

Geographical variation in leaflet morphology of *Cycas revoluta* (Cycadaceae) on the the Ryukyu Islands

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Hiroaki Setoguchi¹, Shigeharu Kyoda¹, Yoshiyuki Maeda² and Naofumi Nomura¹ : **Geographical variation in leaflet morphology of *Cycas revoluta* (Cycadaceae) on the Ryukyu Islands**

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Abstract

Sixteen populations of *Cycas revoluta* (Cycadaceae), covering almost all distribution areas on the Ryukyu Islands and Kyushu, Japan, were examined to determine geographical differences in leaflet morphology. We assessed four morphological characteristics of leaflets and applied one-way analysis of variance (ANOVA) to test the difference in each morphological character. Cluster analysis based on four morphological properties resulted in two clear major clusters comprising northerly and southerly populations that were demarcated north of Yoronou and south of Tokunoshima Islands, respectively. Northern cycads tended to have leaflet margins with shallow recurving and narrower leaflet lamina width, while the southern populations had deeply recurved leaflet margins and wider leaflet lamina width. The morphological differentiation may imply intraspecific differentiation.

Key words : *Cycas revoluta*, geographical variation, Kyushu, leaflet morphology, Ryukyu Islands.

Introduction

Cycas revoluta Thunb. (Cycadaceae) is a cycad that grows on coastal rocky terrain of islands, including southern Kyushu and the most southern Ryukyu Islands of Japan (Jones 1993 ; Yamazaki 1989, 1995). The wide distribution range of *C. revoluta*, i.e., >1,000 km in a north-east to southwest direction, may imply geographical differences in morphology of the populations. Fertile seeds of cycads are generally heavy and sink in water, and only inviable seeds float (Jones 1993). Nishida (1935) compared seed morphology among cycads on three islands, Amamioshima, Okinoerabujima and Okinawa, and suggested the existence of geographical differentiation, i.e., cycads from the former two islands produce seeds with a short and rounded base, whereas seeds from Okinawa Island are longer and have a pointed base. However, Nakamura et al. (2005) did not find any geographical differentiation in seed morphology of cycads from four islands, Amamioshima, Okinawa, Ishigakijima and Iriomotejima.

No comparative study of differentiation in vegetative parts of cycads from different popula-

tions has been conducted. However, one of the authors (Y. Maeda) has noticed differences in leaflet morphology between the north and south Ryukyu Islands during commercial trading in cycads, i.e., the leaflet margin of the southerly cycads tends to recurve on the adaxial side. The recurved (revolute) leaflet margin has been recognized as characteristics to *C. revoluta* among other cycads (e.g., Jones 1993). In this study, we compared leaflet morphology of *C. revoluta* among populations on the Ryukyus and in southern Kyushu.

Materials and methods

Leaf samples of *C. revoluta* were collected from 16 populations (294 individuals) on the Ryukyu Islands and Kyushu, covering almost the whole distribution range (Fig. 1). For each population of *C. revoluta*, all samples were collected from natural habitats located in coastal rocky terrain. A leaflet was collected from the middle part of a mature pinnate compound leaf from each individual, and a transverse section at mid-length of the leaflet was measured (Fig. 2 A). Four morphological characteristics of leaflets

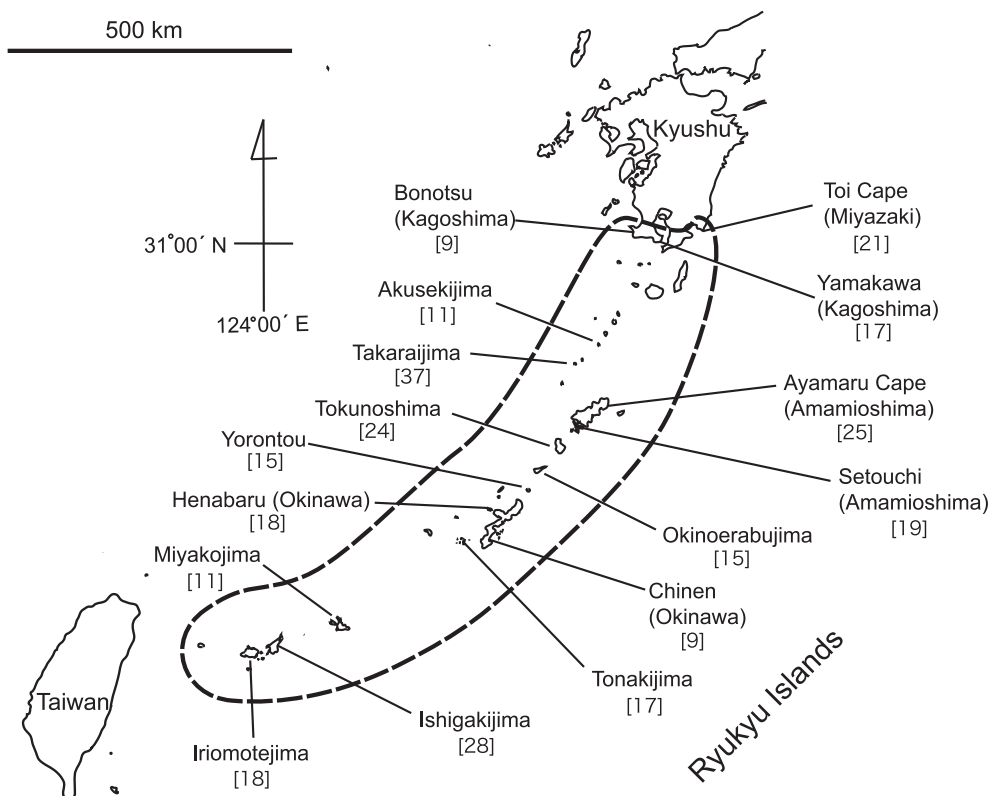


Fig. 1. Locations of the 16 collection sites of *Cycas revoluta* in Kyushu and the Ryukyu Islands. The distribution range is shown by a broken line. Number of samples examined are shown in brackets.

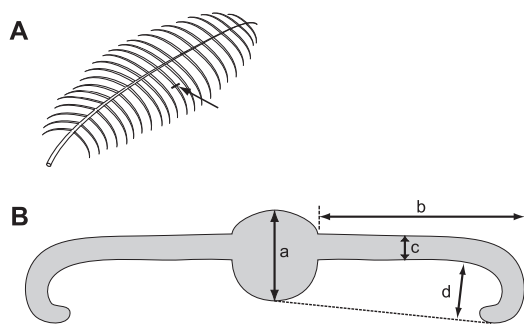


Fig. 2. Measurement of leaflet morphology of *Cycas revoluta*. A: position from which the leaflet section was taken (arrow). B: transverse section of leaflet. a, thickness of the leaflet midrib; b, width of the leaflet lamina; c, thickness of the leaflet lamina; d, height of recurving of the leaflet margin.

were assessed (Fig. 2 B): thickness of the leaflet midrib (a), width of the leaflet lamina (b), thickness of the leaflet lamina (c) and height of recurving of the leaflet margin (d). All dimensions

were measured using eyepiece micrometer on Olympus BX-51 microscope. One-way ANOVA was applied to test the difference in each morphological character, and post-hoc Tukey HSD test was used to determine the significant difference between each population. The statistical analyses were performed using SPSS version 10 (SPSS, Chicago). Distances between populations were estimated with rescaled distances based on mean values, and dendrogram was generated by cluster analysis using group average methods implemented in package PVCLUST (Suzuki, R. and Shimodaira, H. 2006. PVCLUST: Hierarchical clustering with p-values via multiscale bootstrap resampling. <http://www.is.titech.ac.jp/~shimo/prog/pvclust/>) for R software version 2.8.1 (R Foundation, Vienna). Approximately unbiased p-value was computed on each cluster by 1,000 multiscale bootstrap resamplings.

Results and discussion

The four morphological characteristics were normally distributed, and values were significantly different among populations (ANOVA, $p < 0.001$; Table 1). Thickness of the leaflet midrib and thickness of the leaflet lamina (Fig. 3 A, C) varied among populations but with no geographical cline, ranging from 1.02 ± 0.17 mm (mean \pm standard deviation; Yorontou Island) to 0.73 ± 0.15 mm (Akusekijima) and from 0.53 ± 0.06 mm (Okinoerabujima) to 0.39 ± 0.06 mm (Yamakawa of Kagoshima), respectively. Width of the leaflet lamina suggested a weak geographical trend, where some populations from southerly islands (Chinen of Okinawajima, Miyakojima, Ishigakijima, and Iriomotejima) showed a greater width than the other populations (Fig. 3 B, Turkey HSD). The width of the leaflet lamina ranged from 2.42 ± 0.42 mm (Iriomotejima) to 1.40 ± 0.24 mm (Akusekijima). Height of recurving of the leaflet margin implied distinct geographical cline, as height tended to increase in samples from southerly islands (Fig. 3 D, Turkey HSD). The height of leaflet recurving ranged from 0.91 ± 0.20 mm (Miyakojima) to 0.54 ± 0.17 mm (Akusekijima).

Cluster analysis using mean values of each four morphological characters resulted in two clear major clusters comprising northerly and southerly populations that were demarcated north of Yorontou and south of Tokunoshima Islands, respectively (Fig. 4). However, affiliation

of three island populations, Tokunoshima, Okinoerabujima and Yorontou Islands, was overlapped: Okinoerabujima and Yorontou belonged to northerly populations, whereas Tokunoshima belonged to southerly populations, possibly ascribed to the wider leaflet lamina in Tokunoshima that tends to be common in the southerly populations (Fig. 3 B).

Our results indicate that *C. revoluta* shows morphological differentiation in leaflet morphology in terms of recurving of the leaflet margins (and leaflet lamina width). Although its north-south demarcation crosses at central Ryukyus (between Tokunoshima and Yorontou Islands), northern cycads tended to have leaflet margins with shallow recurving (and narrower leaflet lamina width), while the southern populations had deeply recurved leaflet margins (and wider leaflet lamina width). As the distribution range of this cycad exceeds 1,000 km in a northeast to southwest direction, the morphological differentiation may imply intraspecific differentiation. Comparative genetic studies should be undertaken to supplement our understanding of cycad morphological differentiation among the Ryukyu Islands and Kyushu.

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Table 1. Results of ANOVA for four morphological parameters

	Source of variation	SS	dF	MS	F	p
A. Thickness of the leaflet midrib (mm)	Among populations	2.03	15	0.135	5.30	<0.0001
	Within populations	7.09	278	0.025		
	Total	9.11	293			
B. Width of the leaflet lamina (mm)	Among populations	20.9	15	1.392	12.2	<0.0001
	Within populations	31.7	278	0.114		
	Total	52.6	293			
C. Thickness of the leaflet lamina (mm)	Among populations	0.41	15	0.027	7.40	<0.0001
	Within populations	1.02	278	0.004		
	Total	1.42	293			
D. Height of recurving of the leaflet margin (mm)	Among populations	4.38	15	0.292	11.1	<0.0001
	Within populations	7.34	278	0.026		
	Total	11.7	293			

SS, Sum of Squares; dF, degree of Freedom; MS, Mean Squares; F, F-statistics; p, p value for the null hypothesis.

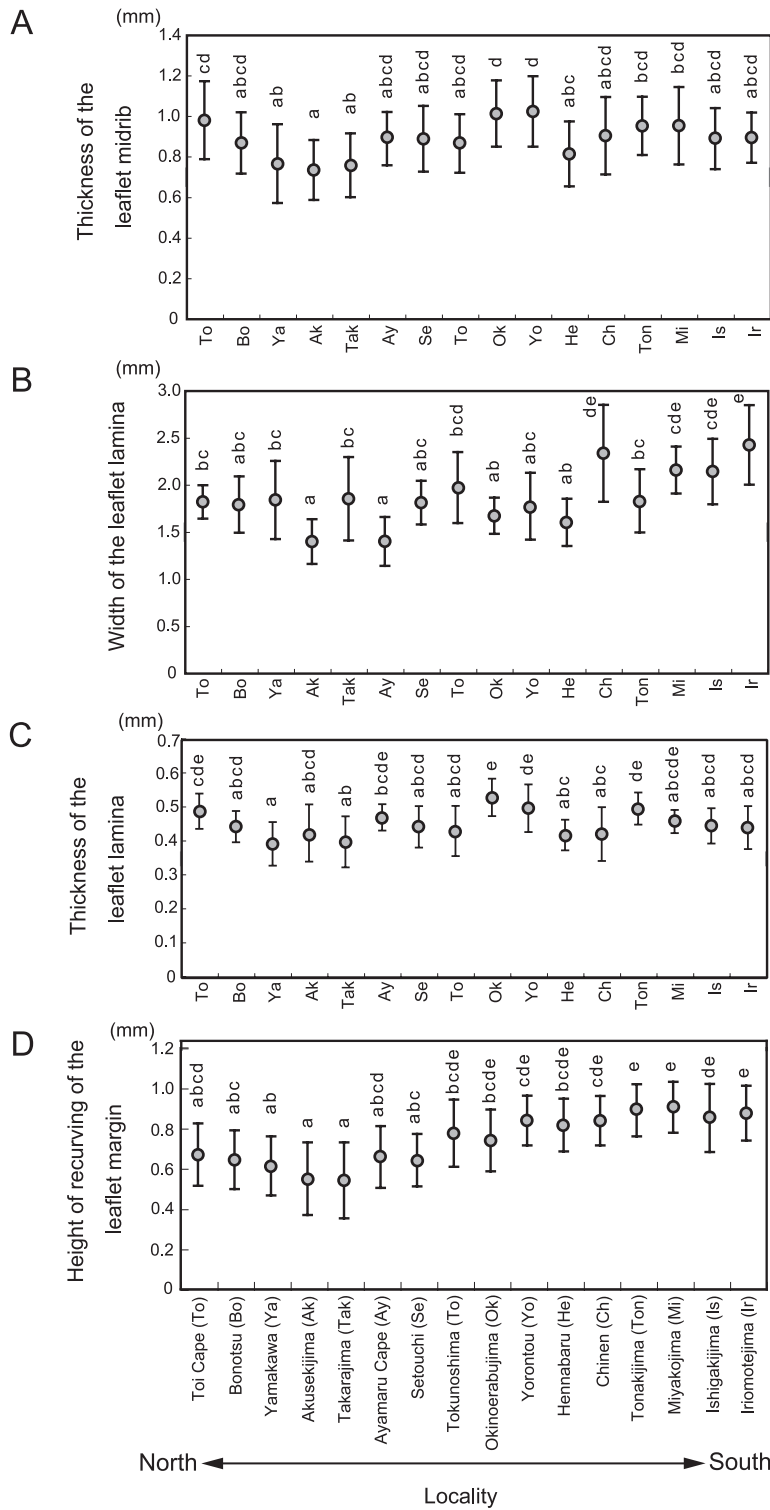


Fig. 3. Means and standard deviations of dimensions of the thickness of the leaflet midrib, leaflet lamina width, leaflet lamina thickness and the height of recurving of the leaflet margin in *Cycas revoluta*. Different abbreviation above the vertical bar means significant difference in post-hoc Tukey HSD test.

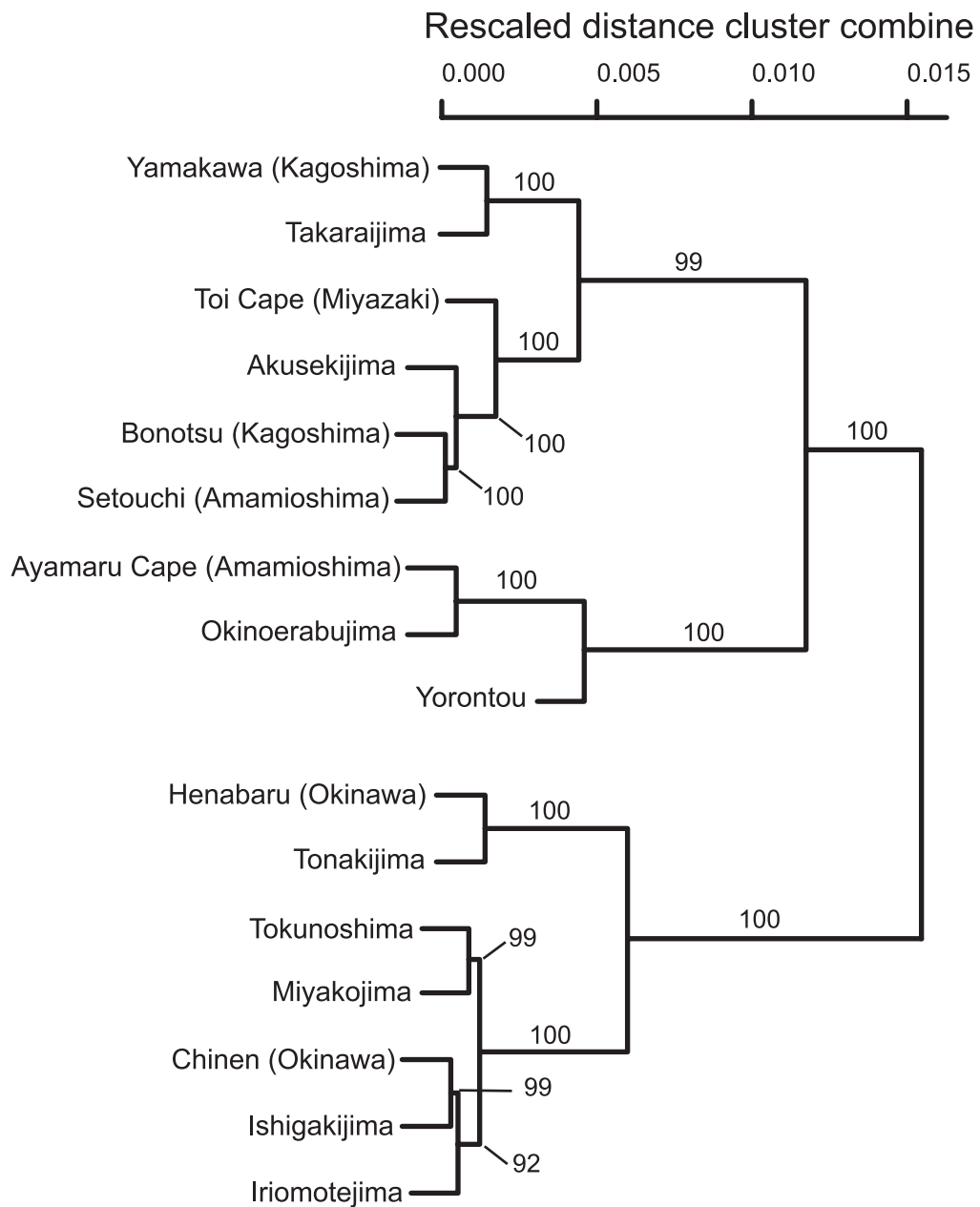


Fig. 4. Population relationships represented by a dendrogram in *Cycas revoluta* based on morphological characteristics of leaflets. Approximately unbiased p-values (%) are shown above the branches.

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- (Received October 15, 2008 ; accepted February 27, 2009)
- 瀬戸口浩彰¹・許田重治¹・前田芳之²・野村尚史¹：
琉球列島におけるソテツ（ソテツ科）の小羽片形態の地理的変異
- 琉球列島から九州にかけて分布するソテツ（*Cycas revoluta* Thunb.）の自生地16集団を対象にして、小羽片の形態の地理的な分化を検証した。小羽片の横断面における小羽片の幅と厚さ、中肋の厚さ、小羽片の縁の巻き込みの高さの4形質を測定してクラスター解析にかけたところ、北方型と南方型の2つのグループに分かれた。北方型には主に与論島以北の集団が含まれ、南方型には主に徳之島以南の集団が含まれる。ただし、この境界にある徳之島は南方型に、沖永良部島は北方型に含まれる。これには、小羽片の幅と縁の巻き込みの高さに南北間で違いがあることが寄与しており、とくに縁の巻き込みの高さは、南側で大きく巻き込む傾向が見られた。
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