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Cytological Studies on *Skimmia arborescens* Gamble subsp. *nitida* N. P. Taylor & Airy Shaw (*Rutaceae*) from Mt. Shiwandashan, Guangxi Autonomous Region, China

Tomoko FUKUDA[a, *], Ji Jiang BAI[b] and Tao CHEN[b]

[a]Department of Zoology, National Museum of Nature and Science,
4-1-1, Amakubo, Tsukuba, 300-0005 JAPAN;
[b]Fairy Lake Botanical Garden, Shenzhen & Chinese Academy of Sciences,
160, Xianhu Road, Liantang, Luohu District, Shenzhen, Guangdong, 518004 CHINA
*Corresponding author: t-fukuda@kahaku.go.jp

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Skimmia (*Rutaceae*) from Mt. Shiwandashan, Guangxi Autonomous Region, China was studied. On the basis of field and herbarium works, all the *Skimmia* found there were determined as *S. arborescens* Gamble subsp. *nitida* N. P. Taylor & Airy Shaw, dioecious tree with black fruits. Its chromosome number was 2n = 30, the first report for this subspecies. The karyotype was similar to that of *S. japonica* subsp. *japonica* var. *japonica* in having large metacentric pairs, no supernumeral chromosomes and sometimes having satellites on the middle chromosome pairs. Its arm ratio was more symmetric than other *Skimmia* species studied until now.

Key words: China, chromosome number, karyotype, *Rutaceae*, *Skimmia arborescens*.

The genus *Skimmia* Thunb. (*Rutaceae*) is evergreen shrub to tree, distributed in temperate to subtropical regions from Himalaya to Japan.

Two *Skimmia* species, *S. arborescens* T. Anderson ex Gamble and *S. kwangsiensis* C. C. Huang has been reported from Mt. Shiwandashan in the western end of Guangxi Autonomous Region, China (Huang 1958, Taylor 1987). *Skimmia arborescens* is dioecious tree with black fruits (Huang 1958, 1997, Taylor 1987). *Skimmia kwangsiensis* is dioecious shrub with red fruits (Huang 1958, Taylor 1987), and it is characterized by grey to whitish powder-coating petioles and sometimes stems. However, Huang (1997) noted that *S. kwangsiensis* may be one geographic variation within *S. arborescens*, and Zhang and Hartley (2008) treated it as a synonym of *S. arborescens*. Taylor (1987) published a comprehensive monograph on the genus *Skimmia*, in which he recognized two subspecies in *S. arborescens*, subsp. *arborescens* from the eastern Himalaya and subsp. *nitida* N. P. Taylor & Airy Shaw from southern parts of China. Taylor (1987) also treated *S. kwangsiensis* as an infraspecific taxon of *S. japonica* Thunb., namely *S. japonica* subsp. *reevesiana* (Fortune) N. P. Taylor & Airy Shaw var. *kwangsiensis* (C. C. Huang) N. P. Taylor. As Taylor (1987) noted, *Skimmia arborescens* and *S. japonica* in China are similar to each other except fruit color. Therefore these *Skimmia* taxa have taxonomic confusion.

Chromosome numbers of *S. arborescens* and *S. japonica* have been reported previously

Table 1. Materials of *Skimmia* plants used for this cytological analysis

Taxon/Locality	Voucher	2n	Karyotype*
S. arborescens subsp. *nitida*			
Lion's Head Peak, Mt. Shiwandashan, Guangxi Autonomous Region, China, alt. 850–920 m	CSS-0001	30	
	CSS-0007	30	
	CSS-0009	30	
	CSS-0015	30	
	CSS-0017	30	○
	CSS-0019	30	○
	CSS-0020	30	
Head of Pearl River, Mt. Shiwandashan, Guangxi Autonomous Region, China, alt. 800–900 m	CSJ-0004	30	○
	CSJ-0009	30	○
	CSJ-0010	30	
	CSJ-0011	30	
S. japonica subsp. *japonica*			
Mt. Takatori-yama, Kochi Pref., Japan, alt. 540 m	—	30	○
Mt. Takao-san, Tokyo Pref., Japan, alt. 500 m	Sk-0378	30	○
S. japonica subsp. *reevesiana* var. *reevesiana*			
Chito, Nantou Pref., Taiwan, alt. 1600 m	Sk-0085	64	○
Yuanyang Lake, Ilan Pref., Taiwan, alt. 1700 m	Sk-0722	60	○
S. arisanensis			
Mt. Ali-shan, Chiayi Pref., Taiwan, alt. 2300 m	Sk-0087	60	○

*Samples with circles were used for karyotype analysis.

by Johnson and Taylor (1989) and Fukuda et al. (2007). Johnson and Taylor (1989) reported the chromosome number of 2n = 30 for *S. arborescens* subsp. *arborescens* from Eastern Nepal, 2n = 30 for *S. japonica* subsp. *japonica* from Japan and Taiwan, and 2n = 60 for *S. japonica* subsp. *reevesiana* from Taiwan and Mainland China. They reported that the basic chromosome number was x = 15 in this genus, and suggested a karyomorphological feature of the genus such as a heteromorphic chromosome pair of *S. japonica*. Fukuda et al. (2007) also reported 2n = 30 for *S. japonica* subsp. *japonica* (as *S. japonica*) from Japan, 2n = 30–32 from Taiwan, 2n = 60–64 for *S. japonica* subsp. *reevesiana* (as *S. reevesiana*) and 2n = 60 for *S. arisanensis* Hayata from Taiwan, and they have a large chromosome pair or quartet (the first pair or quartet) with a median-submedian centromere in the karyotype. Cytological studies have proven useful for understanding the close relationships of *Skimmia* species (Johnson and Taylor 1989, Fukuda et al. 2007). However, there have been no previous cytological studies on *Skimmia arborescens* subsp. *nitida*.

This paper aims to report the karyotype of *Skimmia arborescens* subsp. *nitida* from Mt. Shiwandashan, Guangxi Autonomous Region, China, and to discuss their morphological and cytological relationships with their allied species.

Materials and Methods

Cytological studies were conducted on 11 individuals of two populations of *Skimmia arborescens* subsp. *nitida* from Mt. Shiwandashan, Guangxi Autonomous Region, China (Table 1). Methods for chromosome observation and karyotype analysis followed Fukuda et al. (2007). Karyotype analysis was also performed on four individuals of *S. arborescens* subsp. *nitida*. For comparison of karyotype analysis, we used our previous data of three taxa (Fukuda et al. 2007; Table 1): *S. japonica* Thunb. (*S. japonica* subsp. *japonica*),

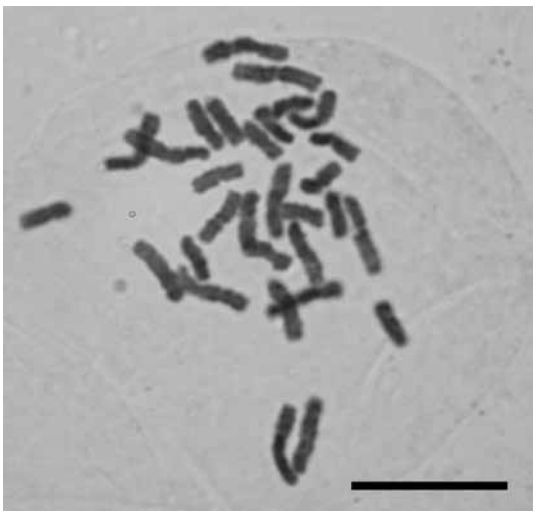

Fig. 1. Photomicrograph of *Skimmia arborescens* Gamble subsp. nitida Taylor & A. Shaw from Lion's head peak, Mt. Shiwandashan (CSS-0019). Scale bar = 10 μm.

S. reevesiana Fortune (*S. japonica* subsp. *reevesiana*) and *S. arisanensis* Hayata which was treated by Taylor (1987) as a synonym of *S. japonica* var. *reevesiana*. Arm ratio and TF% (Huziwara 1962) were calculated for karyotypes. Arm ratio was calculated dividing the length of the long arm by the length of the short arm, and three types (m: 1.0–1.7, sm: 1.7–3.0, st: 3.0–7.0) were determined for every chromosome pair. TF% is the ratio in percentage of the total sum of short arm-lengths to the total sum of chromosome lengths. Voucher specimens are deposited in the Herbarium of the South China Botanical Garden, Guangzhou, Guangdong (IBSC).

Results and Discussion

During the botanical expedition in Mt. Shiwandashan, Guangxi Autonomous Region, China, we collected *Skimmia arborescens* subsp. *nitida* in two populations. *Skimmia kwangsiensis* was not seen, though its type specimen was collected from this mountain (Shi wan da Shan, Feb. 1944, S. H. Chun 4588a, IBSC). *Skimmia kwangsiensis* might have been unknown species, because we could not find a type specimen in IBSC. Further reexamination of the type specimen will be necessary.

Chromosome numbers determined in this study are shown in Table 1. *Skimmia arborescens* subsp. *nitida* showed the chromosome number of 2n = 30 (diploid). This is the first report for this subspecies (Fig. 1). Somatic metaphase chromosomes had a large metacentric pair ranging from 4.6 to 2.5 μm in length with no supernumeral chromosomes (Fig. 2). Though it is difficult to make karyogram of *S. arborescens* subsp. *arborescens* from the photograph of Johnson and Taylor (1989), no large metacentric chromosomes seem to be observed.

Taylor (1987) pointed out the similarity of *S. arborescens* subsp. *nitida* and *S. japonica* subsp. *reevesiana* in growth forms and vegetative characteristics. However, our previous and present data indicated that karyotype of *S. japonica* subsp. *reevesiana* var. *reevesiana* from Taiwan is not very similar to *S. arborescens* subsp. *nitida*, even if we do not consider the difference of diploid and tetraploid (cf. Fukuda et al. 2007). They had no large metacentric chromosomes, and sometimes had supernumeral chromosomes (Fig. 2), though we could not find them in our previous study.

Large metacentric chromosomes with no supernumeral chromosomes are found in *S. japonica* subsp. *japonica* in Honshu/Shikoku to the northern Japan, except those of Taiwan (Fukuda et al. 2007). They are similar to *S. arborescens* subsp. *nitida* in having, though not always, satellites at the short arms of middle-length chromosomes (Fig. 2).

However, they are quite different in growth forms and merocity: growth forms of *S. japonica* subsp. *japonica* in Honshu/Shikoku to the northern Japan are often ca. 1m (at most 2 m at some Pacific coastal localities) or lower, and they sometimes creeps. Its flowers are mostly 4-merous, instead 5-merous for other species and subspecies.

Large metacentric chromosomes are also seen for *S. arisanensis*, endemic to Taiwan,

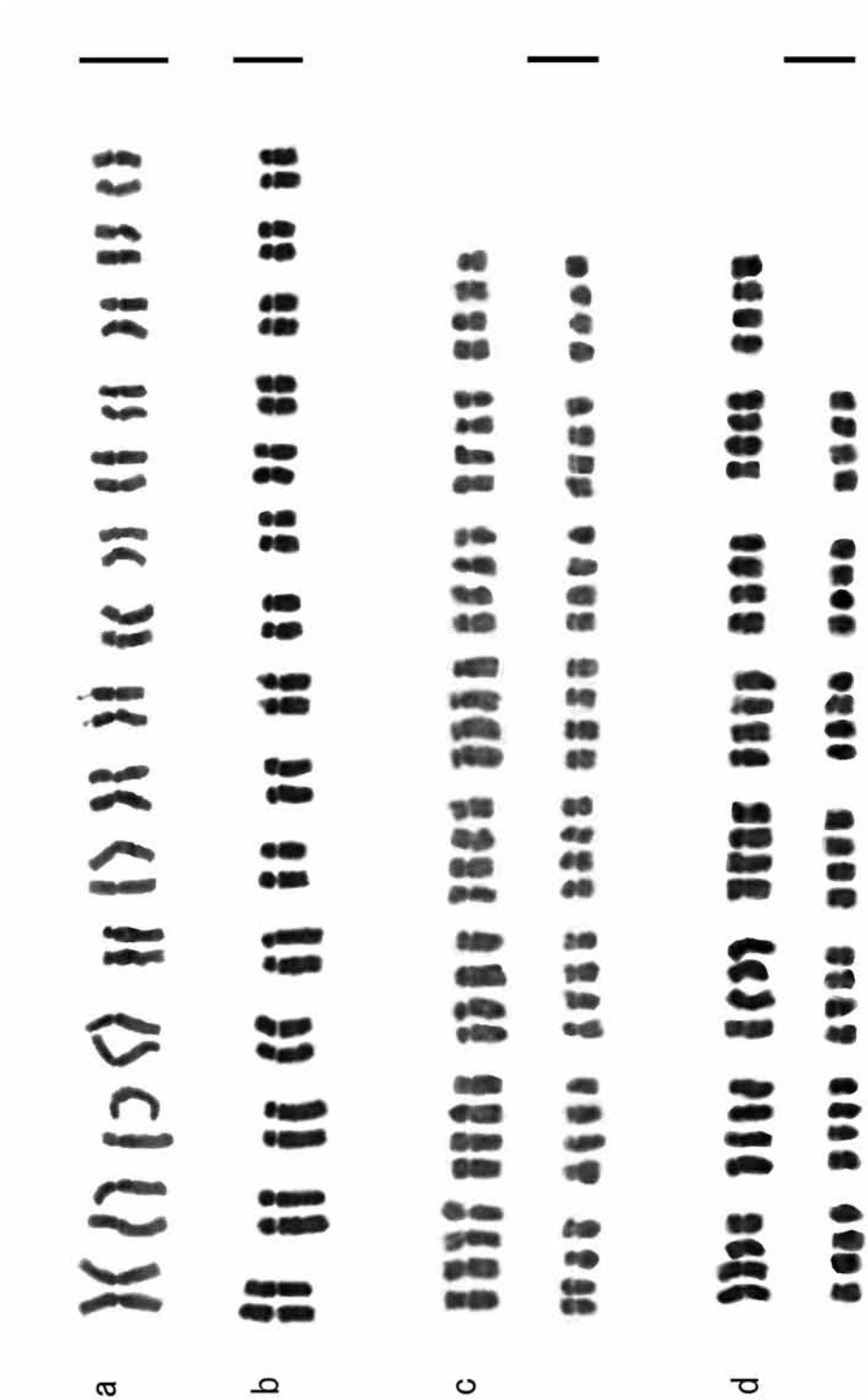

Fig. 2. Karyogram of *Skimmia arborescens* Gamble subsp. *nitida* N. P. Taylor & Airy Shaw (a) in comparison with those of previous results (b–d). a. *S. arborescens* subsp. *nitida* from headstream of Pearl River of Mt. Shiwandashan, Guangxi Autonomous Region, China (CSJ-0004). b. *S. japonica* subsp. *japonica* from Mt. Takatori-yama, Kochi, Shikoku, Japan (Sk-0487). c. *S. japonica* subsp. *reevesiana* var. *reevesiana* (= *S. reevesiana*) from Chitou, Taiwan (Sk-0085). d. *S. arisanensis* from Mt. Ali-shan, Taiwan (Sk-0087). Scale bar = 5 μm.

Table 2. Arm ratio for chromosome pairs (quartets for subsp. reevesiana and S. arisanensis) and average TF% for karyograms of *Skimmia* plants studied

Species	Number of karyograms studied	2n	m	sm	st	Average TF%
S. arborescens subsp. *nitida*	4	30	7–10	3–7	1–2	36.9
S. japonica subsp. *japonica*	2	30	4–5	7	3–4	31.3
S. japonica subsp. *reevesiana* var. *reevesiana*	2	60–64	6–7	6–7	1–3	35.7
S. arisanensis	1	60	5	8	2	34.9

though they are heteromorphic within the first quartet (Fig. 2). It seems to have no supernumeral chromosomes, and satellites were sometimes seen on the smaller (after 10th quartets in length within 15 quartets) quartet (Fukuda et al. 2007). However, this is only one individual observed for this species, and additional study will be needed. The growth form is similar to *S. arborescens* subsp. *nitida*, with erect, tall stems of 3–6 m.

Comparison of arm ratio revealed that *S. arborescens* subsp. *nitida* has 7–10 metacentric chromosome pairs, which was the largest number among karyotypes of compared taxa (Table 2). Though *S. japonica* subsp. *japonica* also had a large metacentric pair, the number of metacentric chromosome pairs was 3–4. *Skimmia japonica* subsp. *reevesiana* var. *reevesiana* had 6–7 quartets, and *S. arisanensis* had 5 quartets of metacentric chromosomes.

Skimmia arborescens subsp. *nitida* also had the largest amount of TF% –36.9% among those species compared (Table 2), which indicates the highest symmetry of arm length. *Skimmia japonica* subsp. *reevesiana* var. *reevesiana* had TF% of 35.7%, *S. arisanensis*–34.9. *Skimmia japonica* subsp. *japonica* had the lowest TF% among these taxa–31.3%.

As a result, we summarize our study as follows.

From this locality, we found only *Skimmia arborescens* subsp. *nitida*, and we could not find *S. kwangsiensis*, though more field researches are required. Karyotype of *S. arborescens* subsp. *nitida* may be different from that of subsp. *arborescens*. Though Taylor (1987) noted the similarity of *S. arborescens* subsp. *nitida* and *S. japonica* subsp. *reevesiana* from morphological aspects, they were not similar in karyotypes. On the other hand, karyotype of *S. japonica* subsp. *japonica* is more similar to that of *S. arborescens* subsp. *nitida* except for the lower karyotype symmetry for the former, and they may be more closely related, in spite of the morphological differences. Further study will be required in karyotype analysis for *S. arborescens* subsp. *arborescens*. In addition, it is necessary to find the type specimen of *S. kwangsiensis* in order to solve the part of taxonomic problems of the genus.

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福田知子[a]，白 積江[b]，陳 涛[b]：**中国広西チワン族自治区十万大山の *Skimmia arborescens* subsp. *nitida*（ミカン科）の染色体解析**

中国広西チワン族自治区十万大山のミヤマシキミ属 *Skimmia*（ミカン科）について調べた．採集したすべての個体は *Skimmia arborescens* Gamble subsp. *nitida* N. P. Taylor & Airy Shaw で，染色体数は 2n = 30 であった．これは亜種としては初めての報告である．既に報告されているミヤマシキミ属の他の分類群の核型との比較を行ったところ，大型の中部動原体型（メタセントリック）染色体を持ち，過剰染色体を持たないこと，サテライトの位置の違いなどの点で，本亜種の核型は日本の本州・四国以北のミヤマシキミの核型に似ていた．また，腕比を調べた結果，本亜種の核型は比較した分類群の中で最も対称的であった．

（[a] 国立科学博物館動物研究部，
[b] 中国広東省深圳市仙湖植物園）