species in Alaska. Thus, the range now includes Alaska and Japan, even though the species has been reported neither from Kuriles nor from Hokkaido.


Literature cited


Explanation of Plate III

Fig. 1. Holotype of Pilophoron nigricaule Sato (×5). Fig. 2. Part of holotype of Pilophoron curtulum Kurok. et Shibuichi (×6). Fig. 3. The same (×12).

日本のカムリゴケ属については佐藤正己博士 (1940, 1941, 1962) がすでに論説を発表しておられ、3種が知られている。すなわち、カムリゴケ (P. hallii), オオカムリゴケ (P. aciculare), マルミカムリゴケ (P. nigricaule) である。カムリゴケに似ているが、成分が異なり、子柄の軸がしばしば暗褐色をおびるものを新種として加え、これをカムリゴケモドキ（新称）(P. curtulum) とした。これら4種について、結晶
外来植物の人为的散布の一例（浅井康宏）Yasuhiro Asai: On the Solidago canadensis group widely spread by bee-keepers in Japan.

近年、我国各地から外来のアキノキリンソウ属 Solidago の爆発的な繁殖、広分布が報ぜられている。その正確な種名の検定については、今後全国的な規模で種々の観点から検討を要するが、いずれにしてもこの大形の Solidago の仲間が第 2 次大戦後にどのようにして急激に拡がり、特に市街地周辺の荒廃地やそれに近接した丘陵地带に生育して、大群を形成するに至ったかについては、外来植物調査上、誠に興味ある事実と云わねばならない。勿論我国に現在渡来している Solidago は、その他の外来植物に比較して草丈も高く、繁殖力も大きく、しかも開花期には米名 Goldenrod の名にそくかね鮮黄色の花穗を一面に波うたせ、群生地の景観を一変させる極めて目立った存在であることも、この仲間が一般的に注目される所以であろう。

従来本種の分布原因としては、その観賞価値に伴う人為的な移植及び地下茎による旺盛な繁殖力などが挙げられていたが、しかしそれも自らの限度があり、その新地域（特に都市周辺の原野、隠蔽、荒廃地など）への、この大形植物の移動、分布要因について、筆者は少なからず疑問と興味を抱いていた次第である。ところが昨年、はからずもその一因とも云える事実を知り得たので、ここに記録し同僚諸氏の御参考に供し度いと思う。ご承知のように養蜂家にとっては、いわゆる蜜源植物は蜜蜂の生産に随伴して欠くことの出来ない存在である。しかしながら近年、我国の自然の破壊及び都市周辺の農耕地の宅地化は、実にめざましいものがあり、これに伴ないいわゆる蜜源植物が年々極度に減少の一途を辿りつつあることは周知の事実である。そこで、これに対応するため、養蜂家は蜜源植物の増殖と獲得に取り出し、種々努力を続けているが、この一環として現在各地で話題となりつつある外来の Solidago がクローズアップされて来たものである。

蜜源植物となるためには種々の条件（増殖率や適応性が大きく、多数のものが一帯所にまとまって生育し、一時に多量の花蜜を供給し得ること、及び開花が長期に亘ることなど）が必要であるが、この中でも晚秋まで開花し、しかも前述の諸条件をも巧成り充分なものとして本種が目指された次第である。その結果、日本養蜂協会の指導者がこれを築上げ、これを各地へ増殖するよう配慮したものである。これに呼応して関係者（全国で約 1 万人の協会員のうち、数千人がこれに関与したと云う）は、極めて
KUROKAWA & SHIBUICHI: Japanese species of Pilophoron.