A Biosystematic Study of *Neohattoria herzogii* (HATT.) KAMIM.

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Introduction

Neohattoria KAMIM. is a monotypic genus of the Jubulaceae (=Frullaniaceae) with a single species, *N. herzogii* (HATT.) KAMIM., known from central to northern Japan and the southern part of the Kurile Islands. The present genus was segregated from *Frullania* by KAMIMURA (1961; sub. nom. *Hattoria* KAMIM. nom. illeg., non SCHUST., 1961) on the basis of the branching type, the shape of the first leaf and underleaf on branch, the total lack of secondary pigmentation, the uniform cell structure of the stem in cross section, and the strongly toothed leaf lobes. The generic concept of *Neohattoria* was greatly expanded by SCHUSTER (1970), who included eight species and classified them into two subgenera, subgen. *Neohattoria* (with a single species) and subgen. *Microfrullania* SCHUST. (with seven species); however, HATTORI *et al.* (1972) transferred all species of subgen. *Microfrullania* to a newly segregated genus *Schusterella* HATT. *et al.*, thus retaining the monotypic status of *Neohattoria.*

As already described and illustrated by HATTORI (1955), KAMIMURA (1961), MIZUTANI (1961), LADYZHENSKAJA (1963), SCHUSTER (1970), and HATTORI *et al.* (1972), *Neohattoria herzogii* is closely related to species of both *Jubula* and *Frullania.* Regarding the taxonomic desposition of *Neohattoria*, MIZUTANI (1961) and MIZUTANI & HATTORI (1969) placed it with *Jubula* in a subfamily Jubuloideae of Lejeuneaceae and HATTORI *et al.* placed it in Jubulaceae (s. lat.). But, KAMIMURA (1961), SCHUSTER (1970, 1979), and GUERCKE (1978) placed it more close to *Frullania*, e.g. in a subfamily Frullanioideae of Jubulaceae (s. lat.); more recently, ASAKAWA *et al.* (1979b), admitting three distinct families, Jubulaceae, Frullaniaceae, and Lejeuneaceae, placed *Neohattoria* and *Jubula* in the Jubulaceae (s. str.) but *Frullania* and *Schusterella* in the Frullaniaceae.

Recently we were able to study several colonies of living plants of *Neohattoria herzogii* from Hokkaido and central Honshu. The present paper is dealing with

biosystematic aspects of this peculiar species based on these living materials. The chemical analysis was made at Tokushima Bunri University and we are indebted to Miss N. TOKUNAGA for technical assistance. The first and third authors (H. INOUE and S. F. GRADSTEIN) are indebted to the Japan Society for the Promotion of Science (JSPS) for financial support which made possible the joint works at the National Science Museum, Tokyo, leading to this publication. Vaucher specimens used for this study are deposited in TNS and U.

Materials

Living plants of *Neohattoria herzogii* were collected as follows:

1. On bark of *Abies* sp., ca. 1,000 m. alt., Mt. Higashi-nupukaushi, south of the Lake Shikaribetsu, Tokachi, Hokkaido; coll. H. INOUE 26320 (June 26, 1979).
2. On log in *Abies veitchii* forest, ca. 2,150 m. alt., northern slope of Mt. Fuji (near 5th Step), Yamanashi Pref.; coll. S. R. GRADSTEIN 3332 (November 7, 1979).
3. On bark of *Abies veitchii*, ca. 1,800 m. alt., between 3rd and 4th Step along Shojiko Route of Mt. Fuji, Yamanashi Pref.; coll. H. INOUE no. 25228 (October 17, 1980).

The methods employed to study the chromosome number and chemical constituents will be given below.

Results and Discussions

1. Chromosomes

Plants from Hokkaido (H. INOUE no. 26320) were cultured in a glass-pot with a moistened filter paper at room temperature for about 20 days; newly developed shoot apices were fixed by acetic-alcohol (1: 3) for about two hours and stained by acetic orcein using the squash method (INOUE, 1975).

At the metaphase, eight chromosomes were counted. Among the eight chromosomes, one is slightly larger than the others and it has a median constriction. At the prophase, the large part of this chromosome is deeply stained, indicating that this chromosome may be compared with the Y chromosome of some species of *Frullania*. TATUNO (1960) reported n=8=7+Y chromosomes for male plants of the subgen.

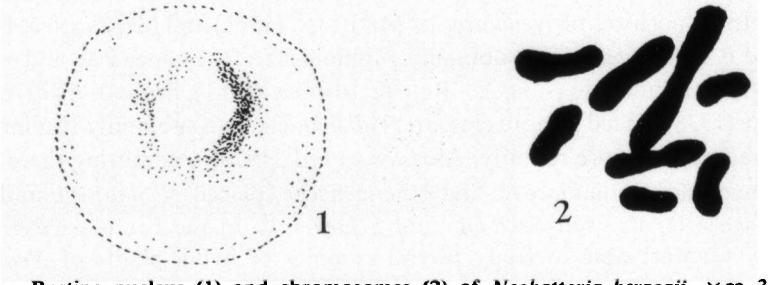

Fig. 1. Resting nucleus (1) and chromosomes (2) of *Neohattoria herzogii*. ×ca. 3,000.

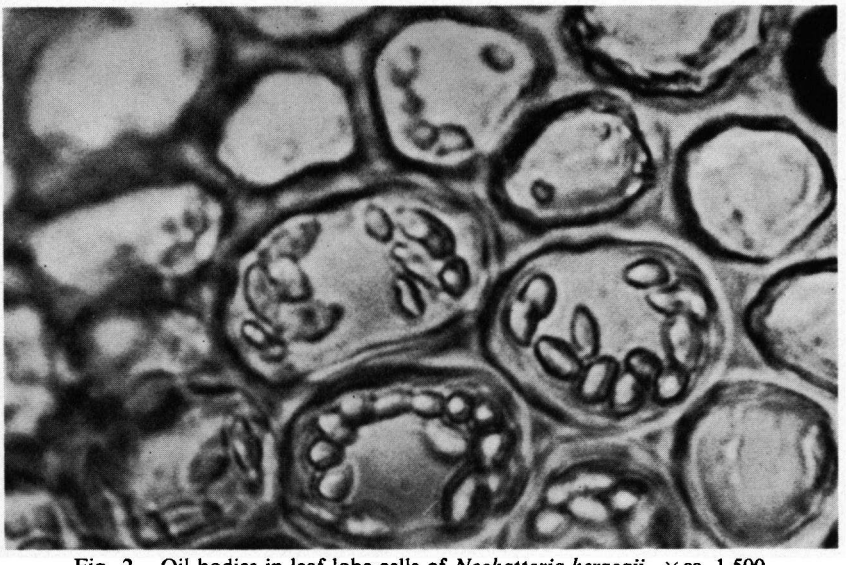

Fig. 2. Oil bodies in leaf-lobe cells of *Neohattoria herzogii*. ×ca. 1,500.

Trachycola of *Frullania*. The shape of the chromosomes found in *Neohattoria herzogii* are indeed very similar to those of subgen. *Trachycolea*.

All plants so far checked of *Neohattoria herzogii*, including those from Hokkaido, do not show any trace of sexual organs and sexual condition of this species is thus unknown. The chromosome data, however, suggest that the plants from Hokkaido, at least, are male plants.

2. Oil bodies

HATTORI *et al.* (1972) described the oil bodies of *Neohattoria herzogii*, basing on plants from Hokkaido (Mt. Meakan), as "hyaline and homogeneous, 10–20 per cell in the leaf-lobe, and almost equal in size to the chloroplasts". We observed essentially similar oil bodies in plants from Mt. Fuji (S. R. GRADSTEIN no. 3332, H. INOUE 25228); they are present in all living cells of leaf-lobes, underleaves, and epidermal cells of stem, variable in number but usually 7–15 per leaf-lobe cell (rarely up to 22), completely colorless and homogeneous, but sometimes (degeneration?) faintly papillose with a few indistinct granules; furthermore, they are ellipsoid to fusiform and 3–5×2–2.5 μ or sometimes globose and about 2–2.5 μ in diam.

The hyaline, homogeneous, rather numerous, small, "*Massula*-type" oil bodies of *Neohattoria herzogii* are rather peculiar, because in *Jubula* and *Frullania* the oil bodies are always distinctly botryoidal, with distinct minute globules; the oil body type in other genera of Jubulaceae is not yet known.

3. Chemical constituents

Air-dried material (7.7 mg) from Mt. Fuji (S. R. GRADSTEIN no. 3332) was extract was filtered through a short column packed with silica gel (70–2301). The solvent

Table 1. Chemical components of *Neohattoria herzogii*.

Peak No.	Components
1	Sesquiterpene hydrocarbon (M^+ 204, base 119)
2	Sesquiterpene alcohol (M^+ 220, base 119)
3	Paraffin C-16
4	Paraffin C-17
5	Paraffin
6	Sesquiterpene alcohol (M^+ 218, base 109)
7	Drimenol
8	(Phthalate)
9	Paraffin C-18
10	Paraffin C-19
11	Sesquiterpene alcohol (M^+ ?, base 109)
12	Alkene (M^+ 280, base 57)
13	Paraffin
14	Paraffin C-20
15	Paraffin C-21
16	Paraffin
17	Paraffin C-22
18	Paraffin C-23
19	Alkene (M^+ ?, base 57)
20	(Phthalate)
21	Paraffin
22	Paraffin C-24
23	Paraffin C-25
24	Paraffin C-25
25	Paraffin
26	Paraffin C-26
27	Paraffin C-27
28	Paraffin
29	Paraffin C-28
30	Paraffin C-29
31	Campesterol
32	Stigmasterol
33	Sitosterol

was evaporated to afford the residue (0.15 mg), which was injected to the GC-MS apparatus. TLC, GC and GC-MS analysis were performed in the same manner as described previously (ASAKAWA *et al.*, 1979 a).

The GC showed a complex gaschromatogram in which at least 50 peaks appeared. The assignment of each peak was carried out by mass spectral analysis and the results were summarized in Table 1. *Neohattoria herzogii* elaborates many paraffinic hydrocarbons together with a few sesquiterpenoids, drimenol, and an unidentified sesquiterpene hydrocarbon, three unidetified sesquiterpene alcohols, and sterol mixture (campesterol, stigmasterol, and sitosterol, 1: 1: 1 in GC), which have been found in all liverworts so far examined chemically. The main components of *Neohattoria herzogii*

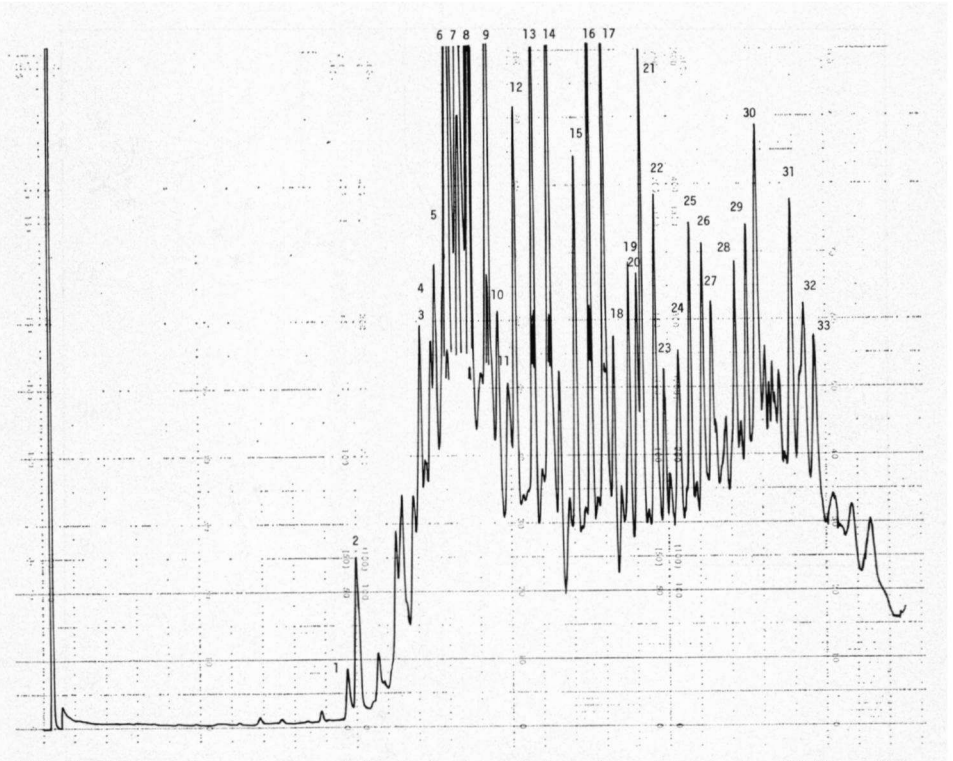

Fig. 3. The gaschromatogram of the crude extract of *Neohattoria herzogii*. GLC condition: Column, SE-30 1%, 3 m×2 mm, column temp. 50–270° at 5°/min, inject. temp. 260°, He 30 ml/min.

are the sesquiterpene alcohol (peak no. 6; MÜ, 218, base 109) and drimenol (peak no. 7).

ASAKAWA *et al.* (1979 b) reported that *Frullania* species in general produce great amounts of sesquiterpene lactones, which are thus considered important chemosystematic markers of Frullaniaceae. But *Jubula* species do not produce sesquiterpene lactones and they contain cyclocolorenone and maalioxide as major components (ASAKAWA *et al.*, 1979 b).

As stated above, *Neohattoria herzogii* produces many paraffinic hydrocarbons, and it appears that there is no particular chemical affinity among *Neohattoria*, *Jubula*, and *Frullania*.

4. Ecology and distribution

As has been emphasized repeatedly in the literature, *Neohattoria herzogii* is a very minute plant, easily overlooked in dried condition. It is a species of the subalpine coniferous forest zone, between 1,600–2,200 m. alt. in central Honshu, decreasing altitude in Hokkaido where it occurs at about 1,000–1,500 m. This species apparently prefers shaded, moist coniferous forest, almost always growing on trunks predomi-

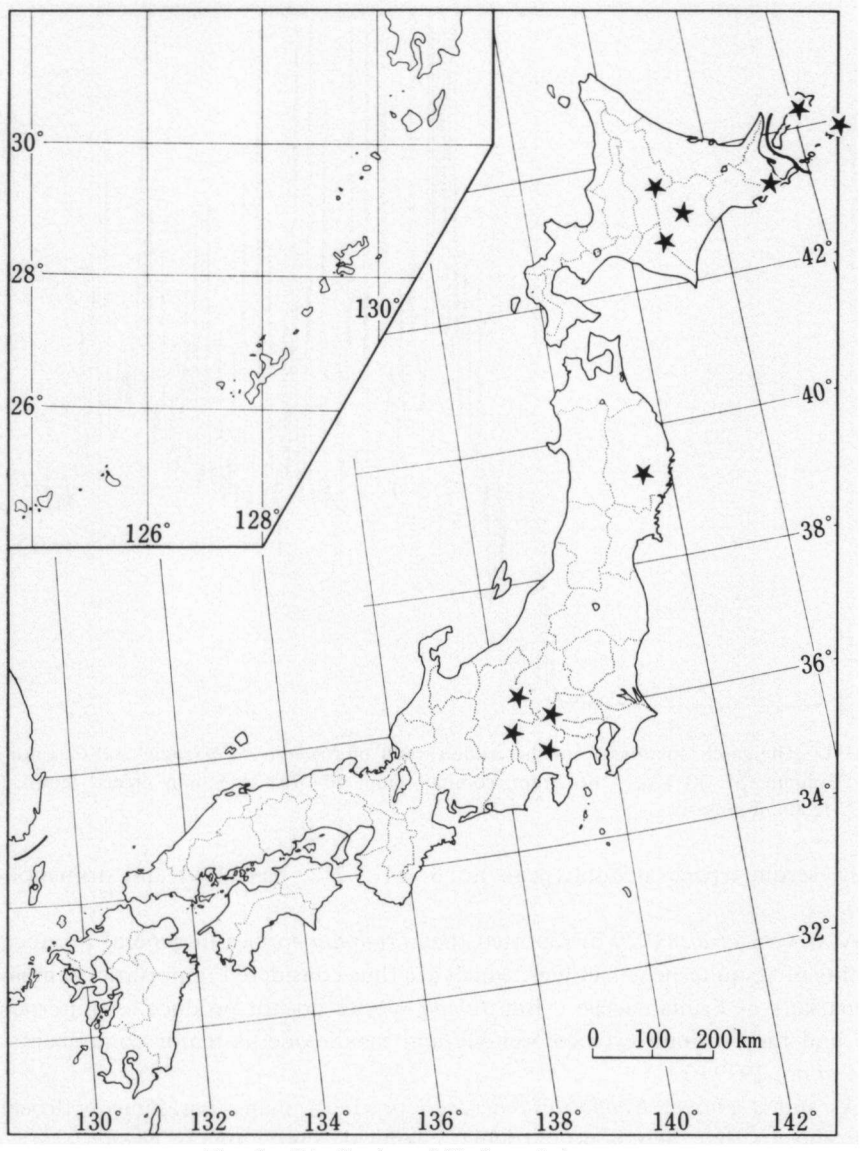

Fig. 4. Distribution of *Neohattoria herzogii*.

nantly of *Abies veitchii* and *Tsuga diversifolia*, sometimes of *Picea* sp. and rarely of *Betula* sp. Usually the species does not form pure mats but grows intermingled among large mats of *Nipponolejeunea pilifera*, *N. subalpina*, *Frullania tamarisci* subsp. *obscura*, *Ptilidium pulcherrimum*, *Herbertus aduncus*, *Bazzania denudata*, etc. It may be very difficult to recognize the minute plants by the naked eye, but by the characteristically pale green color (sometimes with slightly reddish-brown pigmentation) and

irregularly toothed leaves with strongly caducous lobules it may be easily recognized under the microscope.

At present *Neohattoria herzogii* is known from several mountains in Japan (Honshu and Hokkaido) and Shikotan and Kunashiri in the Kurile Islands nearby Hokkaido. This pattern of distribution suggest that *Neohattoria* may possibly be found in the Pacific northwest of North America; similar distributions are known for e.g. *Ptilidium californicum*, *Plagiochila satoi*, etc. The areas of these species usually do not extend into the continental regions of Asia and North America, and they are distributed through the Kurile and Aleutian Islands.

Discussion on the Taxonomic Position of *Neohattoria*

As stated in the introduction, *Neohattoria herzogii* has been variously placed close to *Jubula* in the Lejeuneaceae (MIZUTANI, 1961; MIZUTANI & HATTORI, 1969) or the Jubulaceae (HATTORI *et al.*, 1972; ASAKAWA *et al*, 1979 b), or close to *Frullania* in the Jubulaceae (KAMIMURA, 1961; SCHUSTER, 1970, 1979; GUERCKE, 1978). The linkage to *Jubula* is based particularly on the presence of the *Frullania*-type branches with the *Jubula*-type half-leaves and initial branch leaves, and the total lack of secondary reddish pigmentation. Association with *Frullania* is based on the resemblance of *Neohattoria herzogii* to species of the subgen. *Microfrullania* SCHUST. (which is now placed in the genus *Schusterella* HATT. *et al.*). All taxonomic evidences for the classification of *Neohattoria* has been derived purely from the morphological and anatomical data of the sterile gametophyte, since reproductive structures and sporophytes of *Neohattoria* are still unknown. Thus, a critical comparison of important suprageneric characters such as seta anatomy and sporeling development is impossible.

A first attempt to tackle the suprageneric classification of the "Jubulaceae complex" chemically was made by ASAKAWA *et al.* (1979 b, 1980), who adopted three different families on the basis of the following evidence:

Frullaniaceae .....Sesquiterpene lactones
Jubulaceae .....Cyclocolorenone, Maaloxide
Lejeuneaceae .....Pinguissane-type sesquiterpenes

The results of our chemical analysis of *Neohattoria* are negative in as far as we cannot find any particular chemical affinity to any of the above three families. None of these groups produce paraffinic hydrocarbons characteristic for *Neohattoria*.

As to the oil body, *Neohattoria* only resembles some genera of Lejeuneaceae (e.g. *Acrolejeunea*, *Brachiolejeunea*, *Lopholejeunea*), which also have the *"Massula*-type" oil bodies. Otherwise the relationship to this family is rather remote. *Jubula* and *Frullania* have completely different, finely segmented oil bodies. By its chromosomes, however, *Neohattoria* (n=8=7+Y) is different from *Jubula* but identical to the species of *Frullania* subgen. *Trachycolea*. Thus, there is additional cytological evidence for a close relationshup of *Neohattoria* to *Frullania* than to *Jubula*. As indicated above, the morphological resemblance within the Frullaniaceae is seen

between *Neohattoria* and *Schusterella*, but the latter genus has never been studied chemically or cytologically, and is only known from dead plants. A biosystematic investigation of *Schusterella* would thus be worth-while.

In conclusion, we would like to suggest that our present knowledge of cytology and chemistry is yet insufficient to justify conclusions on the phylogenetic classification of the genera of the "Jubulaceae complex". For the time being, we would therefore prefer to classify *Neohattoria* with *Jubula* in the family Jubulaceae (following ASAKAWA *et al.*, 1979 b), although biosystematic evidence suggests a greater difference between these two genera than has been assumed previously.

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