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Cytotaxonomical studies of the genus *Ligustrum* (Oleaceae) in the Ryukyus of Japan

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Abstract. Cytotaxonomical studies were conducted on three species of the genus *Ligustrum* in the Ryukyus. This is the first report of the chromosome number of *L. tamakii* and *L. liukiuense*, which are endemic and semi-endemic to the Ryukyus, respectively, as $2n=46$. Considering the most common basic chromosome number of the family Oleaceae, *L. tamakii* and *L. liukiuense* were regarded as diploid based on $x=23$. As with these two species, all *L. japonicum* individuals had a chromosome number of $2n=46$. This chromosome number is a new count for *L. japonicum* and is clearly inconsistent with previous studies in which *L. japonicum* has been reported to have a chromosome number of $2n=22$ and $n=22$ (i.e., $2n=44$) based on $x=11$.

Keywords: Chromosome number, Endemic species, *Ligustrum*, Oleaceae, Ryukyu Archipelago

Introduction

The genus *Ligustrum* L. in the olive family (Oleaceae) comprises approximately 50 species of evergreen and deciduous shrubs that are distributed in temperate to tropical areas of the Old World; their distribution is centered in East Asia (Hatusima, 1975; Walker, 1976). In the central and southern Ryukyus, south of the Tokara Gap, three *Ligustrum* species grow spontaneously: *L. tamakii* Hatusima, *L. liukiuense* Koidz., and *L. japonicum* Thunb. (Hatusima and Amano, 1994; Shimabuku, 1997).

Of these three *Ligustrum* species, *L. tamakii* is endemic to the Ryukyu Archipelago (Hatusima, 1977). The growth of this prostrate (up to 25 cm in height) evergreen shrub is restricted to the tops of exposed windy limestone cliffs. Only five populations are known from three islands (Tonaki-jima, Irabu-jima, and Yonaguni-jima) in the central and southern Ryukyus (Hatusima and Amano, 1994; Yokota *et al.*, 2006). One population on Tonaki-jima Island disappeared because of vegetation succession; human activities such as pasturage threaten this rare endemic taxon in certain other populations (Yokota *et al.*, 2006). Thus, *L. tamakii* is listed in the Red Data Books for this area (Environment Agency of Japan, 2000; Yokota *et al.*, 2006).

In contrast, *L. liukiuense* is an evergreen shrub endemic to the Ryukyu-Taiwan region (Hatusima, 1975; Hatusima and Amano, 1994; Yang and Lu, 1998). This species is somewhat common and can be found in shrubby areas or forests on five islands (Amami-ohshima, Tokuno-shima, Okinawa-jima, Ishigaki-jima, and Iriomote-jima) of the Ryukyus. Cytotaxonomical studies regarding endemic taxa such as *L. tamakii* and *L. liukiuense* may help to elucidate their relationship with congeneric relatives. However, no such cytological observations of *L. tamakii* or *L. liukiuense* have been made thus far.

In comparison with *L. tamakii* and *L. liukiuense*, *L. japonicum* has a slightly wider range of distribution in the south of the Korean Peninsula and Taiwan, as well as in the southeastern part of Japan, including the Ryukyu Archipelago (Iwatsuki *et al.*, 1993). The chromosome number of *L. japonicum* has been reported as $n=22$ (Sugiura, 1931) and $2n=22$ (Somego, 1974) based on $x=11$, although Somego (1974) considered $n=22$ (i.e., $2n=44$) by Sugiura (1931) as a miscount. The basic chromosome number of $x=11$ is quite uncommon among the phylogenetically derived genera of the Oleaceae, and it would be interesting to verify how a chromosome number of $x=11$ developed within the genus. However, the previous chromosome counts by Sugiura (1931) and Somego (1974) were estimated based on only a small number of individuals from a few

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localities, and the chromosome number of *L. japonicum* should be reexamined based on more samples from a more extensive area.

Chromosomal data should be available as fundamental information for future studies because it will improve the understanding of the origin and establishment of such a diverse flora in the Ryukyus. Indeed, recent cytotaxonomical studies of species from this area have provided valuable results that have helped to elucidate the evolutionary processes associated with endemic taxa or taxa of phytogeographic interest (e.g., Yamashiro *et al.*, 2000; Tamaki *et al.*, 2001; Denda and Yokota, 2003, 2004; Oginuma *et al.*, 2004a, 2004b; Denda *et al.*, 2006). Thus, our objective was to report the chromosome numbers of *L. tamakii*, *L. liukiuense*, and *L. japonicum* in the Ryukyus; no chromosome numbers are currently available for the first two species.

Materials and methods

Details of the collection localities for *L. tamakii*, *L. liukiuense*, and *L. japonicum* are provided in Table 1. For cytotaxonomical analyses, vigorous and healthy shoots were collected from a total of nine *L. tamakii*

Table 1. Collection localities and diploid ($2n$) chromosome number of three *Ligustrum* species examined in the present study

Taxa	Locality	$2n$ chromosome number (number of individuals observed)
Ligustrum tamakii		
	Tonaki-jima Isl., central Ryukyus	
	Umu-no-saki, Tonaki-son, Okinawa Pref.	46 (1)
	Irabu-jima Isl., southern Ryukyus	
	Funausagibanata, Miyakojima-shi, Okinawa Pref.	46 (5)
	Yonaguni-jima Isl., southern Ryukyus	
	Thindabana, Yonaguni-cho, Okinawa Pref.	46 (1)
	Minami-bokujo, Yonaguni-cho, Okinawa Pref.	46 (2)
Ligustrum liukiuense		
	Okinawa-jima Isl., central Ryukyus	
	Yona, Kunigami-son, Okinawa Pref.	46 (2)
	Iyu-dake, Ogimi-son, Okinawa Pref.	46 (1)
	Nekumachiji-dake, Ogimi-son, Okinawa Pref.	46 (2)
	Tano-dake, Nago-shi, Okinawa Pref.	46 (1)
	Iriomote-jima Isl., southern Ryukyus	
	Yuchin river, Taketomi-cho, Okinawa Pref.	46 (2)
Ligustrum japonicum		
	Honshu Isl.	
	Sendabori, Matsudo-shi, Chiba Pref.	46 (2)
	Chuzankei, Shimonoseki-shi, Yamaguchi Pref.	46 (1)
	Kyushu Isl.	
	Kokura, Kitakyushu-shi, Fukuoka Pref.	46 (1)
	Kamikawa-taki, Kinko-cho, Kagoshima Pref.	46 (2)
	Okinawa-jima Isl., central Ryukyus	
	Nekumachiji-dake, Ogimi-son, Okinawa Pref.	46 (2)
	Matsuda, Ginoza-son, Okinawa Pref.	46 (1)
	Tonaki-jima Isl., central Ryukyus	
	Gurukuno-saki, Tonaki-son, Okinawa Pref.	46 (2)
	Ishigaki-jima Isl., southern Ryukyus	
	Banna-dake, Ishigaki-shi, Okinawa Pref.	46 (1)
	Iriomote-jima Isl., southern Ryukyus	
	Yuchin river, Taketomi-cho, Okinawa Pref.	46 (1)

individuals from four localities on Tonaki-jima, Irabu-jima, and Yonaguni-jima, and eight *L. liukiuense* individuals were collected from five localities on Okinawa-jima and Iriomote-jima. In addition, shoots were also collected from seven *L. japonicum* individuals from five localities on Okinawa-jima, Tonaki-jima, Ishigaki-jima, and Iriomote-jima, and from six individuals from four localities in Honshu and the Kyushu Islands. Of these four localities, which are north of Kyushu, Sendabori in Chiba Prefecture lies outside the natural distribution range of *L. japonicum*, indicating that in this region, this species may be an escaped ornamental.

All of the cuttings collected in the field were planted in a greenhouse at the University of the Ryukyus. Root tips were collected from the cuttings for cytological observations after rooting had occurred. Mitotic metaphase chromosomes were then examined using the aceto-orcein squash method. Voucher specimens for all plant materials analyzed were deposited in the herbarium of the Faculty of Science, University of the Ryukyus (RYU).

Results and Discussion

The somatic chromosome numbers of the three *Ligustrum* species examined are listed in Table 1. All *L. tamakii* and *L. liukiuense* individuals showed a somatic chromosome number of $2n=46$ (Fig. 1A, B). This is the first report of the chromosome number of these two species. Accumulated cytological data from preceding studies provided evidence that the most frequent basic chromosome number in the genus *Ligustrum* is $x=23$ (e.g., Sax and Abbe, 1932; Taylor, 1945; George *et al.*, 1989). Thus, the chromosome number of $2n=46$ found in *L. tamakii* and *L. liukiuense* represents a diploid based on $x=23$. Hatusima (1977) mentioned that *L. tamakii*, as well as *L. morrisonense* Hay. in Taiwan, might be treated as subspecies within *L. quihoui* Carr., which is distributed in China and South Korea. *Ligustrum quihoui* has a somatic chromosome number of $2n=46$ (Weng and Zhang, 1992), identical to that of *L. tamakii*. However, chromosome number agreement does not necessarily imply a close relationship between *L. tamakii* and *L. quihoui* because $2n=46$ is the most common chromosome number shared by the phylogenetically derived genera of Oleaceae, including

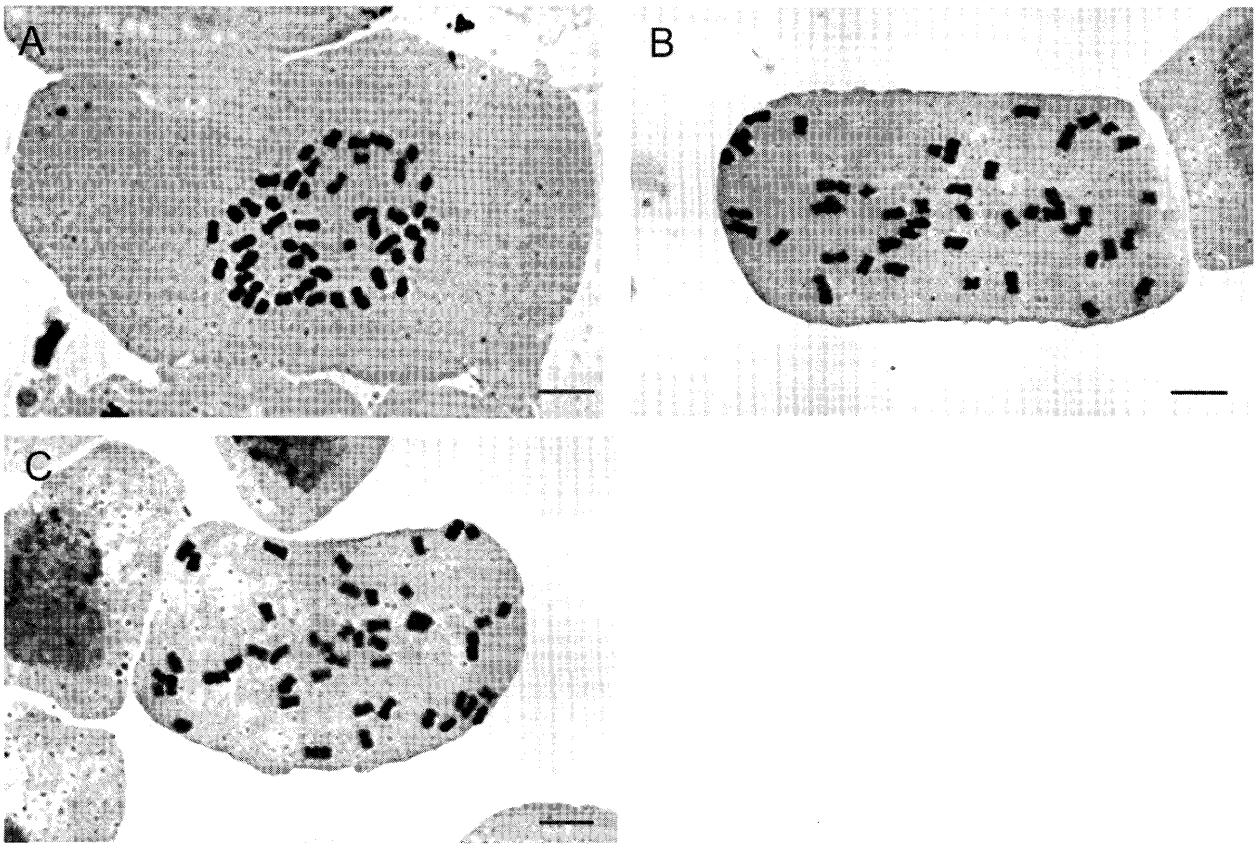

Figure 1. Somatic chromosomes at mitotic metaphase of three *Ligustrum* species in the Ryukyus. A; *L. tamakii* (Thindabana, Yonaguni-jima Isl.), B; *L. liukiuense* (Nekumachiji-dake, Okinawa-jima Isl.), C; *L. japonicum* (Gurukuno-saki, Tonaki-jima Isl.). Scale bars are 5 μm.

the genus *Ligustrum* (Taylor, 1945; George *et al.*, 1989; Wallander and Albert, 2000).

Concerning *L. japonicum*, the somatic chromosome number of all individuals examined was $2n=46$ (Table 1, Fig. 1C), indicating that this species is also a diploid based on the basic chromosome number of $x=23$. This chromosome number of $2n=46$ is a new count for *L. japonicum* and is clearly inconsistent with previous reports in which the chromosome number of this species was estimated as $n=22$ (Sugiura, 1931) or $2n=22$ (Somego, 1974) based on $x=11$. In addition, the basic chromosome number was found to be $x=11$ for *L. lucidum* and *L. ovalifolium* (Somego, 1974), although these two species have been reported elsewhere as $2n=46$ (Taylor, 1945; George *et al.*, 1989). George *et al.* (1989) considered that the chromosome number $x=11$ found in the genera *Menodora* and *Myxopyrum* of the ancestral subfamily Jasminoideae was the original basic chromosome number of the Oleaceae. In contrast, a basic chromosome number of $x=23$, which is assumed to have an allopolyploid origin (Taylor, 1945), is a synapomorphic characteristic shared among the genera of the derived subfamily, Oleoideae, including *Ligustrum* (Taylor, 1945; George *et al.*, 1989; Wallander and Albert, 2000). Taking into consideration our results in which all *L. japonicum* individuals from an extensive area appear to have $2n=46$, it seems unlikely that an ancestral basic chromosome number of $x=11$ would be found within the genus *Ligustrum*, which is primarily $x=23$, although it is possible that the basic chromosome number in the genus has been reduced from $x=23$ to $x=11$. Further and careful chromosome number counts are necessary to confirm the basic chromosome number in the genus *Ligustrum*.

The gross morphology of *L. japonicum* is somewhat similar to that of *L. liukiuense*, from which it differs in terms of larger leaves and fruits. However, several authors have mentioned that *L. liukiuense* is morphologically variable, and many intermediate forms occur between *L. liukiuense* and *L. japonicum* (Hatusima, 1975; Walker, 1976). Walker (1976) suggested that natural hybridization might occur between these two species based on the morphological continuity. In addition, *L. japonicum* is used as a synonym for *L. liukiuense* in the Flora of Taiwan, 2nd edition (Yang and Lu, 1998). We expected that *L. liukiuense* and *L. japonicum* would be distinguishable on the basis of cytotaxonomical features. However, these two species had the same chromosome number of $2n=46$, and their karyotypes could not be determined reliably because of the small chromosome size ranging from ca. 1.0 to 3.0 μm. No cytotaxonomical evidence was obtained supporting the taxonomic delimitation between *L. liukiuense* and *L. japonicum*. The relationship between these two species remains problematic and should be reexamined based on detailed morphological and molecular comparisons.

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