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Karyotype of *Ophiopogon reversus* (Convallariaceae) from Taiwan and the Southern Ryukyus

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ABSTRACT: Karyotypes of *Ophiopogon reversus* (Convallariaceae) collected from four localities in Taiwan and one locality on Yonaguni-jima Island in the southern Ryukyus of Japan were reported for the first time. Those of *O. jaburan* collected from three major islands (Amami-oshima, Tokuno-shima and Okinawa-jima) of the central Ryukyus were also investigated for comparison. All plants of *O. reversus* and *O. jaburan* investigated were diploid ($2n = 36$) based on the basic chromosome number of $x = 18$. Among 36 chromosomes of *O. reversus*, one pair of short chromosomes with submedian centromeres had secondary constrictions at the proximal region of the long arms. Based on this character, *O. reversus* is cytologically distinguishable from *O. jaburan*, in which one pair of short metacentric chromosomes had secondary constrictions at the proximal region of the short arms.

KEY WORDS: Chromosome number, Convallariaceae, Karyotype, *Ophiopogon jaburan*, *Ophiopogon reversus*, The Ryukyu Archipelago, Taiwan.

INTRODUCTION

The genus *Ophiopogon* (Convallariaceae, Ophiopogoneae) is an evergreen perennial herb that forms attractive clumps of simple, linear, and lush leaves with spikes of showy white flowers and has bright bluish seeds. This genus comprises approximately 55 species that are widely distributed in temperate to tropical Asia from Japan, west to India through China and the Himalayas, and south to Java through Indochina. In the Ryukyu Archipelago of Japan, besides *O. jaburan* (Kunth) Lodd. and *O. japonicus* (Thunb.) Ker-Gawl., which have been listed in the floras of this area (Hatusima, 1975; Walker, 1976; Shimabuku, 1997), *O. reversus* C. C. Huang was recently reported from Yonaguni-jima in the southern Ryukyus (Tanaka, 2001a, b). Apart from the Ryukyus, *O. reversus* is distributed in southern China (Hainan Island, western Guangxi and Hong Kong) and Taiwan (Tanaka, 2000, 2001b). In other words, Yonaguni-jima is located at the northeastern limit of this species' range. *Ophiopogon jaburan* has also been recorded from Yonaguni-jima (e.g., Fujimoto, 1972; Walker, 1976; Niiro and Shinjyo, 1989). However, Tanaka (2001a, b) mentioned that this might be the result of misidentification of *O. reversus*. Indeed, *O. reversus* is morphologically resemblant to *O. jaburan*, but its flowers are small, with shorter pedicels and creamy anthers (Tanaka, 2001b).

Previous cytological works (e.g., Nagamatsu and Noda, 1964, 1971; Hasegawa, 1968; Yang et al., 1990; Kondo et al., 1992; Yamashita and Tamura, 2001) have revealed chromosome numbers for 26 of 55 *Ophiopogon* species; the basic chromosome number of this genus is estimated as $x = 18$ (Tamura, 1995). Accumulation of cytological data is very important to deepen our understanding of the cytotaxonomical features of the genus *Ophiopogon*. With regard to *O. reversus*, however, no cytological observations have been made to date. This study is intended to reveal the respective karyomorphologies of *O. reversus* collected in Taiwan and the southern Ryukyus of Japan for accumulating cytotaxonomical information of the genus. *Ophiopogon jaburan* collected from the central Ryukyus was also examined for comparison.

MATERIALS AND METHODS

A total of 14 individuals of *O. reversus* were collected from four localities (Urai, Taroko National Park, Mt. Hontou-shan and Tienchih Lake) on the main island and Lanyu Island of Taiwan and one locality (Mt. Kubura) on Yonaguni-jima in the southern Ryukyus of Japan (Table 1, Fig. 1). All

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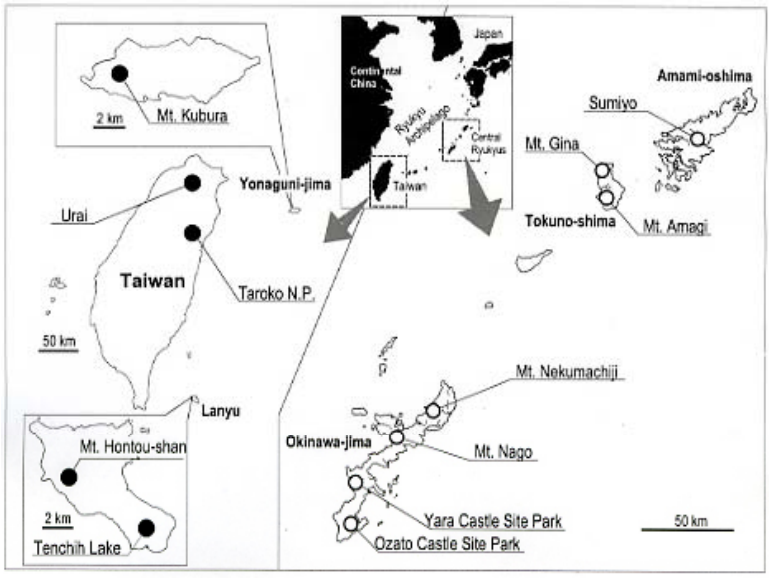

Fig. 1. Collection localities of *Ophiopogon reversus* and *O. jaburan* in Taiwan and the Ryukyus. Solid circles are *O. reversus* and open circles are *O. jaburan*. For details on localities, see also Table 1.

Table 1. Collection localities and diploid chromosome number of *O. reversus* and *O. jaburan* used in this study.

Taxa	Locality	Diploid chromosome number (number of individuals examined)	Voucher
Ophiopogon reversus C. C. Huang	Yonaguni-jima Island, the southern Ryukyus		
	Mt. Kubura, Yonaguni-cho, Okinawa Pref., Japan	36 (4)	DT050081
	The main island of Taiwan		
	Urai, Taipei County, Taiwan	36 (2)	NK061362
	Taroko N. P., Fushih Village, Hualien County, Taiwan	36 (3)	NK061363
	Lanyu Island, Taiwan		
	Mt. Hontou-shan, Taitung County, Taiwan	36 (3)	NK061364
	Tenchih Lake, Taitung County, Taiwan	36 (2)	NK061365
O. jaburan (Kunth) Lodd.	Amami-oshima Island, the central Ryukyus		
	Sumiyo, Sumiyo-son, Kagoshima Pref., Japan	36 (1)	DT050121
	Tokuno-shima Island, the central Ryukyus		
	Mt. Gina, Isen-cho, Kagoshima Pref., Japan	36 (1)	NK061360
	Mt. Amagi, Tokuno-shima-cho, Kagoshima Pref., Japan	36 (2)	NK061361
	Okinawa-jima Island, the central Ryukyus		
	Mt. Nekumachiji, Ogimi-son, Okinawa Pref., Japan	36 (2)	DT050078
	Mt. Nago, Nago-shi, Okinawa Pref., Japan	36 (1)	DT050072
	Yara Castle Site Park, Kadena-cho, Okinawa Pref., Japan	36 (2)	DT050102
	Ozato Castle Site Park, Ozato-son, Okinawa Pref., Japan	36 (1)	DT050101

these plants bloomed relatively small flowers with shorter pedicels and creamy anthers under cultivated conditions (Fig. 2), confirmed their taxonomical identity as *O. reversus*. In addition to *O. reversus*, 10 individuals of *O. jaburan* were also collected from one locality (Sumiyo) of Amami-oshima, two localities (Mt. Amagi and Mt. Gina) on Tokuno-shima, and four localities (Mt. Nekumachiji, Mt. Nago, Yara Castle Site Park and Ozato Castle Site Park) on Okinawa-jima (Table 1, Fig. 1).

For cytological observations, root tips were collected from plants that had been transplanted to a greenhouse at the University of the Ryukyus and mitotic chromosomes were then examined using the aceto-orcein squash method. Descriptions for the position of centromeres on metaphase chromosomes were in accordance with those specified by Levan et al. (1964). Vouchers were deposited in the herbarium of the Faculty of Science, University of the Ryukyus (RYU).

RESULTS AND DISCUSSION

All plants of *O. reversus* collected from Taiwan and Yonaguni-jima showed the chromosome number of $2n = 36$ (Table 1, Fig. 3). Because the basic chromosome number of the genus *Ophiopogon* is $x = 18$ (Tamura, 1995), $2n = 36$

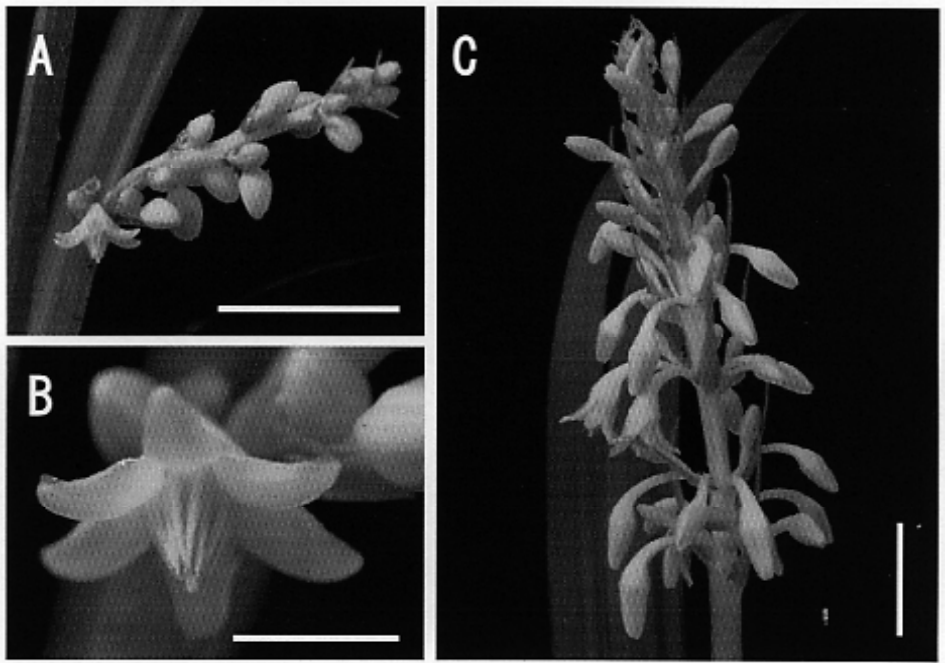

Fig. 2. Inflorescence and flower of *Ophiopogon reversus* and *O. jaburan*. A and B: *O. reversus* in Mt. Kubura (Yonaguni-jima). C: *O. jaburan* in Mt. Nekumachiji (Okinawa-jima). Scale bars are 2 cm in A and C, and 5 mm in B.

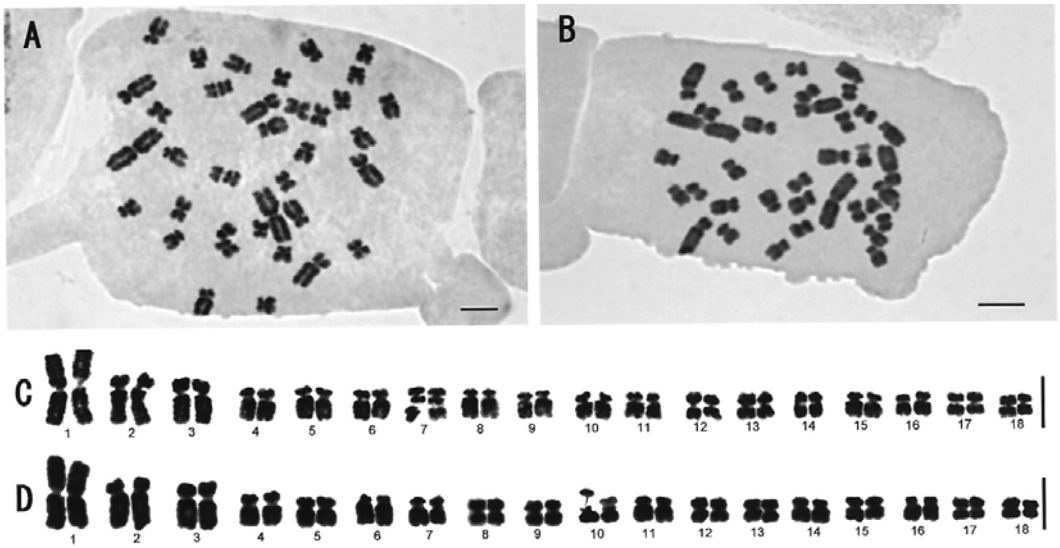

Fig. 3. Somatic chromosomes at mitotic metaphase of *Ophiopogon reversus* and *O. jaburan*. A and C: *O. reversus* in Mt. Hontou-shan, (Lanyu Island) and Mt. Kubura (Yonaguni-jima), respectively. B and D: *O. jaburan* in Mt. Amagi (Tokuno-shima). Scale bars are 5 µm.

represents a diploid. Somatic chromosomes of *O. reversus* consisted of 12 metacentric chromosomes (Figs. 3A, C-1, C-12 to 14, C-17, 18), 22 submetacentric chromosomes (Figs. 3A, C-2 to 7, C-9 to 11, C-15, 16) and two subtelocentric chromosomes (Fig. 3A, C-8). Of these 36 chromosomes, a pair of metacentric chromosomes (Fig. 3A, C-1) was long (ca. 7.5 µm), two pairs of submetacentric chromosomes (Figs. 3A, C-2, 3) had medium length of ca. 5.4 to 4.9 µm, and the other pairs of metacentric, submetacentric or subtelocentric chromosomes (Figs. 3A, C-4 to 18) were short, with length ranging from ca. 3.6 to 2.3 µm. In addition, one pair of short chromosomes with submedian centromeres had secondary constrictions at the proximal region of the long arms (Fig. 3A, C-7).

On the other hand, karyological features of *O. jaburan* investigated in this study corresponded to those reported in previous studies (Yamashita and Tamura, 2001). Somatic metaphase chromosomes of *O. jaburan* collected in the central Ryukyus consisted of one pair of long (ca. 7.8 μm) metacentric chromosomes (Figs. 3B, D-1), two pairs of medium-sized (ca. 5.7 to 4.8 μm) submetacentric chromosomes (Figs. 3B, D-2, 3), and 15 pairs of short (ca. 3.8 to 2.0 μm) metacentric, submetacentric or subtelocentric chromosomes (Figs. 3B, D-4 to 18). Among these chromosomes, one pair of short (ca. 2.4 μm) metacentric chromosomes had secondary constrictions at the proximal region of the short arms (Figs. 3B, D-10). These results indicate that *O. reversus* is cytotaxonomicaly distinguishable from *O. jaburan*, especially on the basis of morphological differences in chromosomes with secondary constriction.

Yamashita and Tamura (2001) categorized the karyotypes of six species of the genus *Ophiopogon* into two groups: the Jaburan-group and the Brevipes-group. The Jaburan-group, comprising four species (*O. jaburan*, *O. planiscapus*, *O. siamensis* and *O. sylvicola*) that are distributed in temperate zones, is characterized by two pairs of medium-sized chromosomes. On the other hand, the Brevipes-group, comprising only *O. brevipes*, which is distributed in a tropical monsoon zone, is characterized by three pairs of medium-sized chromosomes. The karyotype of *O. reversus*, which is characterized by a pair of large metacentric chromosomes, two pairs of medium-sized chromosomes and a pair of short chromosomes with secondary constriction, clearly corresponds to those of the Jaburan-group. Tanaka (2001b) mentioned that *O. reversus* is considered to be related closely to *O. jaburan* and *O. japonicus* because these three species share distinctive characteristics such as thick fibrous roots and diurnal flowers. Based only on the morphology of chromosomes with secondary constrictions, however, *O. reversus* seems to show some similarity with *O. planiscapus* (Jaburan-group), which has a pair of short submedian chromosomes with secondary constrictions at the proximal region of the long arms (Yamashita and Tamura, 2001). Karyomorphological features were not correlated with morphological characters in the genus *Ophiopogon*. Yamashita and Tamura (2000) showed a sister relationship between *O. jaburan* and *O. japonicus* based on chloroplast DNA sequence data, but no information has been obtained to date about a phylogenetic position of *O. reversus* in the genus. Molecular phylogenetic analysis of *O. reversus* is desirable to reveal phylogenetic relationships among congeneric species, and to evaluate karyological information of this genus against the phylogenetic background.

As indicated by Tanaka (2001b), misidentification between *O. reversus* and *O. jaburan* might cause problems in identifying the distribution range and abundance of *O. reversus* in the southern Ryukyus. Indeed, despite several records of *O. jaburan* in Yonaguni-jima (e.g., Fujimoto, 1972; Walker, 1976; Niiro and Shinjo, 1989), we have not found any *Ophiopogon* species except *O. reversus* at Mt. Kubura through our field researches on this island in March and August, 2005. Although *O. jaburan* has also been reported on other islands of the southern Ryukyus (e.g., Chinen, 1993), it remains unclear whether *O. reversus* and *O. jaburan* occur sympatrically in the southern Ryukyus or whether their distribution does not overlap in this area. For further study to reveal the relationship between *O. reversus* and *O. jaburan* and for deepening our understanding of the flora of the Ryukyus and Taiwan, distribution of these two species in the southern Ryukyus must be reinvestigated through careful and precise identification.

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臺灣與琉球南部產鈴蘭科高節沿階草之核型分析

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摘　　要

本文首次報導採自臺灣 (四個採集點) 與日本琉球南部與那國島 (一個採集點) 之鈴蘭科高節沿階草 (*Ophiopogon reversus*, Convallariaceae) 之核型；同時與採自琉球中部三個主要島嶼 (奄美大島、德之島、冲繩島) 之闊葉沿階草 (*O. jaburan*) 之核型做比較。根據本屬植物之染色體基數 $x = 18$ 可知，本研究中所調查的高節沿階草與闊葉沿階草的個體，均屬於二倍體 ($2n = 36$)。高節沿階草的 36 條染色體，在其中一對短的近端中結染色體上，位於長臂基部的區域具有二次狹窄(或稱次級收縮區)；而闊葉沿階草則在其中一對短的中央中結染色體上，位於短臂基部的區域具有二次狹窄。依據此一特徵，高節沿階草可以和闊葉沿階草從細胞學上加以區分。

關鍵詞：染色體數、鈴蘭科、核型、闊葉沿階草、高節沿階草、琉球羣島、臺灣。

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