A Biosystematic Study of *Nipponolejeunea* HATT. (Hepaticae)

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The genus *Nipponolejeunea* HATTORI was established by HATTORI (1944) based on *Pycnolejeunea pilifera* STEPH. from Japan. Its primary important generic characters include 1) the two gynoecial innovations, 2) the triplicate perianth, and 3) the long cilia on leaf- and underleaf-margin. HATTORI (1944) also assigned *Pycnolejeunea subalpina* HORIK. to *Nipponolejeunea*, thus admitting two Japanese species in that genus. Since then, the genus *Nipponolejeunea* has been repeatedly discussed, especially by MIZUTANI (1961) and SCHUSTER (1963) from taxonomic points of view. The branching and innovation types of *Nipponolejeunea* were described by MIZUTANI (1970); INOUE (1976) proposed the subgeneric separation of the two species, as subgen. *Nipponolejeunea* (with *N. pilifera*) and subgen. *Mizutania* (with *N. subalpina*). Surprisingly, GROLLE (1981) recently found a fossil species of this genus in Europe, *N. europaea* GROLLE, embedded in an amber, from the southern part of Scandinavia. The species belongs in the subgen. *Mizutania* and, according to GROLLE (1981), might even prove to be conspecific with *N. subalpina.*

Regarding the taxonomic position of *Nipponolejeunea,* MIZUTANI (1961) placed the genus in the subfamily Jubuloideae together with *Jubula* and *Neohattoria,* but SCHUSTER (1963) proposed an independent subfamily in the Lejeuneaceae for this genus, Nipponolejeuneoideae SCHUST. & KACHROO, which has now become generally accepted (cf. GRAGSTEIN, 1979; SCHUSTER, 1979).

Although the two living species of *Nipponolejeunea* have been repeatedly described and illustrated as to their morphology (cf. INOUE, 1974, 1976), little attention has been given to the biosystematic characters. The present paper focusses on these aspects.

Materials

Living plants of *Nipponolejeunea pilifera* and *N. subalpina* were collected as follows:

Nipponolejeunea pilifera (STEPH.) HATT.

1. On bark of *Abies veitchii* in subalpine forest, northern slope of Mt. Fuji, Yamanashi Pref., ca. 1900 m. alt., coll. S.R. GRADSTEIN & H. INOUE no. 3337 (Nov. 8, 1979).

2. On bark of *Cryptomeria japonica* in natural *Cryptomeria* forest, Yakushima Isl., Kagoshima Pref., ca. 1000 m. alt., coll. S. R. GRADSTEIN no. 3214 (Oct. 27, 1979).

3. On rock covered with humus, with several mosses, Mt. Kimpu, Yamanashi Pref., ca. 2200–2500 m. alt., coll. H. INOUE no. 24714 (July 28, 1979).

Nipponolejeunea subalpina (HORIK.) HATT.

1. On *Abies veitchii* trunk in subalpine forest, northern slope of Mt. Fuji, Yamanashi Pref., ca. 1900 m. alt., coll. S. R. GRADSTEIN & H. INOUE no. 3338 (Nov. 8, 1979).

2. On trunk of *Abies veitchii*, between Shibunoyu and Kuroyuri-daira, Mts. Yatsugatake, Nagano Pref., ca. 2300 m. alt., coll. H. INOUE no. 25100 (Sept. 2, 1980).

3. On bark of *Abies veitchii*, along the Gotenba Route, southern slope of Mt. Fuji, Shizuoka Pref., ca. 2100 m. alt., coll. H. INOUE no. 25199 (Oct. 16, 1980).

Voucher specimens are all deposited in TNS and U. The methods employed to study the chromosome number and chemical contents will be given below.

Results

Chromosomes

Living plants of *Nipponolejeunea pilifera* from Mt. Kimpu (H. INOUE no. 24714 and *N. subalpina* from Mt. Fuji (H. INOUE no. 25109) were used to study the chromosomes. Culture, fixation, and staining methods were similar to those employed for *Neohattoria herzogii* (INOUE et al., 1981).

INOUE (1980) reported 9 chromosomes of *Nipponolejeunea pilifera* based on INOUE no. 24714. The same chromosome number was also demonstrated for *N. subalpina* based on H. INOUE no. 25109 from Mt. Fuji. Chromosome number and formulae of *Nipponolejeunea pilifera* and *N. subalpima* are almost similar to those in most other species of the Lejeuneaceae so far studied (cf. TATUNO & NAGATOMO, 1969).

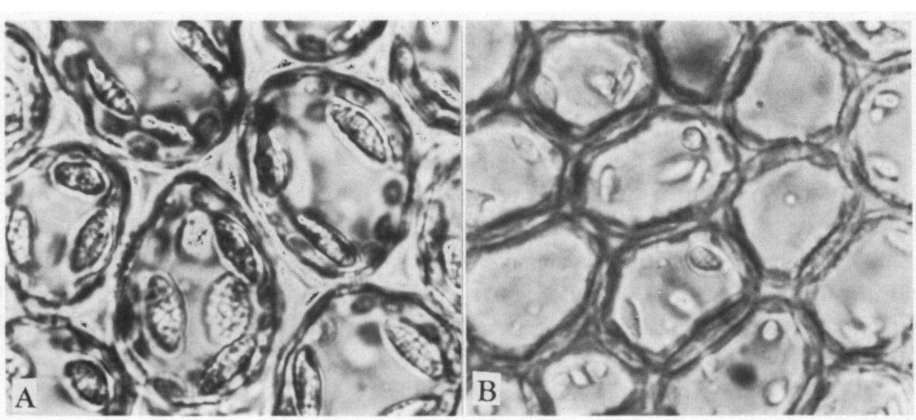


Fig. 1. Oil bodies of *Nipponolejeunea pilifera* (A) and *N. subalpina* (B). ×ca. 1500.

Table 1. Chemical components of *Nipponolejeunea subalpina*.

Peak No.	Components
1	Borneol (1)
2	Bornyl acetate (2)
3	δ-Elemene (3)
4	β-Cubebene (4)
5	Sesquiterpene hydrocarbon (M^+ 204, base 161)
6	Sesquiterpene hydrocarbon (M^+ 204, base 95)
7	γ-Cadinene (5)
8	Sesquiterpene alcohol (M^+ 222, base 121)
9	Sesquiterpene alcohol (M^+ 222, base 161)
10	Sesquiterpene alcohol (M^+ 222, base 81)
11	Sesquiterpene ketone (M^+ 218, base 79)
12	Sesquiterpene alcohol (M^+ 222, base 79)
13	M^+ 248 (base 79)
14	M^+ 238 (base 41)
15	Sesquiterpene acetate (M^+ 260, base 43)
16	M^+ 266 (base 165)
17	M^+ 278 (base 68)
18	M^+ 274 (base 173)
19	Phthalate
20	M^+ 296 (base 121)
22	M^+ 308 (base 134)
23	M^+ 290 (base 134)
24	M^+ 308 (base 134)
25	M^+ 290 (base 161)
26	M^+ ? (base 187)
27	Paraffin (base 57)
28	M^+ 430 (base 83)
29	Campesterol
30	Stigmasterol
31	Sitosterol

Oil Bodies

The oil bodies of the two species of *Nipponolejeunea* were described by MIZUTANI (1961), as "3–4 (7) per cell, hyaline, ovate, 5–10×4–6 μ, flat, containing about 20 spherules within, which are loosely but regularly arranged, the inner spherules less distinct or almost inperceptible" for *N. pilifera*, and "3–4, occasionally 5, per cell, somewhat grayish, elliptical, 4.5×25 μ, formed of 15–20, somewhat indistinct granules, each less than 1 μ in diameter" for *N. subalpina*. However, his observations seem to include some errors for *N. subalpina* (may be due to degeneration of oil bodies). In fresh *N. subalpina* the oil bodies appear to be almost always homogeneous or very rarely with a few, very indistinct spherules (probably by degeneration), showing a clear, strong contrast to those of *N. pilifera* which has distinctly segmented oil bodies. We repeatedly observed the oil bodies of *N. pilifera* to be 2–5–(6) per cell, fusiform to elliptical and 5–13×4–8 μ or globose to subglobose and 4–6×4–7 μ, distinctly seg-

Table 2. Chemical components of *Nipponolejeunea pilifera.*

Peak No.	Components
1	Borneol (**1**)
2	Bornyl acetate (**2**)
3	Sesquiterpene hydrocarbon (M^+ 204, base 161)
4	Sesquiterpene hydrocarbon (M^+ 204, base 121)
5	Sesquiterpene hydrocarbon (M^+ 204, base 121)
6	Germacrene D (**8**)
7	Sesquiterpene alcohol (M^+ 222, base 105)
8	Sesquiterpene alcohol (M^+ 222, base 161)
9	Sesquiterpene alcohol (M^+ 222, base 81)
10	Sesquiterpene alcohol (M^+ 222, base 43)
11	Sesquiterpene alcohol (M^+ 220, base 41)
12	Sesquiterpene alcohol (M^+ 220, base 43)
13	M^+ 236 (base 95)
14	M^+ 236 (base 109)
15	M^+ 238 (base 136)
16	M^+ 282 (base 43)
17	M^+ ? (base 109)
18	Sesquiterpene acetate (M^+ 260, base 43)
19	M^+ ? (base 43)
20	M^+ 278 (base 68)
21	M^+ 278 (base 81)
22	M^+ 278 (base 81)
23	Phthalate
24	M^+ 310 (base 58)
25	M^+ 310 (base 109)
26	M^+ 310 (base 121)
27	M^+ ? (base 79)
28	M^+ ? (base 71)
29	M^+ ? (base 257)
30	Paraffin (M^+ ?, base 57)
31	Alkene (M^+ ?, base 55)
32	Alkene (M^+ ?, base 57)
33	Alkene (M^+ ?, base 43)
34	Alkene (M^+ ?, base 57)
35	M^+ 430 (base 83)
36	Campesterol
37	Stigmasterol
38	Sitosterol

mented and composed of 13–22 globules of 1–1.5 μ in diameter; those of *N. subalpina* were observed to be 3–6 per cell, fusiform or elliptical and 3–3×4–5 μ or globose to subglobose and 2–3 μ in diameter, almost completely homogeneous.

Chemical constituents

The living plants of *Nipponolejeunea pilifera* (from Mt. Fuji, GRADSTEIN & INOUE no 3337 and from Isl. Yakushima, GRADSTEIN no. 3214) and *N. subalpina* (from Mt.

Fuji, GRADSTEIN & INOUE no. 3338), each 100 mg. were air-dried for five days, then grounded; the ground materials were extracted with ether for one week. The crude extracts were filtered off with silica gel packed in a short glass column and the solvent was evaporated *in vacuo* at 28°C. The crude extracts after dilution with ether in a suitable concentration were directly analysed by GC, TLC and GC-MS. The terpenoid compounds were confirmed by comparison of the mass spectra of the authentic samples and published materials; TLD, GLC and GC-MS analysis were performed in the same manner as described previously (ASAKAWA et al., 1980).

Nipponolejeunea subalpina emits fragrant odor when it is crushed. The gaschromatogram of the crude extract showed the presence of two major peaks, which were confirmed to be bornyl acetate (**2**) and an unknown diterpene alcohol (M^+ 290). In addition to the above major components, borneol (**1**), δ-elemene (**3**), β-cubebene(**4**), γ-cadinene (**5**) have been detected together with several unknown sesqui- and diterpenoids, as the minor components. The fragrant order of *N. subalpina* is due to the mixture of bornyl acetate and borneol.

Nipponolejeunea pilifera emits fragrant odor, though not so strong as in *N. subalpina.* The GC-MS analysis of the crude extract showed the presence of an

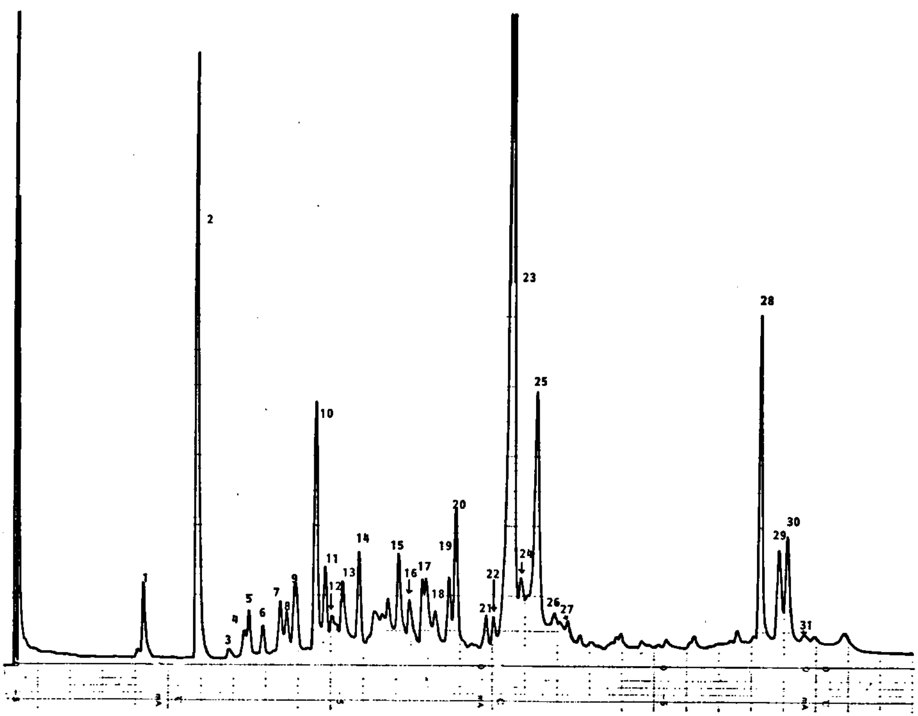


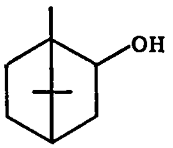
Fig. 2. Gaschromatogram of the crude extract of *Nipponolejeunea subalpina.* GLC condition: Column, SE-30 1% 3 m×2 mm, column temp. 50–270° at 5°/min, inject. temp. 260°, He 30 ml/min.

Fig. 3. Gaschromatogram of the crude extract of *Nipponolejeunea pilifera*. GLC condition: Column SE-30 1% 3 m×2 mm, column temp. 50–270° at 5°/min, inject. temp. 260°, He 30 ml/min.

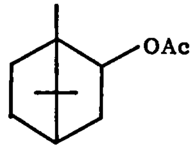
unidentified sesquiterpene alcohol and a sesquiterpene acetate as the major components. The small quantities of borneol and bornyl acetate, and of various unknown sesquiterpene alcohols have also been detected. The whole gaschromatogram of *N. pilifera* was quite different from that of *N. subalpina* as seen in Fig. 1 and 2. One of the most important differences is that *N. pilifera* mainly elaborates the sesquiterpene alcohols whilst *N. subalpina* does bornyl acetate and diterpene alcohol. However, *N. pilifera* and *N. subalpina* biosynthesize the same terpenoids; bornyl acetate and borneol, an unknown sesquiterpene alcohol (M^+ 222, base 81: peak No. 9 in *N. pilifera* and peak No. 10 in *N. subalpina*), an unknown sesquiterpene acetate (M^+ 260, base 43: peak No. 18 in *N. pilifera* and peak No. 15 in *N. subalpina*) and the compound (M^+ 430, base 83: peak No. 35 in *N. pilifera* and peak No. 28 in *N. snbalpina*) although their contents are different.

Ecology and Distribution

Nipponolejeunea subalpina is restricted in distribution to the subalpine forest zone in Japan and Sakhalin, where it grows on barks of various trees, mainly including



(1)



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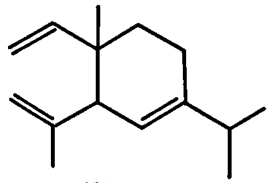

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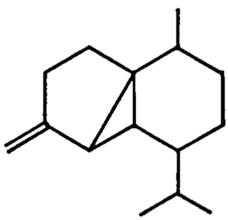

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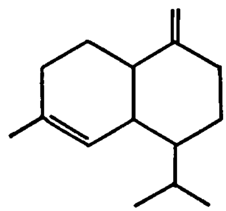

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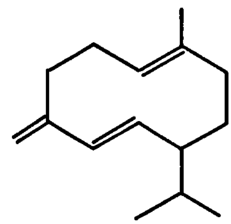

(6)

Fig. 4. Chemical components of *Nipponolejeunea subalpian* and *N. pilifera*. (1) Borneol. (2) Bornyl acetate. (3) δ-Elemene. (4) β-Cubebene. (5) γ-Cadinene. (6) Germacrene D.

Tsuga diversifolia, *Abies veitchii*, and *A. mariesii*, or sometimes even on more or less shaded rocks in coniferous forest (mainly on granite or sandstone, never on calcareous rocks). It usually forms pure mats on bark sometimes associated with *Frullania tamarisci* (subsp. *obscura*), *Ptilidium pulcherrimum*, *Nipponolejeunea pilifera*, and several small mosses. Although the plants of *N. subalpina* are very small, filiform, with several branches, usually less than 1 cm long and 0.7 mm wide, of pale to bright green color, it can easily be detected in the field by its rather pale green, sponge-like, dense mats when moist; but when dry, it may be difficult to find the plants due to their rather whitish or pale yellowish color, which resembles the color of the bark.

Because this species primarily prefers the bark of coniferous trees as *Abies* spp. and is more common in the northern district (lacking in Kyushu and western Honshu), it may possibly be found also in hte Siberian taiga or more westwards; this assumption may further be supported by the presence of a fossil species in southern Scandinavia, *N. europaea*, which is rather closely allied to *N. subalpina*.

On the other hand, *Nipponolejeunea pilifera* is rather commonly found throughout Japan, Korea, and Formosa, but not reported from Sakhalin, becoming scarcer in northern districts. Vertically it is distributed from lowland areas (as low as ca. 500 m. alt.) up to the subalpine zone (as far as up about 2500 m. alt.), growing on the bark of broad-leaved or coniferous trees or shrubs, or on rocks intermingled among various, large mosses on humus (including *Polytrichum commune*, *Hylocomium splendens*, *Dicranum* spp., etc.); on coniferous trees in the subalpine zone it usually forms rather pure mats or sometimes grows associated with *Nipponolejeunea subalpina*, *Frullania tamarisci* (subsp. *obscura*), *Ptilidium pulcherrimum*, *Herbertus aduncus*, *Bazzania denudata*, *Scapania ampliata* (at trunk base), etc.; on broad-leaved trees in lowland areas it is usually associated with various species of Lejeuneaceae and *Frullania*, or sometimes forms loose, pure mats. The plants of *N. pilifera* are much larger than those of *N. subalpina* (usually about 2–4 cm long and 1.3–1.6 mm wide), with yellowish or bright green color, and it is very easily recognizable in the field by the conspicuous, ciliar teeth on the leaf-tip.

Discussion

Since the establishment of *Nipponolejeunea* (HATTORI, 1944), both *N. pilifera* and *N. subalpina* had been included in the same genus, but INOUE (1976) recognized both species to belong to different subgenera, subg. *Nipponolejeunea* (*N. pilifera*) and subg. *Mizutania* (*N. subalpina*). INOUE's concept for the separation of those two subgenera was based on 1) the 13–15 cortical cell-rows in *N. subalpina* (20–25 cell-rows in *N. pilifera*) and 2) the almost homogeneous oil bodies in *N. subalpina* (distinctly segmented oil bodies in *N. pilifera*). The vegetative branching type of *Nipponolejeunea* was analyzed by MIZUTANI (1970), who found the *Frullania-Jubula* type in *N. subalpina* and the *Frullania-Nipponolejeunea* type in *N. pilifera*; the pattern of branching type was schematically represented in GRADSTEIN (1979).

As is obvious from the above biosystematic data, *N. pilifera* and *N. subalpina* are quite different from each other by the nature of the oil bodies and their constituents, almost suggesting a generic rather than subgeneric differentiation. However, the structure of the gynoecia, androecia, and sporophytes of both species are quite similar; the type of gynoecial innovations is also rather similar in both species. We would, therefore, retain both species in the same genus, though admitting subgeneric separation.

Nipponolejeunea, the only genus in the subfam. Nipponolejeuneoideae, has been considered to be "one of the most strongly isolated genera within the entire Lejeuneaceae" (SCHUSTER. 1963). This fact seems to be supported from the chemical data, too. Both *N. pilifera* and *N. subalpina* elabolate borneol (**1**) and bornyl acetate (**2**), which were not yet known from the Lejeuneaceae (bornyl acetate has only been isolated from the thallose *Conocephalum conicum* and *Wiesnerella denudata* (ASAKAWA et al., 1980)]. However, it is still impossible to make conclusive remarks on the chemotaxonomy of the genera of Lejeuneaceae due to scarcity of observation on their chemistry (cf. GRADSTEIN et al., 1981). We would, therefore, like to postpone such discussions until more data on the chemistry of the Lejeuneaceae become available.

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