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Chromosome Number of *Microlepia hookeriana* (Dennstaedtiaceae) and Chromosome Number Evolution in the Genus *Microlepia*

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Abstract The chromosome number $2n=156$ ($x=39$, tetraploid) is newly recorded in *Microlepia hookeriana* collected from Okinawa, Japan. This base number is new to the genus *Microlepia* and is not concordant with previous reports based on a conspecific plant of Taiwan. The species often has been placed in an independent genus, and the present chromosome number and molecular phylogeny support its isolated position within the genus.

Key words: chromosome number, Dennstaedtiaceae, *Microlepia.*

The genus *Microlepia* (Dennstaedtiaceae) comprises ca. 45–70 species mostly distributed in the Old World tropics (Copeland, 1947; Ching, 1959). Its chromosome base number is considered $x=40$, 42, 43 and 44 (Walker, 1984) based on previous reports of approximately 22 species from India, Sri Lanka, China, Taiwan, Japan and New Guinea.

Microlepia hookeriana (Wall. ex Hook.) C. Presl is the only species having unipinnate fronds with entire pinnae in the genus, and is distributed in Nepal, India, SE Asia, China, Taiwan and Japan (Okinawa Prefecture). Smith (1875) established a monotypic genus *Scypholepia* J. Sm. considering the importance of the combination of characters of its entire margins of segments and articulated pinnae to rachis. However, Copeland (1947) pointed out that the species does not have any articulation at the base of the pinna. Still subsequent researchers recognize *Scypholepia* as a genus or a section of *Microlepia* on the basis of its exceptional unipinnate frond with auriculate pinnae (Tagawa, 1951; Kramer, 1958). Cytologically, Tsai and Shieh (1983), Yang *et al.* (1988) and Tsai (1992) reported $x=43$ (diploid, sexual reproduction), one of the aneuploid series, in *M. hookeriana* collected in Taiwan. In this study, we reexamined the chromosome number of *M. hookeriana* using Japanese material, and also compared its base number with the result of molecular phylogeny.

Materials and Methods

An individual (*N. Nakato 2602*) collected in Mt. Yonaha-dake, Kunigami-son, Okinawa Prefecture, Japan was used for chromosome observation, and a voucher specimen is deposited in Department of Botany, National Museum of Nature and Science (TNS). The method of chromosome observation followed Nakato and Serizawa (1981).

Results and Discussion

Chromosome number of M. hookeriana

Somatic chromosomes observed in two independent cells were $2n=156$ (Fig. 1). This number does not match any previously known base numbers of the genus, $x=40$, 42, 43 and 44 (Walker, 1984), and a previously counted number of *M. hookeriana* [$x=43$, Tsai and Shieh (1983), Yang

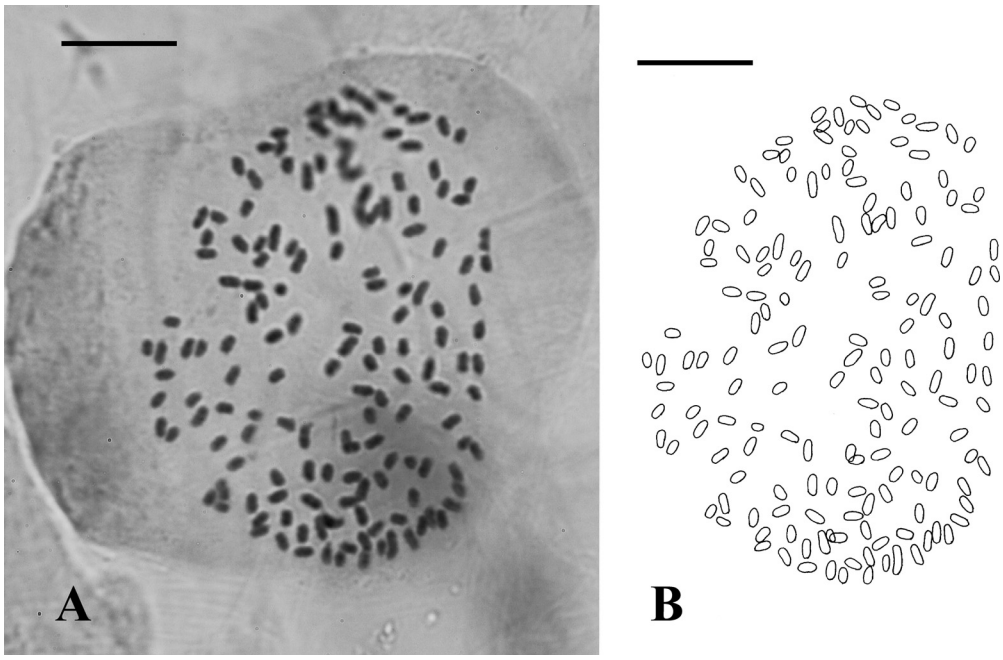

Fig. 1. Somatic chromosomes of *Microlepia hookeriana*, $2n=156$. A: a photomicrograph, B: an explanatory drawing. Scale bars = 10 μm.

et al. (1988) and Tsai (1992)]. Considering previous countings of the genus, the number $2n=156$ counted on a Japanese individual could be considered as a tetraploid based on a novel basic number $x=39$, so far unknown to the genus.

The chromosome numbers reported from Taiwan and Japan are different. We had several doubts about the reliability of previous reports from Taiwan [Tsai and Shieh (1983), Yang *et al.* (1988) and Tsai (1992)]. First, there are several overlaps of bivalent chromosomes in the picture of Tsai and Shieh (1983, p. 152, Fig. 22), so it seems difficult to count the exact number in the picture. Second, we could not count more than 40 bivalent chromosomes in the picture indicated as "$n=43$II" chromosomes in Yang *et al.* (1988) and the chromosome sketch does not match the chromosome picture in their paper but match that of Tsai and Shieh (1983). Finally, Tsai (1992) reported the newly counted somatic chromosome number of $2n=86$, but he did not provide any figure. We therefore considered that reexamination of chromosomes is necessary for Taiwanese *M. hookeriana*. Nonetheless, the individuals observed by Tsai and Shieh (1983) and Yang *et al.* (1988) are undoubtedly diploids, suggesting the presence of infraspecific polyploidy.

Chromosome number evolution in Microlepia

Figure 2 shows known chromosome base numbers of Dennstaedtiaceae on a phylogenetic tree of the family using chloroplast *rbcL* sequences, that is a part of the all Japanese pteridophyte phylogeny by Ebihara (2011). Monophyly of both *Microlepia* and *Dennstaedtia* is supported and they are sister groups to each other. *Microlepia hookeriana* is placed at the most basal position of *Microlepia* species to be examined. The chromosome base number of *Microlepia* is reported $x=40$, 42, 43 and 44 (Walker, 1984) as already noted, although the base number $x=44$ seems rare in the genus, and has only been observed in Indian *M. speluncae* (L.) T. Moore (Abraham *et al.*, 1962; Ghatak, 1977). We regard *M. pseudostrigosa* with $2n=160$ (Kurita, 1963) as an infraspecific aneuploid derived from $x=42$

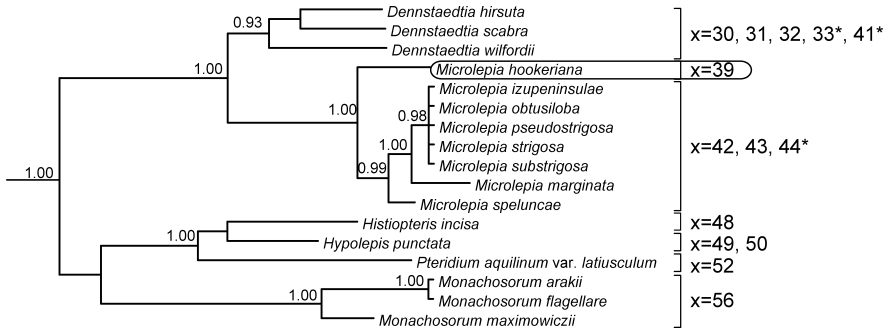

Fig. 2. Phylogeny and the chromosome base numbers of the Dennstaedtiaceae. See discussion about the base numbers with asterisks.

or 43, because chromosome numbers based on $x=42$ and 43 are known both in *M. pseudostrigosa* Makino (Mitui 1975; Nakato and Serizawa, 1981) and its related species (Takamiya, 1996). We also do not include the unconfirmed report of $n=$ca. 70 ($x=35$?) in *M. strigosa* by Manickam and Irudayaraj (1988). Base numbers of the other genera of Dennstaedtiaceae in Fig. 2 are in accordance with the list by Takamiya (1996) except for $x=33$ and 41 in *Dennstaedtia scabra* counted outside Japan (Mehra and Khanna, 1959; Weng, 1985).

The tree shows that Dennstaedtiaceae consists of two large clades. One comprises *Monachosorum*, *Pteridium*, *Histiopteris* and *Hypolepis*, and the other comprises *Dennstaedtia* and *Microlepia*. The chromosome base number of the former clade ranges from $x=48$ to $x=56$, and that of the latter is lower ($x=30$ to $x=43$). *Dennstaedtia* and *Microlepia* in the latter clade, each are monophyletic as far as Japanese taxa are concerned, and the base number of *Dennstaedtia* is known to be $x=30$ to $x=33$, and $x=41$, while the base numbers $x=34$, 46 and 47 are also known in non-Japanese species (Walker, 1973, 1984; Lovis, 1977).

Microlepia hookeriana ($x=39$) occupies the most basal position in the clade of genus *Microlepia*, and is sister to all the other Japanese *congeners* ($x=42$ and 43). Genetic distances among the Japanese *congeners* with $x=42$ and 43 seem relatively small, which suggests recent speciation possibly involving allopolyploidization. Although the chromosome base number of the ancestral stock of *Microlepia* is uncertain, an isolated systematic position of *M. hookeriana* within *Microlepia* is supported by cytological and molecular data.

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References

Abraham, A., Ninan, C. A. and Mathew, P. M. 1962. Studies on the cytology and phylogeny of the pteridophytes VII. Observation on one hundred species of south Indian ferns. Journal of the Indian Botanical Society 41: 339–421.

Ching, R.-C. 1959. Flora Reipublicae Popularis Sinicae 2. Pteridophyta, Ophioglossaceae—Oleandraceae. Science Press, Beijing.

Copeland, E. B. 1947. Genera Filicum. Waltham, Mass.

Ebihara, A. 2011. *RbcL* phylogeny of Japanese pteridophyte flora and implications on infrafamilial systematics. Bulletin of the National Museum of Nature and Science, Series B 37: 63–74.

Ghatak, J. 1977. Biosystematic survey of pteridophytes from Shevaroy Hills, south India. Nucleus 20: 105–108.

Kramer, K. U. 1958. The taxonomic position of *Saccoloma wercklei* Christ. American Fern Journal 48: 111–118.

Kurita, S. 1963. Cytotaxonomical studies on some leptosporangiate ferns. Journal of the College of Arts and Sciences, Chiba University 4: 43–52.

Lovis, J. D. 1977. Evolutionary patterns and processes in

ferns. Advances in Botanical Research 4: 229–415.
Manickam, V. S. and Irudayaraj, V. 1988. Cytology of ferns of the Western Ghats (South India). Today & Tomorrows, New Delhi.
Mehra, P. N. and Khanna, K. R. 1959. Cytology of some Himalayan ferns. Journal of Genetics 56: 1–14.
Mitui, K. 1975. Chromosome numbers of Japanese pteridophytes. Bulletin of Nippon Dental College, General Education 4: 221–271.
Nakato, N. and Serizawa, S. 1981. Chromosome numbers of the genus *Microlepia* in Japan with descriptions of a new hybrid. Journal of Japanese Botany 56: 161–168.
Smith, J. 1875. Historia Filicum. Macmillan, London.
Tagawa, M. 1951. Fern miscellany (5). Journal of Japanese Botany 26: 185–188.
Takamiya, M. 1996. Index to Chromosomes of Japanese Pteridophyta (1910–1996). Japan Pteridological Society, Tokyo.
Tsai, J.-L. 1992. Cytotaxonomic studies on the fern genus *Microlepia* in Taiwan. In: Tsai J.-L. and Shieh, W.-C. (eds.), Proceedings of the Second Seminar on Asian Pteridology, Taiwan. pp. 55–71. National Chung Hsing University and National Science Council, Taichung.
Tsai, J.-L. and Shieh, W.-C. 1983. A cytotaxonomic survey of the pteridophytes in Taiwan. Journal of Science and Engineering (National Chung Hsing University) 20: 137–159.
Walker, T. G. 1973. Evidence from cytology in the classification of ferns. In: Jermy, A. C., Crabbe, J. A. and Thomas, B. A. (eds.), The Phylogeny and Classification of the Ferns. pp. 91–110, supplement no. 1 to Botanical Journal of the Linnean Society vol. 67, London.
Walker, T. G. 1984. Chromosomes and evolution in pteridophytes. In: Sharma, A. K. and Sharma, A. (eds), Chromosome Evolution of Eukaryptic Groups 2. pp. 103–141. CRC Press, Boca Raton.
Weng, R.-F. 1985. Observation on chromosomes of some Chinese ferns. Journal of Wuhan Botanical Research 3: 367–370 (in Chinese).
Yang, H.-C., Tsai, J.-L. and Shieh W.-C. 1988. Chromosome studies on the fern family Dennstaedtiaceae in Taiwan. Journal of Science and Engineering (National Chung Hsing University) 25: 69–82 (in Chinese).