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Chromosome Numbers of *Zannichellia* L. (Zannichelliaceae) in Japan

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Abstract Chromosome numbers of *Zannichellia* L. (Zannichelliaceae) found in Japan was studied. Plants collected from six localities had a chromosome number of 2n=24. The chromosome numbers of *Zannichellia* found in Japan were firstly reported with collecting information. According to existing taxonomic keys, *Zannichellia* plants used in this study were determined to be *Z. palustris* or *Z. pedunculata*, a species previously not reported in Japan.

Key words: Chromosome number, flow cytometry, Japan, *Zannichellia palustris*, *Zannichellia pedunculata*.

Introduction

Zannichellia L. (Zannichelliaceae) is a genus of annual or perennial submerged aquatic plants, which inhabit brackish and fresh waters. It is widely distributed in tropical and temperate latitudes (Tomlinson, 1982). The taxonomy of *Zannichellia* has not been studied entirely and has been confused not only in Japan but throughout the world (Cook, 1996; Kadono, 1994). Japanese *Zannichellia* plants have been identified as *Z. palustris* L. (Ohwi, 1972; Yamashita, 1982; Kadono, 1994).

The chromosome numbers of *Zannichellia* have been previously reported as 2n=12, 14, 24, 28, 32, 34, and 36 (Van Vierssen, 1982; Talavera *et al.*, 1986; Talavera and Garcia Murillo, 1992; Montgomery *et al.*, 1997; Mesicek and Javurkova-Jarolimova, 1992). *Zannichellia palustris* was also found to have variations as follows: 2n=24 from Europe, China and Japan; 28 from Europe; 34 from Europe and China; 36 from Europe (Scheerer, 1940; Tarnavschi, 1948; Harada, 1956; Reese, 1961, 1963, 1967; Hedberg and Hedberg, 1964; van Vierssen, 1982; van Vierssen and van Wijk, 1982; Uotila *et al.*, 1983; Mesicek and Javurkova-Jarolimova, 1992; Sun, 1992; Montgomery *et al.*, 1997).

Harada (1956) reported 2n=24 as the chromosome number of *Z. palustris* in Japan. However, the collecting site and voucher specimens are not documented. Therefore, observation of chromosome numbers from locations with collection data is necessary to provide basic information in order to revise the taxonomic treatment. In this paper, we report chromosome numbers of *Z. palustris* from six localities in Japan.

Materials and Methods

Six of the eleven localities of *Zannichellia* plants reported by Tanaka *et al.* (2006) were used in this study (Table 1). Some individuals from each location were studied. Voucher specimens are deposited in the National Museum of Nature and Science (TNS).

Root tips of each plant were cut and soaked in 8 mM aqueous hydroxyquinoline solution at 4°C overnight. After fixation with a 3 : 1 (v/v) mixture of ethanol and acetic acid at room temperature for three hours, they were macerated in 1 N HCl for 10 min at 60°C, stained with 1% aceto-orcein

Table 1. Localities and voucher specimens of populations investigated in japan.

Population	Locality	Voucher
Tofutsu	Lake Tofutsu, Koshimizu, Hokkaido Pref.	TNS 9528810-9528818
Yudo	Lake Yudo, Toyokoro, Hokkaido Pref.	TNS 9528819, 9528820
Takahoko	Lake Takahoko, Rokkasyo, Aomori Pref.	TNS 9528835, 9528836
Ogawara	Lake Ogawara, Kamikita, Aomori Pref.	TNS 9528778
Nishiki-Hama	Nishiki-Hama, Higashi-Izumo, Shimane Pref.	TNS 9528826-9528831
Nobeoka	Nobeoka, Miyazaki Pref.	TNS 9528833, 9528834

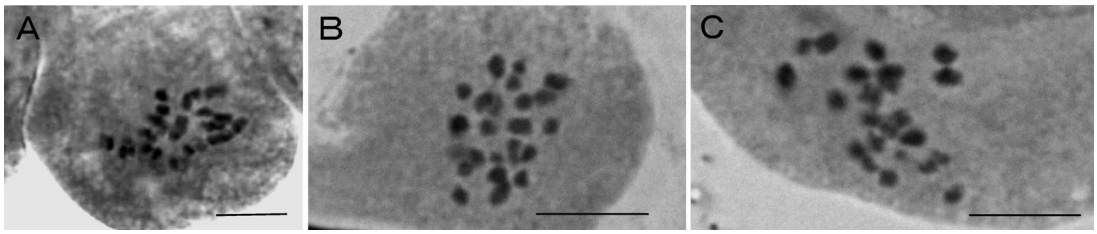

Fig. 1. Somatic chromosomes of *Zannichellia* at metaphase. A: Tofutsu, B: Takahoko, C: Ogawara. Bar indicates 5 μm.

for 1 min at room temperature, and squashed on a glass slide.

Flow cytometry (FCM) analyses were conducted to indirectly estimate the chromosome number of *Zannichellia* plants whose roots could not be collected. Samples were prepared using CyStain UV precise P kit (Partec, Germany) for nuclei extraction and staining of nuclear DNA. The genome size was determined by a basic procedure of Ploidy Analyzer PA (Partec, Germany). *Oryza sativa* L. cv. Nihonbare was used as an internal standard.

Results and Discussion

The chromosome numbers of *Zannichellia* plants from Tofutsu, Takahoko, and Ogawara were 2n=24 by microscopic observation (Fig. 1). By FCM analyses, the genome size of the plants in Yudo, Takahoko, Nishiki–Hama and Nobeoka, were shown to be nearly equal to each other (Fig. 2, Table 2). Although the peak indices of each samples had minor differences (1.209–1.241, Table 2), these samples were estimated to be 2n=24 on the presumption that their chromosome numbers are an even number. These results showed that the *Zannichellia* plants from all localities of this study have a chromosome number of 2n=24. This is consistent with the only data on chromosome numbers of Japanese *Zannichellia* (Harada, 1956).

Table 2. Peak indices of flow cytometry analyses.

Population	Peak index*		
	1	2	3
Yudo	1.239	2.489	—
Takahoko	1.241	2.499	—
Nishiki-Hama	1.209	2.408	4.855
Nobeoka	1.221	2.388	—

*Peak index shows the relative location when the peak of internal standard is assumed to be 1.0.

Some *Zannichellia* in this study were not determined to be *Z. palustris*, which is the only known *Zannichellia* species in Japan (Ohwi 1972; Yamashita, 1982; Kadono, 1994). According to Talavera *et al.* (1986), who used features of leaf and fruit as key characters, plants of Tohfutsu, Yudo and Ogawara were determined to be *Z. palustris* L., while Takahoko, Nishiki-Hama, and Nobeoka, had intermediate characters between *Z. palustris* and *Z. pedunculata* Reichenb.

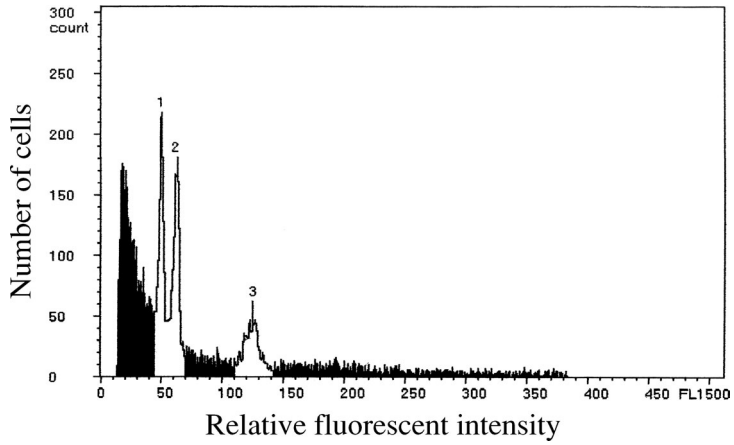

Fig. 2. Peaks in fluorescence of *Zannichellia* plants of Takahoko (Peak 2 and 3), from analyses with flow cytometer. The horizontal scale indicates fluorescent intensity (calibrated by the internal standard, *Oryza sativa*: Peak 1) and the vertical scale indicates number of cells.

(not previously reported in Japan). According to van Vierssen (1982), who used leaf and fruit as key characters, Takahoko, Nishiki-Hama and Nobeoka, were identified as *Z. pedunculata*. However, this study described the chromosome numbers of *Z. palustris* and *Z. pedunculata* to be 2n=24 and 2n=36, respectively. Van Vierssen's taxonomic description of morphological characters and chromosome numbers are inconsistent with our results. In the future, a molecular phylogenetic approach would be necessary to identify and delineate taxa in this genus.

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References

Cook, C. D. K. 1996. Aquatic Plant Book. 228 pp. SPB Academic Publishing, Amsterdam.

Environment Agency of Japan (ed.). 2000. Threatened Wildlife of Japan-Red Data Book 2nd ed.–vol. 8, Vascular Plants. 664 pp. Japan Wildlife Research Center, Tokyo. (In Japanese).

Harada, I. 1956. Cytological studies in Helobiae, 1. Chromosome idiograms and a list of chromosome numbers in seven families. *Cytologia* **21**: 306–328.

Hedberg, I and Hedberg, O. 1964. Documented chromosome numbers of Swedish Plants. *Svensk Botanisk Tidskrift* **58**(1):125–128.

Kadono, Y. 1994. Aquatic Plants of Japan. 179 pp. Bunichi Sogo Shuppan, Co. Ltd, Tokyo (in Japanese).

Mesicek, J. and Javurkova-Jarolimova, V. 1992. List of Chromosome Numbers of the Czech Vascular Plants. 49 pp. Academia, Praha.

Montgomery, L., Khalaf, M. Baailey, J. P. and Gornal, K. J. 1997. Contributions to a cytological catalogue of the British and Irish flora, 5. *Watsonia* **21**: 365–368.

Ohwi, J. 1972. Flora of Japan. 1560 pp. Shibundo, Tokyo (in Japanese).

Reese, G. 1961. Chromosome numbers of Central and Northwest European plant species. *Opera Botaica* **5**: 1–581.

Reese, G. 1963. Uber die deutschen Ruppia-und Zannichellia-Kategorien und ihre Verbreitung in Schleswig-Holstein. *Schriften des Naturwissenchaften Vereins für Shleswig-Holstein* **34**: 44–70.

Reese, G. 1967. Cytologische und taxonomische Untersuchungen an *Zannichellia palustris* L. *Biolologisches Zentralblatt* **86**: 277–306.

Scheerer, H. 1940. Chromosomenzahalen aus der Schreswig-Hols-Teinischen Flora II. *Planta* **32**: 716–725.

Sun, X.-Z. 1992. Flora Reipublicae Popularis Sinicae

Tomus 8. 218 pp. Science Press, Beijing (in Chinese).

Talavera, S., Garcia Murillo, P. and Smit, H. 1986. Sobre el genero *Zannichellia* L. (Zannichelliaceae). *Lagascalia* **14** (2): 241–271.

Talavera, S. and Garcia Murillo, P. 1992. Numeros cromosomaricos de plantas occidentals, 661–667. *Anales del Jardin Botanicode Madrid* **50**: 83.

Tanaka, N., Ito, Y. and Uehara, K. 2006. Distribution and new localities of *Zannichellia palustris* L. (Zannichelliaceae) in Japan. *Annals of Tsukuba Botanical Garden* **25**: 1–6.

Tarnavschi, I. T. 1948. Die chromosomenzahlen der Anthophyten-Flora Von Rumänien mit einen Ausblick auf das Polyploide-Problem. *Bululetinul Gradinii Botanice si al Muszeulvi Botanic de la Universitatea* **28**: 1–130.

Tomlinson, P. B. 1982. Anatomy of the Monocotyledons VII. Helobiae (Alismatidae). Clarendon Press, Oxford.

Uotila, P., van Vierssen, W. and van Wijk, R. J. 1983. Notes on the morphology and taxonomy of *Zannichellia* in Turky. *Annales Bototanici Fennici* **20**: 351–356.

Van Vierssen, W., 1982. The ecology of communities dominated by *Zannichellia* taxa in western Europe. I. Characterization and autecology of the *Zannichellia* taxa. *Aquatic Botany* **12**: 103–155.

Van Vierssen, W. and van Wijk, R. J. 1982. On the identity of *Zannichellia peltata* Bertol. in western Europe. *In*: van Vierssen, W. (ed.), The Ecology of Communities Dominated by *Zannichellia* Taxa in Western Europe. pp. 199–215. Stichting Studentenpers, Nijmegen.

Yamashita, T. 1982. Zannichelliaceae. *In*: Satake, Y., Ohwi, J., Kitamura, S., Watari, S. and Tominari, T. (eds.), Wild Flowers of Japan I. Herbaceous Plants - Monocotyledoneae. Heibonsha Ltd., Tokyo (in Japanese).