

研究報告

臺灣產天門冬科綿棗兒之核型研究

謝侑廷¹ 趙建棣² 曾彥學^{1,3*}

【摘要】綿棗兒 (*Barnardia japonica* (Thunberg) Schultes & J. H. Schultes) 廣泛分布於東亞，其生長於各類型棲地，並以核型高度變異著稱。本研究分析此物種於北台灣各族群的核型。結果顯示，臺灣的綿棗兒具有三種不同的核型—AA型 ($2n = 2x = 16$)、BB型 ($2n = 2x = 18$) 及AABB型 ($2n = 4x = 34$)，其中BB型為首次在臺記錄之核型。本研究未於任何族群中發現B染色體。根據核型的分布，二倍體族群與多倍體族群間存在一明顯的地理界線，顯示不同核型間存在棲地上的區隔。AABB型為最廣佈的核型，此核型分布於海濱區域。相較之下，二倍體族群僅發現於較內陸的丘陵。這些發現顯示多倍體化可能造成族群間的棲位分化。

【關鍵詞】染色體、基因體、核型、*Scilla scilloides*、*Barnardia japonica*。

Research paperThe karyotype of *Barnardia japonica* (Asparagaceae) in TaiwanYou-Ting Hsieh¹ Chien-Ti Chao² Yen-Hsueh Tseng^{1,3*}

【Abstract】*Barnardia japonica* (Thunberg) Schultes & J. H. Schultes grows in a wide range of habitats across East Asia and is known to have highly variable karyotypes. In this study, the karyotype of different populations from northern Taiwan was analyzed. Among the three karyotypes identified—abbreviated as AA ($2n = 2x = 16$), BB ($2n = 2x = 18$), and AABB ($2n = 4x = 34$)—BB was recorded for the first time in Taiwan. B chromosomes F and f were not found in all materials collected. The results suggested the presence of a clear geographic boundary between diploid and polyploid populations, and revealed that

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different karyotypic populations occupied different habitats. AABB was the most widespread karyotype as all populations of this type were observed in the island's coastal regions. In contrast, types AA and BB were present only on inland hills. These findings indicate the existence of niche differentiation by polyploidy.

【Key words】 chromosome; genome; *karyotype*; *Scilla scilloides*; *Barnardia japonica*.

Introduction

Polyploidy, the process by which an individual doubles its genome copy number, is a recurrent and important strategy used by flowering plants in order to increase genetic variation (Stebbins 1950; Grant 1981; Wood et al. 2009). With double the amount of genetic material, polyploids improve their chances of survival when faced with environmental changes, through a more flexible gene regulation (Paqion & Adams 1983; Parisod et al. 2010; Van de Peer et al. 2017). Several studies showed that diploid and polyploid species occupy different geographic and climatic niches (Mandákov & Münzbergov 2008; Ramsey 2011; Mcintyre 2012; Theodoridis et al. 2013; Thompson et al. 2014), and that the polyploid lineage of introduced plants may result particularly invasive because of the broader ecological niche these species can occupy (Treier et al. 2009).

The genus *Barnardia* Lindley comprises two species: *Barnardia numidica* (Poir.) Speta, distributed in Europe and northern Africa, and *B. japonica* (Thunberg) Schultes & J. H. Schultes distributed in East Asia (Figure 1)(Chen & Tamura 2000). Before, this genus was often treated as part of *Scilla s. l.* (see review in Speta 1998a), but recent studies seem to consider it as an independent genus of the Asparagaceae or Liliaceae *s. l.* (Speta 1998ab; Chen & Tamura 2000; Tamura 2016). *Barnardia japonica* has a complicated taxonomic history, as a large

number of names have been assigned to this species, including: *B. scilloides* Lindley; *Scilla chinensis* Benth., nom. illeg.; *S. japonica* auct. non Thunb.: (Thunberg) Baker, nom. illeg.; *S. scilloides* (Lindley) Druce; *S. sinensis* (Lour.) Merrill; and *S. thunbergii* Miyabe et Kudo (Liu & Ying 1978; Chen & Tamura 2000; Ying 2000). Although these names have generally appeared in related researches, Chen & Tamura (2000) recognized *Barnardia japonica* (Thunberg) Schultes & J. H. Schultes as the correct name of this species, and such treatment was followed by Yang et al. (2001) and Tamura (2016), as well as in the present study. Researchers were also attracted by the highly variable karyotypes *B. japonica* displayed. Morinaga (1932) first reported three karyotypes from different populations of this species near Fukuoka in Japan, providing additional notes on their genome constitution. Two genomes, A ($n = 8$) and B ($n = 9$), were further defined (Araki 1971; Noda 1974; Haga & Noda 1976), and the morphology of each was described by Araki (1971). The A genome was defined by the longest metacentric a_1 and subtelocentric a_2 chromosomes. In AA diploids, the a_2 chromosomes of the A genome presented a secondary constriction at the proximal part of the short arm, while this was not the case in AABB plants. The B genome was defined by the longest subtelocentric b_1 chromosomes, which presented a secondary constriction at the

proximal part of the short arm, and four pairs of short metacentric b_6 - b_9 chromosomes. To identify the geographic distribution of the different karyotypes, populations across East Asia were analyzed (see review in Hizume & Shibata 2016). The results showed that diploid types AA and BB, and tetraploid type AABB were the three most common types (Shibata et al. 2017b). Also, various karyotypes seemed to have originated from allopolyploidy and hybrid states, including diploid and amphidiploid types (Ding et al. 1998; Shibata et al. 2017a). Araki (1971) artificially hybridized AA and BB types, producing AB karyotype offsprings. Therefore, it has been suggested that the AABB karyotype might have originated from the polyploidization of the AB type.

Taiwan is a mountainous island, located southeast of China and south of Japan, and *B. japonica* is only distributed in its northern regions (Ying 2000). Five populations were studied cytologically and two karyotypes—AA and AABB—were recorded (Chang & Hsu 1974; Uchino et al. 1992; Ding et al. 1998; Hizume & Shibata 2016). Shibata et al. (2017a, b) concluded that the polyploid type AABB originated at least twice in different areas. The maternal parent of the Taiwanese AABB is karyotype BB of Japan, and the paternal parent is an undiscovered karyotype AA population (Shibata et al. 2017b).

It must be noted that these analyses often relied on a small number of plant samples, and to explore the evolutionary history of this species complex from the perspective of genome differentiation and speciation, more information on the karyotypes of different populations is necessary. Reports on karyotypes from the Taiwanese *B. japonica* are sparse and need to be updated. Therefore, the aim of this study was to

conduct a survey of the karyotypes distributed in Taiwanese populations, and to discuss them in relation to their corresponding habitats.

Materials and methods

Plant material

A total of 38 bulbs were collected from six populations across the whole distribution range of *B. japonica* in Taiwan. Sample details are shown in Table 1. The bulbs were planted in plastic pots in the experimental garden of the Department of Forestry, National Chung Hsing University, under natural daylight. Voucher specimens were deposited in the herbarium of National Chung Hsing University (TCF).

Chromosome observation

Root tips were collected from individuals cultivated and pre-treated with 2 mM of 8-hydroxyquinoline for 6 hours at 0°C. Then, the samples were fixed in Carnoy's solution (absolute ethanol: acetic acid = 3:1, v:v) and stored in a freezer overnight. Fixed root tips were treated with 1M HCl at 60°C for 60 s to digest celluloses, and were then stained with 2% acetic orcein overnight at room temperature. After staining, root tips were dissected, each on a glass slide, covered their surface with a few drops of 2% acetic orceins, and then squashed with a coverslip. Slides were examined and photographed using an optical microscope (ACCU-Scope 3025) equipped with a CCD camera (ProgRes C14plus). The interpretation of the chromosomes of A and B genomes was based on Araki (1971). Two different small supernumerary chromosomes (i.e., B chromosomes), designated as F and f based on Ding et al. (1998), were also counted.

Distribution map

The distribution map of the different karyotypes was generated using QGIS ver. 3.10.6 (QGIS.org, 2021). Su (1984ab; 1985) proposed

a scheme of regional climates that summarized the range of climatic variation existing between areas with different vegetation types on the island. The scheme was also digitalized using QGIS ver. 3.10.6, and was used in this study for the identification of any relationship between climate and karyotype distribution.

Results

By their chromosome composition, we clarified that all 38 *B. japonica* plants collected from the six populations in this study belonged to three karyotypes (Figure 2; Table 1). Each genome could be identified by the characteristic chromosomes. The A genome was mainly identified by the longest metacentric a_1 chromosomes, and the B genome was mainly identified by the longest subtelocentric b_1 chromosomes, which has a secondary constriction at the proximal part of short arm. The AA ($2n = 2x = 16$) and BB ($2n = 2x = 16$) diploid

types were found in the Tongxiao and Dagu Mountain populations, respectively. The AABB ($2n = 4x = 34$) tetraploid type was found in Fuguijiao, Bitoujiao, Heping Island, and Elephant Trunk Rock Scenic Area populations. No B chromosomes were observed in Taiwan.

Our study identified different karyotypes and their distribution on the island (Figure 3). Results showed that AABB populations are distributed along the coastline of northern Taiwan, while AA populations are distributed in Miaoli County and Taoyuan city, which are located further south than the distribution areas of the AABB type. The unique BB population is also distributed in Taoyuan city. Based on the scheme proposed by Su (1984a, 1984b, 1985), the diploid populations are found in the northwest region (NW) and central west region (CW), while all polyploid populations are found in the northeast region (NE), except for the Bitoujiao population.

Table 1. Habitat information and karyotypes of eight *Barnardia japonica* populations in Taiwan.

Locality	GPS (WGS84)	Date of bulb collection	Voucher	Sample number	Habitat	Karyotype
New Taipei City: Fuguijiao	25.292543, 121.538746	2016.10.22	<i>Y. T. Hsieh 2</i>	5	Seashore	AABB ($2n=4x=34$)
New Taipei City: Bitoujiao	25.122112, 121.919773	2016.10.22	<i>Y. T. Hsieh 1</i>	17	Seashore	AABB ($2n=4x=34$)
New Taipei City: Elephant Trunk Rock Scenic Area	25.135172, 121.824491	2016.10.22	<i>Y. T. Hsieh 3</i>	8	Seashore	AABB ($2n=4x=34$)
Keelung City: Heping Island	25.161850, 121.765662	2016.10.22	<i>Y. T. Hsieh 4</i>	6	Seashore	AABB ($2n=4x=34$)
Miaoli County: Tongxiao	24.536929, 120.704727	2012.09.06	<i>C. T. Chao 2452</i>	1	Hill near coastline	AA ($2n=2x=16$)
Taoyuan City: Dagu Mountain	25.101322, 121.290310	2015.09.02	<i>C. T. Chao 3826</i>	1	Hill near coastline	BB ($2n=2x=18$)

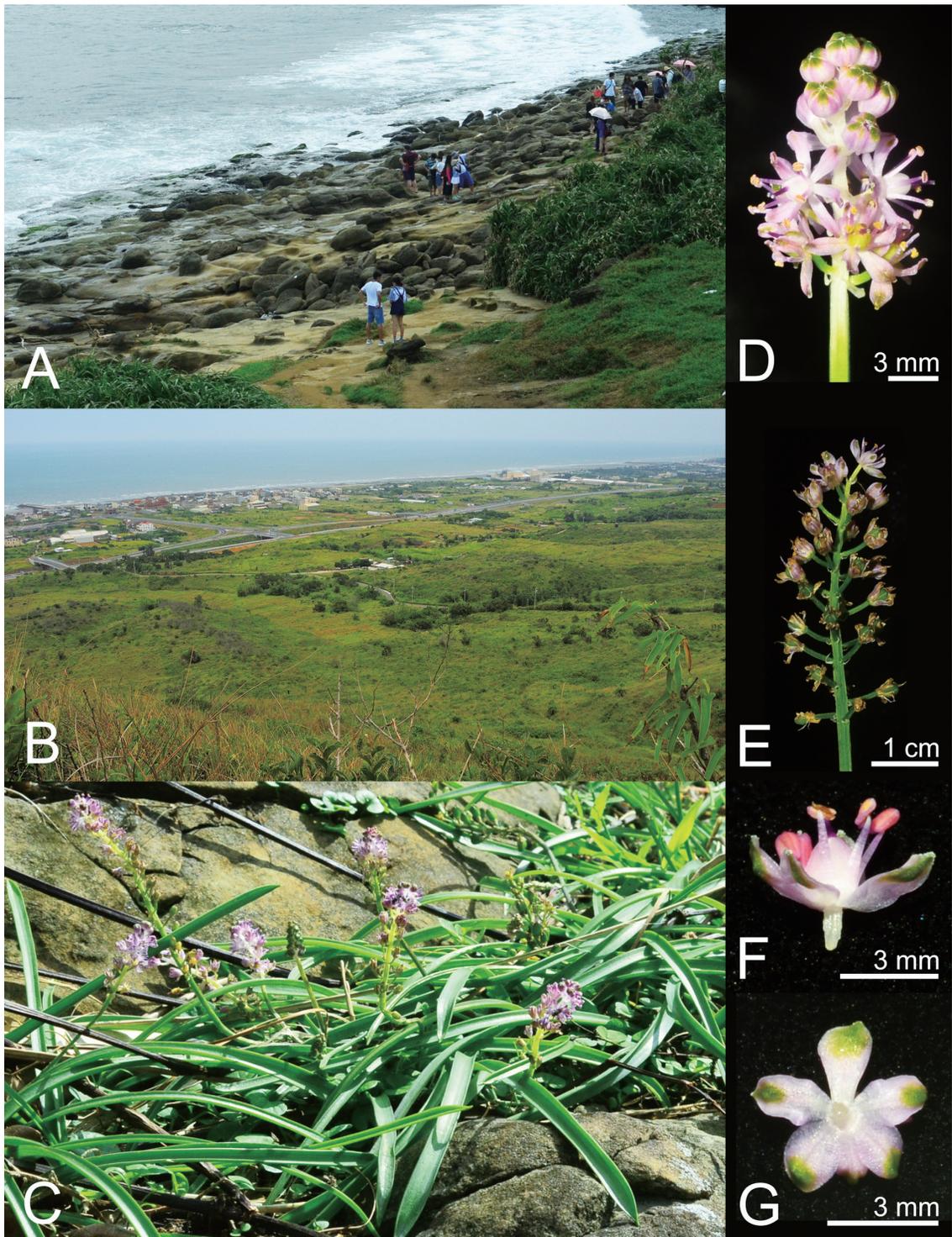


Figure 1. Photographs of *Barnardia japonica* (Thunberg) Schultes & J. H. Schultes. A: Habitat of seashore; B: Habitat of hill near coastline; C: Habit; D: Inflorescence; E: Young infructescence; F-G: Flower, lateral and posterior view, respectively.

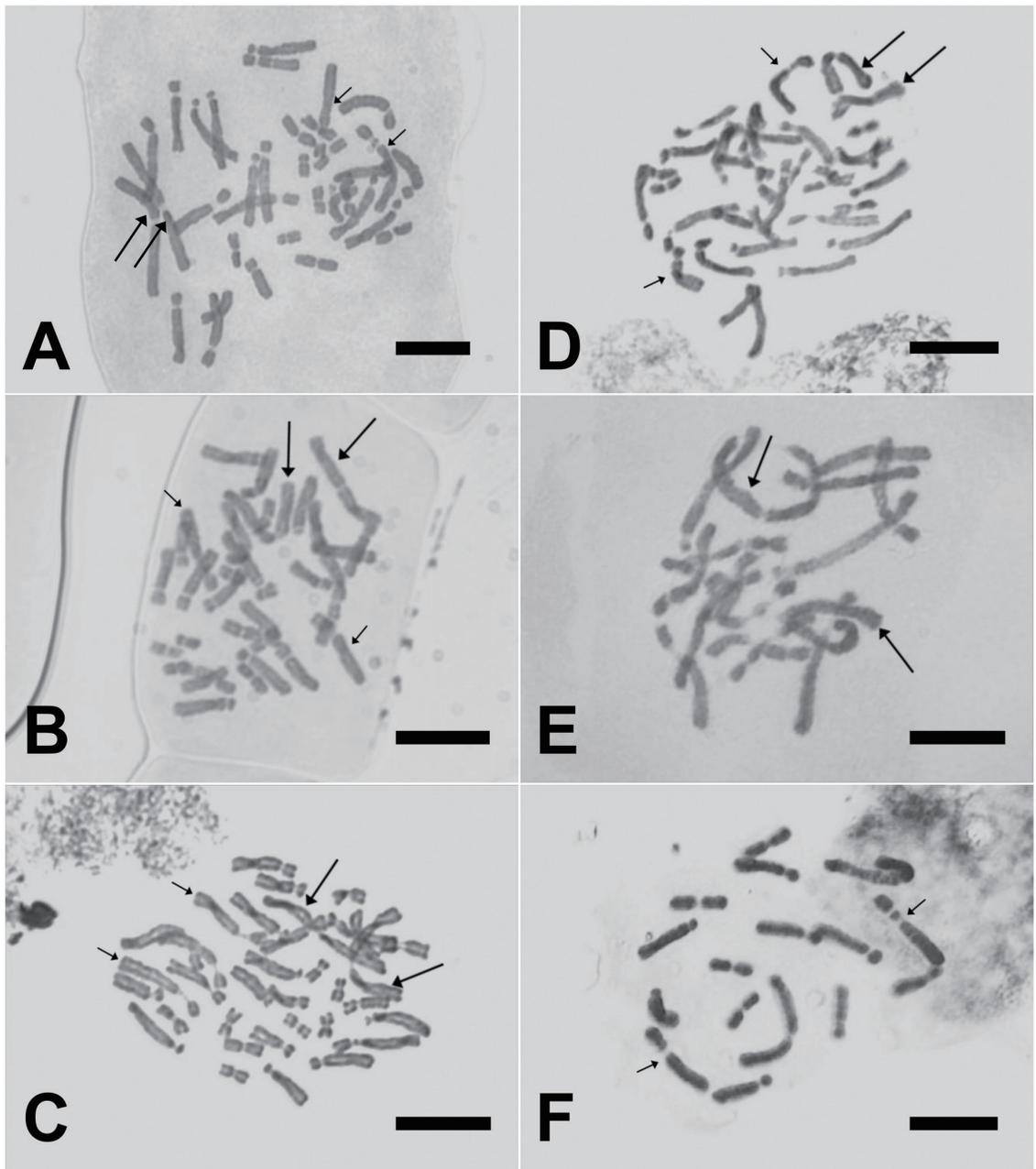


Figure 2. Chromosomes of different populations of *Barnardia japonica* (Thunberg) Schultes & J. H. Schultes in Northern Taiwan (see Table 1 for detailed information about each population). A: Fuguijiao (AABB). B: Bitoujiao (AABB). C: Elephant Trunk Rock Scenic Area (AABB). D: Heping Island (AABB). E: Tongxiao (AA). F: Dahu Mountain (BB). Long arrows show the a1 chromosome, and short arrows show b1 chromosome. Scale bar: = 10 μ m.

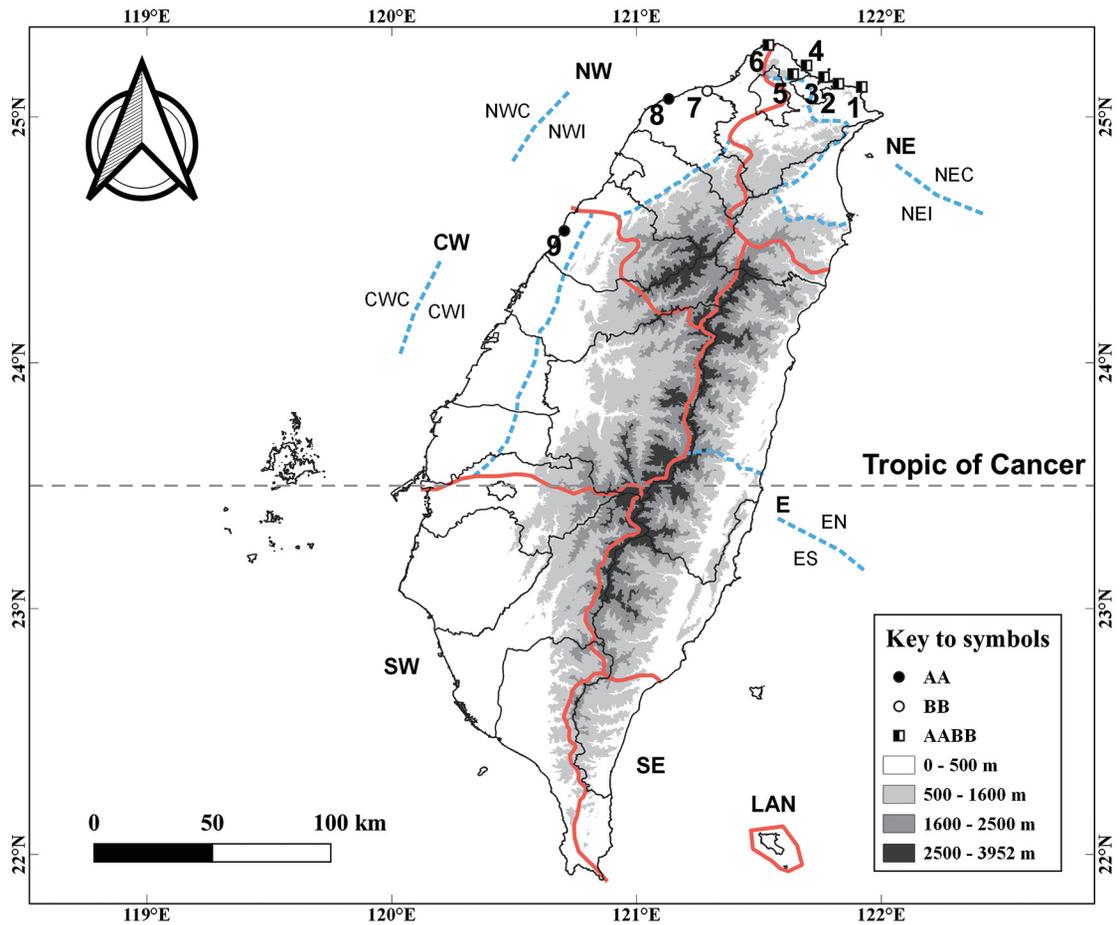


Figure 3. The distribution map of populations of *Barnardia japonica* in Taiwan with their karyotypes. Population No. are as below: 1, Fuguijiao. 2, Elephant Trunk Rock Scenic Area. 3, Heping Island. 4, Yehliu. 5, Wan-li. 6, Bitoujiao. 7, Dagu Mountain. 8, Taoyuan. 9, Tongxiao. Detailed GPS coordinates and the cited references used to plot this map are listed in the Appendix table 1. Red solid lines indicate major climate regions in Su (1985): NW, northwest region. CW, central west region. SW, southwest region. SE, southeast region. E, east region. NE, northeast region. LAN, Lanyu region. Blue dot lines indicate sections in each climate region: C, for coastal section. I, for inland section. N, north section. S, south section.

Discussion

In Taiwan, the karyotypes of *B. japonica* were reported by Chang & Hsu (1974), Uchino et al. (1992), Ding et al. (1998), and Hizume & Shibata (2016). The AA diploid type was reported in Taoyuan (Uchino et al. 1992) and Tongxiao, the latter being a new population with

the southernmost distribution recorded for the AA type in Taiwan. The AABB polyploid type was reported in Wan-li (Chang & Hsu 1974), Bitoujiao (Ding et al. 1998), Heping Island (Hizume & Shibata 2016), and Yehliu (Hizume & Shibata 2016). Our survey confirms previous results obtained from the karyotypes of Heping

Island and Bitoujiao (Ding et al. 1998; Hizume & Shibata 2016), and in addition, it records two new populations of AABB karyotype in Fuguijiao and Elephant Trunk Rock Scenic Area. Also, the BB karyotype from Dagu Mountain is a novel type recorded for the first time in Taiwan.

Current karyotype records suggest a clear geographic boundary between diploid and polyploid populations. Su (1985) argued that the difference in climate between the NE region and NW + CW regions could be mostly explained by the ratio of winter rain to annual precipitation. NW and CW regions present a relatively dry climate with rainy summers; in contrast the NE region is characterized by a wet climate all year around, with higher humidity compared to the NW + CW regions. *B. japonica* usually grows in diverse habitats, including forest margins, hillsides, open slopes, and grasslands, from near sea level to 2,600 m of altitude (Noda 1976; Chen & Tamura 2000). On islands, this species is often found near the seashore (Araki 1985; Haga & Noda 1976; Hizume & Shibata 2016). The distribution pattern of *B. japonica* in different climate regions in Taiwan could be related to karyotypes and their habitat preference. Polyploid plants generally present various physiological characteristics, which are different from those of their diploid progenitors (Warner et al. 1993; Dhawan et al. 1996; Vyas et al. 2007; Hull-Sanders et al. 2009; Ni et al. 2009), and empirical studies showed that polyploids have a better tolerance to water-stress (Bottini et al. 1999; Manzaneda et al. 2012). Therefore, it is interesting that polyploid AABB populations in Taiwan seem to colonize humid regions more than diploid populations do. However, AABB populations were mainly discovered in coastal dune environments near the seashore, while AA and BB populations were

observed only on the inland hills near coastal areas in Taiwan. Coastal dunes are particularly extreme environments for most plants (Hesp 1991); stresses include salt spray, dryness, high light intensity, and wind exposure, which represent strong selection pressures for many species (Hesp 1991). Although climatic differences are moderate for polyploidy populations on the island, the dune microhabitat might still be arid for *B. japonica*. Ding et al. (2005) suggested that auto-polyploidization in this species might be associated with the arid climatic conditions. Ultimately, the polyploid AABB population might survive and colonize such extreme habitats by evolving physiological adaptations.

The scenario in which *B. japonica* with different karyotypes colonizes different habitats has been argued before. Ding et al. (1998) reported that AA and BB types are the main karyotypes distributed in China, while the AABB type is common in Japan and Korea. Although AABB plants are also found in China, they are mainly limited to the overlap zone between the distributions of AA and BB diploids in southeastern China (see reviews in Ding et al. 1998; Hizume & Shibata, 2016). Ding et al. (1998) suggested that the recent discontinuity in the distribution of AABB populations may be a result of multiple origins, and this hypothesis has been confirmed by the analysis of molecular data (Shibata et al. 2017b). In addition, two kinds of subtypes within the AABB karyotype—the Okinawa-type and common-type—were identified (Hizume & Araki 1994). Compared to the common type, the Okinawa-type presented a secondary constriction only at chromosome a_2 , and it was only discovered in Okinawa (Hizume & Araki 1994) and Taiwan (Hizume & Shibata 2016). Hizume & Shibata (2016) suggested that

this type, given its geographical distribution, might successfully adapt to the island environment.

After combining the results of more recent and previous studies of karyotypes and their distribution (Ding et al. 1998; Hizume & Shibata 2016), we suggest that polyploid plants are potentially able to adapt to coastal environments more than diploid plants can. It is possible that polyploidy was a key feature in the adaptation of *B. japonica* in East Asia islands. The relationship of each karyotype to its habitat might be explained by further physiological studies of *B. japonica*.

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Appendix table 1. Detailed GPS coordinates and the cited references used in Figure 3.

No. of sample	Location	Karyotype	GPS (WGS84)		Reference	True GPS
			Latitude	Longitude		
1	New Taipei City: Fuguijiao	AABB	25.292543	121.538746	This study; Ding et al. 1998	Y
2	New Taipei City: Elephant Trunk Rock Scenic Area	AABB	25.135172	121.824491	This study	Y
3	Keelung City: Heping Island	AABB	25.161850	121.765662	This study; Hizume & Shibata 2016	Y
4	Yeliu	AABB	25.2093270	121.694422	Hizume & Shibata 2016	N ¹
5	Wan-li	AABB	25.1741500	121.639810	Chang & Hsu 1974	N ¹
6	New Taipei City: Bitoujiao	AABB	25.122112	121.919773	This study	Y
7	Taoyuan City: Dagu Mountain	BB	25.1054810	121.288349	This study	Y
8	Taoyuan	AA	25.0733600	121.131151	Uchino et al. 1992	N ¹
9	Miaoli County: Tongxiao	AA	24.536929	120.704727	This study	Y

¹ Precise GPS coordinate was not mentioned in the literature. Instead, general GPS coordinate was used to indicate the location of population.