Morphological and Cytological Observations on *Lindsaea trichomanoides* (Lindsaeaceae), a Fern Species from New Zealand

Su-Juan Lin\(^a\), Atsushi Ebihara\(^b\) and Kunio Iwatsuki\(^c\)

\(^a\)Department of Biological Science, Faculty of Life and Environmental Science, Shimane University, 1060, Nishikawatsu-cho, Matsue, Shimane, 690-8504 JAPAN; E-mail: sjlin@life.shimane-u.ac.jp

\(^b\)Department of System Sciences (Biology), Graduate School of Arts and Sciences, the University of Tokyo, 3-8-1, Komaba, Meguro-ku, Tokyo, 153-8902 JAPAN;

\(^c\)The Museum of Nature and Human Activities, Hyogo, 6, Yayoigaoka, Sanda, Hyogo, 669-1546 JAPAN

(Received on September 28, 2005)

Morphological and cytological observations were carried out on *Lindsaea trichomanoides* (Lindsaeaceae), a fern species from New Zealand. The frond buds were used for mitotic chromosome observation. A chromosome number 2n = 76 is newly recorded in the genus. The basic number x = 38 is a new count for the Lindsaeaceae as well.

**Key words:** apical meristem, chromosome number, frond bud, *Lindsaea.*

About 150 species of *Lindsaea* are distributed in tropical and warm temperate regions of the world. It is a large but poorly understood genus. The taxonomic characters are generally not distinct and chromosome numbers of Lindsaeoid ferns are known only for a few species. Moreover, some of these chromosome counts are only approximate, and in some cases the identification of the material sampled is uncertain (Kramer 1971). Kramer (1971), and Löve et al. (1977) enumerated all of the available cytological data for *Lindsaea*, where only about twenty species were recorded. The basic chromosome numbers counted were 34, 42, 43, 44, 47, and ca. 50; as a result, the ploidy of some polyploid plants cannot yet be determined in this genus.

During the last thirty years, little progress has been made in biosystematic research in the Lindsaeaceae. The technical problems of the genus are compounded by the delicate nature of the roots (root tip material cannot be obtained easily in the field) and difficulty of cultivation. Except for some species in sect. Schizoloma, most of the Lindsaeoid ferns have few sporangia in each sorus and the small chromosome size also causes considerable difficulty in obtaining successful meiotic counts. The cytology of *Lindsaea* has thus lagged behind research into other aspects of its systematic biology. Recently, Lin et al. (1990, 1996) and Lin (2000) reported a 32-spored sexual reproductive mode, several combinations of natural hybridization, and speciation in the polyploid series of Lindsaeoid ferns, and they made every effort to obtain data to elucidate the biodiversity of this genus. In addition, Lin et al. (1996) have been successful in obtaining materials for mitotic observation from spore cultivation.

In this study, *Lindsaea trichomanoides* Dryander, a species with a narrow
distribution in New Zealand and Australia, was observed. The authors were successful, using young frond buds for cytological observations, in obtaining a new chromosome record of this species.

**Materials and Methods**

One sample of *Lindsaea trichomanoides* collected from New Zealand (Loopline Road, near Turiwhate, Westland, South Island, A. Ebihara 040117-05) was used for morphological observation and spore counting. The voucher specimen is deposited in

---

the Department of Biological Science, Faculty of Life and Environmental Science, Shimane University.

The living sample was kept in a moist plastic bag at 20°C for two months, and we could harvest several new frond buds, although no new roots were produced to die.

Three young frond buds (about 5 mm in length) were pretreated with 0.002 M 8-hydroxyquinoline solution for 5 hours at 20°C, fixed with acetic acid-ethanol (1:3) solution for more than 15 minutes, macerated in a mixed solution of 1N HCl–45% acetic acid (3:1) for 1.5 minutes at 58°C, and stained with 2% aceto-orcein. The tissue of the apical meristematic region (about 1–2 mm) was squashed for somatic observation. Photographs were taken under both light and phase-contrast microscopes.

Results and Discussion

The sample used in this study was observed morphologically (Fig. 1). The following characters were found to be consistent with previous descriptions given for the species: rhizome creeping, with elongate-triangular scales; fronds ca. 25 cm long, lamina ca. 10 cm long, obovate, pinnae or pinnules entire or lobed; sori continuous or sometimes interrupted along the outer margin. However, it was noticed that the indusia are membranaceous, slightly erose, but falling short of the margins of pinna or pinnules by a distance of about 1–2 mm; this observation differs from that of Kramer (1971) Kramer and Tindale (1976), Betty and Isaac (1986), Chinnock (1974), and Jones and Clemesha (1976) (Fig. 1B). It is necessary to observe more specimens from local populations to elucidate variation in these characters.

Fig. 2. A. Somatic chromosomes of *Lindsaea trichomanoides*, 2n = 76. Bar = 5 μm. B. Explanatory drawing of photo A.
Thirty-two trilet spores per sporangium were counted (Fig. 1D). Thirty-two s/s (thirty-two spores per sporangium) indicates sexual reproduction in the Lindsaeoid ferns (Lin et al. 1990), and the presence of normal trilet spores suggested that this sample was fertile, rather than a sterile hybrid.

Although there were few cells in the apical meristem of frond buds, in which mitotic divisions occurred, the chromosome number was confirmed from six cells to be precisely 2n = 76 (Fig. 2).

Based on the chromosome number of 2n = 76 and on spore observations, this individual was presumed to be a normal sexual diploid (Lin et al. 1990) and the presence of normal trilet spores suggested that this sample was fertile rather than a sterile hybrid.

Although there were few cells in the apical meristem of frond buds in which mitotic divisions occurred the chromosome number was confirmed from six cells to be precisely 2n = 76 (Fig. 2).

Based on the chromosome number of 2n = 76 and on spore observations, this individual was presumed to be a normal sexual diploid (Lin et al. 1990) and the presence of normal trilet spores suggested that this sample was fertile rather than a sterile hybrid.

We thank Professors M. Hasebe and M. Ito for helping us in the field in New Zealand. We are thankful to Ms. Emily Wood, Harvard University Herbaria, who read this paper for a linguistic check.

References

林　蘇娟  海老原　淳  岩槻邦男‘：ニュージーランド産シダ植物 Lindsaea trichomanoides（ホングウシダ科）の形態的・細胞学的研究
ニュージーランド産のホングウシダ科 Lindsaea trichomanoides の形態的・細胞学的観察を行った。栽培が困難で有根端材料を入手することが難しいため、葉芽を用いて体細胞分裂の染色体を観察した。その結果、染色体数は 2n = 76 であることが分かった。この数はホングウシダ属では初めての報告であり、基本数 x = 38 も、ホングウシダ科の新記録であった。
（鳥取大学生物資源科学部生物科学科、*東京大学大学院総合文化研究科、‘兵庫県立人と自然の博物館）