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Karyomorphological Study of *Nertera yamashitae* (Rubiaceae) from Amami Island

Goro KOKUBUGATA[1], Sadamu MATSUMOTO[1] and Yuichi KADOTA[2]

國府方吾郎[1]・松本　定[1]・門田裕一[2]：アマミアワゴケ（アカネ科）の核形態及び核型に関する研究

Nertera yamashitae Yamazaki, Rubiaceae, has recently been described as a new species from Amami Island, the Ryukyus. (Yamazaki 1998). However, there are no reports on the chromosomal characters, even, chromosome number of *N. yamashitae.* In the present investigation, morphology of somatic chromosomes at interphase, mitotic prophase and mitotic metaphase are observed with the standard acetic orcein staining method. The aim of this report is to show the chromosomal characters of *Nertera yamashitae* for the first time.

Material and Methods

The material was collected from the type locality of this species, Sumiyo River, Amami Island, Kagoshima Prefecture by Mr. H. Yamashita on 13, July 1998 (Fig. 1). It was introduced into the Tsukuba Botanical Garden, National Science Museum (Accession no. TBG123041) by Y. Kadota, and was cultivated by S. Matsumoto for the present cytotaxonomical study.

Root tip was pretreated in 2mM 8-hydroxyquinoline at 20℃ for two hours. Then, it was fixed in 45% acetic acid at 4℃ for two hours, and was macerated in a mixture of 1N hydrochloric acid and 45% acetic acid (2 : 1) at 60℃ for ten sec. It was stained with 2% acetic orcein at room temperature for two hours. After staining, it was prepared with the squash method for standard karyotype analysis.

Morphological classification of chromosomes at interphase and mitotic prophase followed Tanaka (1977). Chromosomes at mitotic metaphase were classified according to centromeric position defined arm ratio (Levan *et al.,* 1964). Karyotype asymmetry value of arm ratio [$=1-(\Sigma b_i/B_i)/n$; b_i: the length of short arm; B_i: the length of long arm; n: chromosome number] and karyotype asymmetry of chromosome length ($=s/x$; s: standard deviation of chromosome length; x: mean chromosome length) followed Zarco (1986) with some modification.

Results and Discussion

The chromosomes of *Nertera yamashitae* showed the round prochromosome type at interphase, and the proximal type at mitotic prophase (Figs. 2A and B). Twenty-two round prochromosomes were counted in the nucleus at interphase (Fig. 2A). The combination of the round prochromosome

[1]Tsukuba Botanical Garden, National Science Museum, Tsukuba, 305-0005. 国立科学博物館 筑波研究資料センター 筑波実験植物園.
[2]Department of Botany, National Science Museum, Tsukuba, 305-0005. 国立科学博物館 植物研究部 〒305-0005 茨城県つくば市天久保4-1-1.

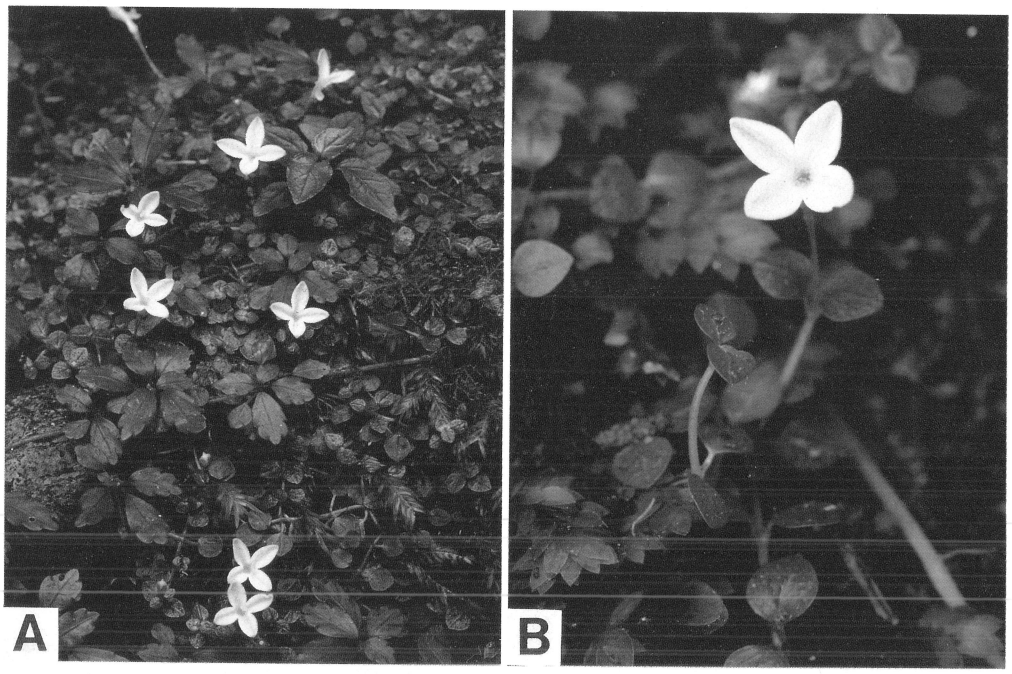

Fig. 1. Habit of *Nertera yamashitae* at the type locality, Sumiyo River, Amami Island, the Ryukyus. **A.** Habit. **B.** Plant.

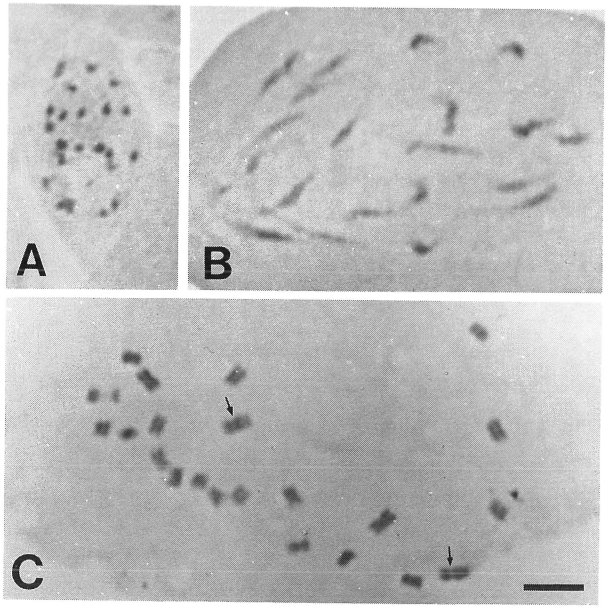

Fig. 2. Somatic chromosomes *of Nertera yamashitae.* **A.** interphase. **B.** mitotic prophase. **C.** mitotic metaphase. Arrows show submedian-centromeric chromosome. Bar shows 10 μm.

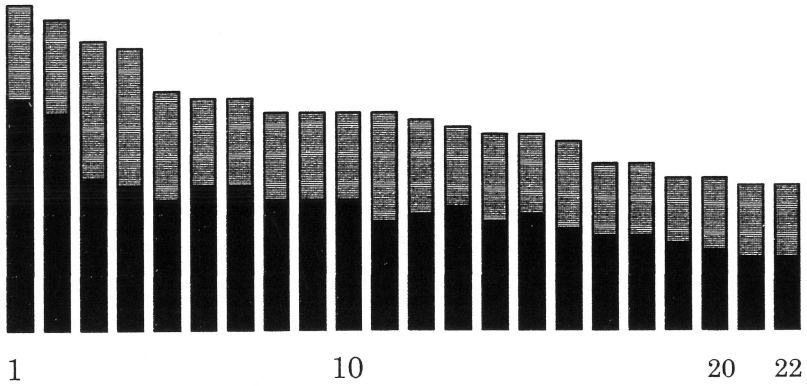

Fig. 3. Ideogram of mitotic chromosome of *Nertera yamashitae.* Soiled areas show long arm. Stippled areas show short arm.

Table 1. Arm ratio and chromosome length in the complement at mitotic metaphase in *Nertera yamashitae*

Chromosome in order	Long arm (μm)	Short arm (μm)	Chromosome length(μm)	Arm ratio	Centromeric position
1	3.2	1.3	4.5	2.5	sm
2	3.0	1.3	4.3	2.3	sm
3	2.1	1.9	4.0	1.1	m
4	2.0	1.9	3.9	1.1	m
5	1.8	1.5	3.3	1.2	m
6	2.0	1.3	3.3	1.5	m
7	2.0	1.3	3.3	1.5	m
8	1.8	1.2	3.0	1.5	m
9	1.8	1.2	3.0	1.5	m
10	1.8	1.2	3.0	1.5	m
11	1.5	1.5	3.0	1.0	m
12	1.6	1.3	2.9	1.2	m
13	1.7	1.1	2.8	1.5	m
14	1.5	1.2	2.7	1.3	m
15	1.6	1.1	2.7	1.5	m
16	1.4	1.2	2.6	1.2	m
17	1.3	1.0	2.3	1.3	m
18	1.3	1.0	2.3	1.3	m
19	1.2	0.9	2.1	1.3	m
20	1.1	1.0	2.1	1.1	m
21	1.0	1.0	2.0	1.0	m
22	1.0	1.0	2.0	1.0	m
Total length(μm)			64.9		

m: median-centromeric chromosome. sm: submedian-centromeric chromosome.

type at interphase and the proximal type at mitotic prophase in *N. yamashitae* was similar to that of *Spiranthes sinensis* (Orchidaceae) reported by Tanaka (1971). At mitotic metaphase, *N. yamashitae* showed the chromosome number of 2n=22 (Fig. 2A), which is reported here for the first time. The chromosome length varied from 4.5 μm to 2.0 μm, and mean and standard deviation of the chromosome length was 3.0 μm $\pm$0.7 μm (Table 1). The karyotype consisted of 20 median- and two submedian-centromeric chromosomes (Figs. 2C and 3). A couple of submedian-centromeric chromosomes were aligned in the first and the second each on the basis of the chromosome length (arrows in Fig 1C, Table 1). The asymmetry values of arm ratio and chromosome length in the karyotype of *N. yamashitae* were 0.24 and 0.24.

Sun *et al.* (1990) reported the chromosome number of n=22 in *N. granadensis,* and suggested the basic chromosome number of X=11 in the genus similar to most members of Rubiaceae (*e. g.,* Kiehn, 1985). The present count of the chromosome number of 2n=22 in *N. yamashitae* supports the basic chromosome number of X=11 in the genus *Nertera* reported by Sun *et al.* (1990). It also suggests that *N. yamashitae* must be a diploid cytotype with the basic chromosome number of X=11.

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Summary

Somatic chromosomes of *Nertera yamashitae* (Rubiaceae) which collected from the type locality in Amami Island were investigated with the standard acetic orcein staining method. The karyomorphology at interphase is the round prochromosome type, and that at mitotic prophase is the proximal type. At mitotic metaphase, *N. yamashitae* showed the chromosome number of 2n=22, and the karyotype consisted of 20 median- and two submedian-centromeric chromosomes. The karyomorphology at interphase and mitotic prophase, and the chromosome number of 2n=22 in *N. yamashitae* is reported here for the first time.

摘　要

奄美大島産アマミアワゴケ（アカネ科）の体細胞染色体を観察し，間期核及び前期染色体の形態と染色体数を初めて報告した。本種の染色体は，間期では球形前染色体型に分類され，体細胞有糸分裂前期では基部型に分類された。体細胞有糸分裂中期において，本種は染色体数2n=22を示し，その核型は20個の中部動原体型染色体と2個の次中部動原体型染色体からなっていた。染色体数2n=22は，本属植物において従来報告された基本数X=11と一致する。

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