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Ribosomal DNA Distribution Patterns on Somatic Chromosomes of Diploid Species in Three Sections in Genus *Aster* (Asteraceae)

Goro Kokubugata[1], Yukiko Saito[2], Makoto Ogawa[3] and Masatsugu Yokota[4]

[1] Department of Botany, National Museum of Nature and Science, 4–1–1 Amakubo, Tsukuba, Ibaraki 305–0005, Japan
E-mail: gkokubu@kahaku.go.jp
[2] United Graduate School of Agricultural Science, Tokyo University of Agriculture and Technology, 3–5–8 Saiwai, Fuchu, Tokyo 183–8509, Japan
[3] Tokushima Prefectural Museum, Bunka-no-mori Park, Hachiman, Tokushima 770–8070, Japan
[4] Laboratory of Ecology and Systematics, Faculty of Science, University of the Ryukyus, 1 Senbaru, Nishihara, Okinawa 903–0213, Japan

Abstract Somatic chromosomes of nine diploid species in three sections, sect. *Aster*, *Pseudocalimeris* and *Teretiachenium*, in genus *Aster* were investigated by the conventional aceto-orcein staining method and the fluorescent *in situ* hybridization using 18S ribosomal DNA (rDNA) probes. The nine species commonly exhibited an rDNA site at a secondary constriction between the short arm and the satellite in a pair of chromosomes. Total chromosome lengths in a complement of *A. asa-grayi* and *A. miyagii* were shorter than those of the other seven species. In sect. *Teretiachenium*, an rDNA site was detected in the longest pair of chromosomes of *A. komonoensis* and *A. savatieri*, while it was detected in shorter pair of chromosomes of *A. rugulosus* and *A. scaber*.

Key words : *Aster*, Asteraceae, chromosomes, FISH, rDNA.

Introduction

Genus *Aster* L. *sensu lato* (*s. l.*) primarily distributes in the North America, the Eurasia Continent and the Far East Asia, and is consisted of about 250 species (Xiang and Semple, 1996) or 500 (Ito and Soejima, 1995). Previously Kitamura (1937) classified Japanese members of *Aster s. l.* to four genera, namely *Aster sensu strict*, *Kalimeris* Cass., *Gymnaster* Kitam. (=*Miyamayomena* Kitam.) and *Hetelopappus* Less. Thereafter Ito and Soejima (1995) treated all of the four genera as a single genus of *Aster* consisted of five sections of *Aster*, *Asteromoea*, *Pseudocalimeris*, *Teretiachaenium* and *Tripolium*. However, taxonomic treatment of *Aster s. l.* has been confusing, and thus further studies concerning taxonomy and phylogeny in this genus has been required for its taxonomy (*e.g.,* Ito *et al.*, 1998).

Recently the florescent *in situ* hybridization (FISH) method has been applied to cytological studies, especially hybrid species in *Aster s. l.*, and verified a useful method (*e.g.,* Kokubugata *et al.*, 2003; Saito and Kokubugata, 2004; Matoba *et al.*, 2005, 2007). To analyze these hybrid species, cytological information in diploid species is required as essential data to estimate its parent species. In the present study, we report the rDNA distribution patterns on somatic chromosomes of nine diploid species in genus *Aster s. l.* by the FISH method using 18S ribosomal DNA (rDNA) probes.

Materials and Methods

Plant materials

Somatic chromosomes of nine species of *Aster s. l.* were investigated in the present study (Table 1). The plant materials were collected, and then were cultivated in the experimental greenhouse

Table 1. Plant materials of *Aster* investigated following taxonomic treatment of Ito and Soejima (1995).

Species	Section	Locality (Voucher in TNS)	Chrom. complement (Total length; μm*)	Chrom. with rDNA (karyotype)
A. glehni F. Schmidt	*Aster*	Hokkaido: near Saroma-ko Lake, Monbetsu (*GK 4595*).	16 m+2 sm (96.7)	8th (m)/10th (m)
A. taiwanensis Kitam. var. *lucens* (Kitam.) Kitam.	*Aster*	Ryukyus: Shirahama, Okinawa (*GK1509*)	14 m+4 sm (127.0)	1st (m)/2nd (m)
A. asa-grayi Makino	*Pseudocalimeris*	Ryukyus: Honohoshi Setouchi, Amami, Kagoshima (*GK 4018*).	14 m+4 sm (44.0)	1st (sm)/2nd (sm)
A. miyagii Koidz.	*Pseudocalimeris*	Ryukyus: Anya, Nago, Okinawa Is. (*GK 1839*).	12 m+6 sm (48.9)	1st (sm)/2nd (sm)
A. spathulifolius Maxim.	*Pseudocalimeris*	cultivated in TBG (*GK 4667*).	16 m+2 sm (90.2)	1st (sm)/2nd (sm)
A. komonoensis Makino	*Teretiachenium*	Shikoku: Mt. Tsurugi-san, Tokushima (*GK 4662*).	12 m+6 sm (81.7)	1st (sm)/2nd (sm)
A. rugulosus Maxim.	*Teretiachenium*	Honshu: Kagamiyama, Higashi-hiroshima, Hirosima (*GK 1319*).	14 m+4 sm (115.6)	15th (sm)/17th (sm)
A. scaber Ell.	*Teretiachenium*	Honshu: Nakasone-daira, Inawashiro, Fukushima (*GK 4665*).	16 m+2 sm (110.2)	15th (m)/16th (m)
A. savatieri Makino	*Teretiachenium*	cultivated in TBG (*GK 1724*).	16 m+2 sm (139.5)	1st (sm)/2nd (sm)

*Total length＝total of 18 chromosome lengths.

of Tsukuba Botanical Garden for the present cytological observation. These voucher specimens were deposited in the herbarium of the National Museum of Nature and Science (TNS; Table 1). Taxonomic treatment of Ito and Soejima (1995) was taken up for the present study.

Conventional aceto-orcein staining squash method

Root tips of the nine species were harvested, pretreated in 2 mM 8-hydroxyquinoline at 15°C for 3 h and fixed in acetic ethanol (1 : 3) at 4°C for 2 h at least. The root tips were macerated in a mixture of 1N hydrochloric acid and acetic acid (2 : 1) at 60°C for 10 sec, put on glass slides, and then were stained in 2% aceto–orcein at room temperature for 4 h and squashed.

FISH method

FISH protocol in the present cytological experiment was following Kokubugata *et al.* (2003). An 18S ribosomal DNA (rDNA) sequence, being a part of 45S rDNA, amplified from the total genomic DNA of *A. ageratoides* (*GK 2810*; Kokubugata *et al.*, 2003) by the polymerase chain reaction (PCR) using the primers of NS1 and NS4 designed by White *et al.* (1990). The PCR-amplified rDNA was labelled with digoxigenin- (DIG-) dUTP. Metaphase chromosomes were observed by the FISH method. The labeled probes on chromosomal DNA were detected using Anti-digoxigenin-fluorescein, Fab-framentavidin as yellow fluorescence with PI revealing non-hybridized regions with orange or dark red fluorescence.

Chromosome classification

Chromosomes at mitotic metaphase were classified by arm ratio (R＝long arm length/short arm length) following Levan *et al.* (1964). Median-centromeric (1.0≦R＜1.7), submedian-centromeric (1.8≦R<3.0), subterminal-centromeric (3.1≦R<7.0), and terminal-centromeric chromosomes (7.1≦R) are respectively abbreviated as

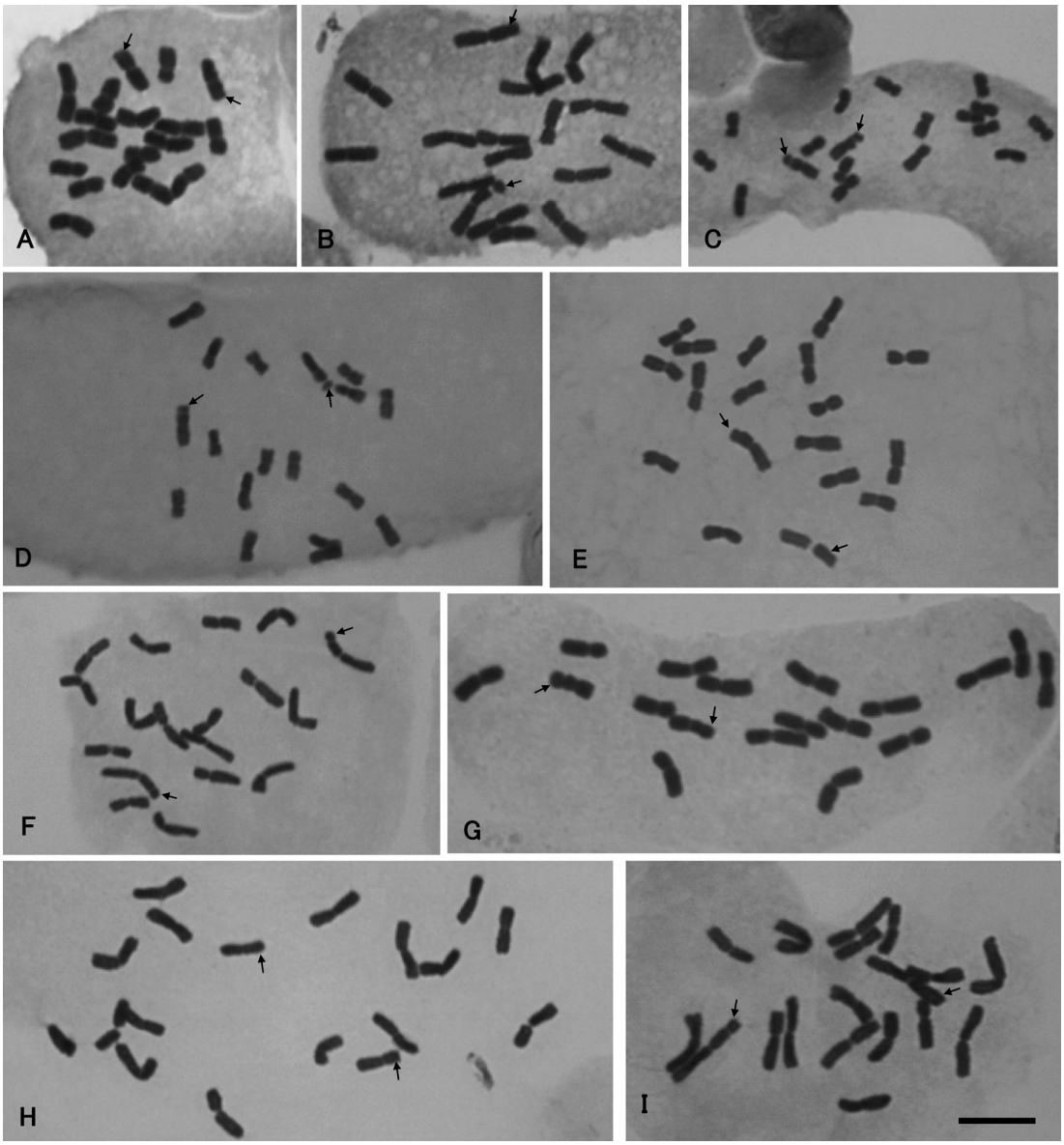

Fig. 1. Orcein-stained chromosomes at mitotic metaphase of nine species in *Aster s. l.* A. *A. glehni*. B. *A. taiwanensis* var. *lucens*. C. *A. asa-grayi*. D. *A. miyagii*. E. *A. spathulifolius*. F. *A. komonoensis*. G. *A. rugulosus*. H. *A. scaber*. I. *A. savatieri*. Arrows show satellite. Bar indicates 10 μm.

m, sm, st and t chromosomes in this paper. Total lengths of eighteen chromosomes were compared among the nine species.

Results and Discussion

Nine species of *Aster* commonly showed a chromosome number of 2n=18 (Fig. 1) being consist with the previous reports of *e.g.,* Shimotomai and Huziwara (1942) and Kanemoto (2000) for *A. asa-grayi*; those of *e.g.,* Tahara and Shimotomai (1926) and Shimotomai and Huziwara (1942) for *A. glehni*, *A. komonoensis*, *A. savatieri* (=*Gymnaster savatieri* (Makino) Kitam.) and *A.scaber*; those of Shimotomai and Huzi-

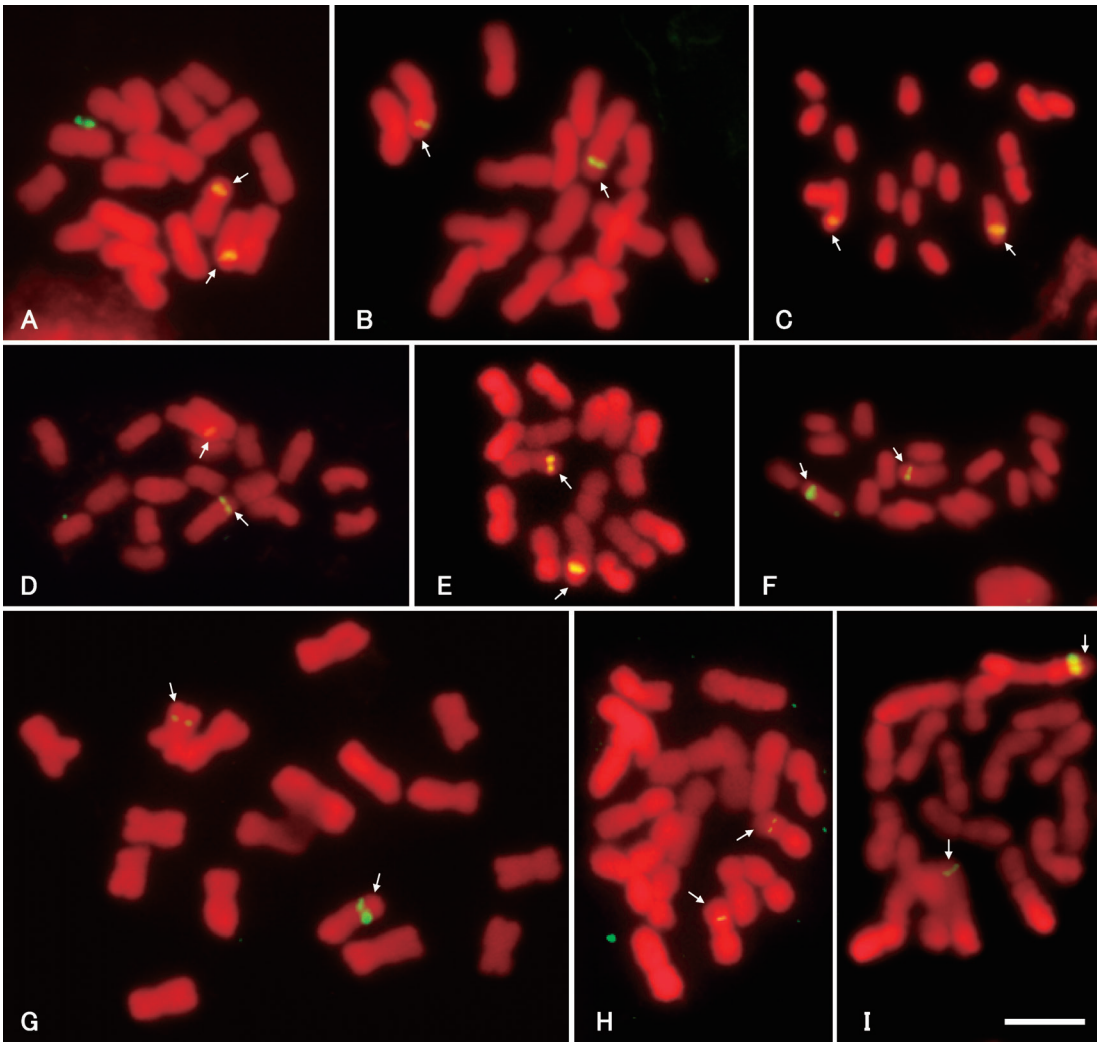

Fig. 2. FISH-detected chromosomes at mitotic metaphase of nine species in *Aster s. l.*. A. *A. glehni*. B. *A. taiwanensis* var. *lucens*. C. *A. asa-grayi*. D. *A. miyagii*. E. *A. spathulifolius*. F. *A. komonoensis*. G. *A. rugulosus*. H. *A. scaber*. I. *A. savatieri*. Arrows show satellite. Bar indicates 10 μm.

wara (1942), Huziwara, (1953) and Saito and Kokubugata (2004) for *A. scaber* and *A. rugulosus*; those of Matsuda (1970) and Kanemoto (2000) for *A. miyagii*; those of Shimotomai and Huziwara (1942) and Huziwara (1956) for *A. spathulifolius*; and those of Huziwara (1965) and Kanemoto (2000) for *A. taiwanensis* var. *lucens*. Karyotypes of the nine *Aster* species were respectively similar to those in the previous reports (*e.g.,* Shimotomai and Huziwara, 1942; Huziwara, 1956; Matsuda, 1970; Huziwara, 1965; Arano, 1962).

The total lengths of *A. asa-grayi* and *A. miyagii*, being endemic to the Ryukyus, in sect. *Pseudocalimeris* were shorter than those of the other seven species (Table 1). Previously it was reported that *A. iinumae* Kitam. (=*Kalimeris pimmatifida* (Matsum.) Kitam.) had smaller chromosomes per haploid karyotype than the other diploid species of *Aster* (*e.g.,* Huziwara, 1955; Tara, 1972; Matoba *et al.*, 2005, 2007). The present results implicate that not only *A. iinumae* but

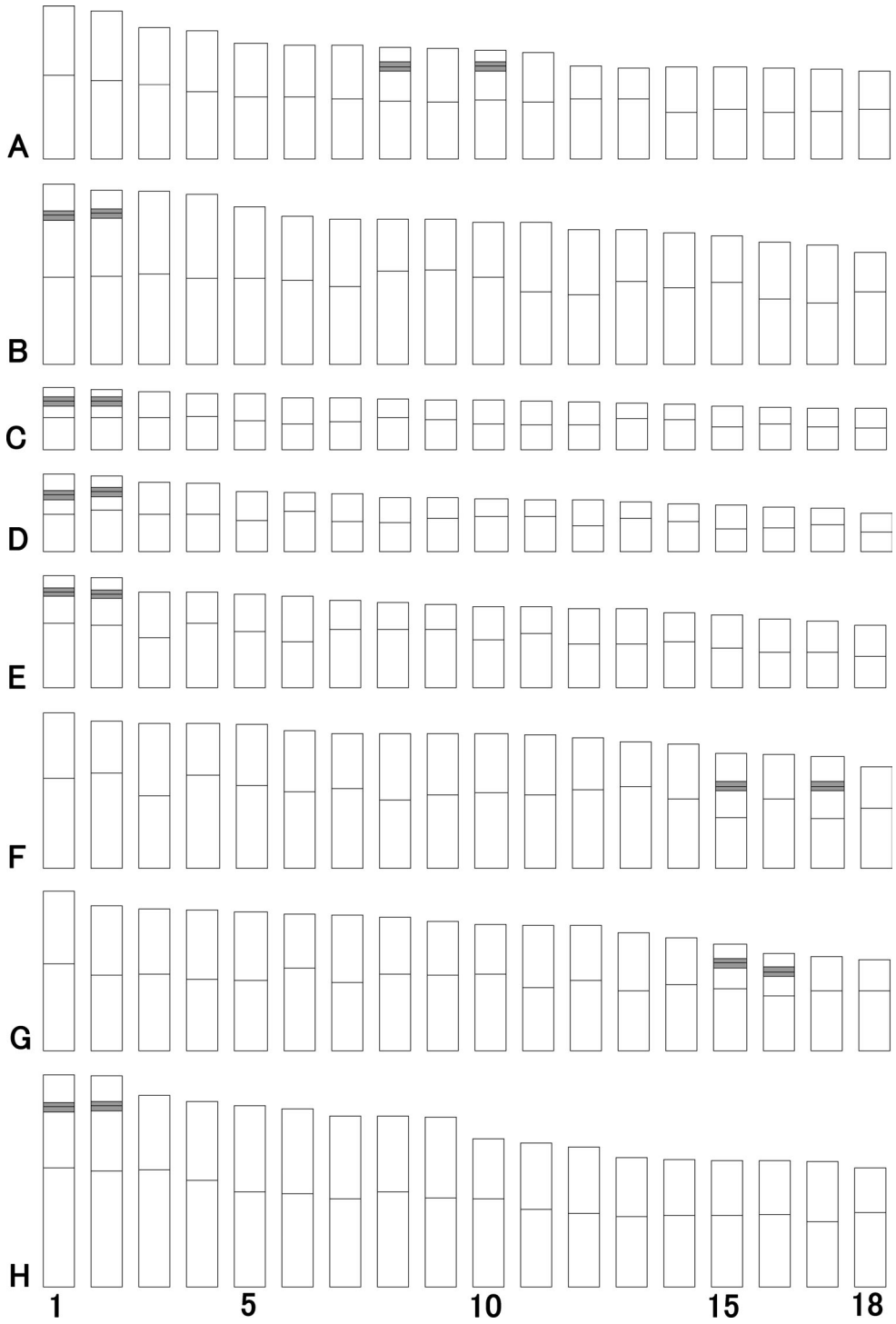

Fig. 3. Ideograms of nine species in *Aster s. l.* A. *A. glehni*. B. *A. taiwanensis* var. *lucens*. C. *A. asa-grayi*. D. *A. miyagii*. E. *A. spathulifolius*. F. *A. komonoensis*. G. *A. rugulosus*. H. *A. scaber*. I. *A. savatieri*. Grayish areas show rDNA sites.

also *A. asa-grayi* and *A. miyagii* had possess smaller chromosomes than the other diploid species of *Aster*.

Ribosomal DNA distribution Pattern

Section *Pseudocalimeris* was consisted of three diploid and three tetraploid species in Japan (Ito and Soejima, 1995). In the present study, all of the three diploid species of *A. asa-grayi*, *A. miyagii* and *A. spathulifolius* were investigated. The three diploid species commonly exhibited the rDNA site on the longest pair of sm chromosomes (Fig. 3C, D, E).

Section *Teretiachaenium* was consisted of four diploid and a polyploid species (Ito and Soejima, 1995), and four of the five species, namely *A. komonoensis*, *A. rugulosus*, *A. scaber* and *A. savatieri* were investigated here. In a complement at mitotic metaphase, an rDNA site was detected on two shorter sm chromosomes aligned in a range from the 15th to the 17th in *A. rugulosus* and *A. scaber* (Fig. 3G, H), while it was detected on the longest pair of chromosomes in *A. komonoensis* and *A. savatieri* (Fig. 3F, I).

Section *Aster* is the largest in the genus *Aster s. l.* consisted of ten diploid, two high polyploid and three polyploid-complex species (Ito and Soejima, 1995). The present study leveled that the two species of *A. glehni* and *A. taiwanensis* var. *lucens* commonly had the rDNA site on two m chromosomes differing from the other seven species having the rDNA site on two sm chromosomes (Table 1). The m chromosomes with the rDNA site were middle chromosome length (the 8th and the 10th) in a complement of *A. glehni*, while they were the longest pair in a complement of *A. taiwanensis* var. *lucens* (Fig. 3).

Ito *et al.* (1998) suggested that three sections of *Aster*, *Pseudocalimeris* and *Teretiachaenium* were not monotypic taxa based on chloroplast DNA restriction data. In the present study, we examine only nine diploid species of *Aster s. l.*, and have not enough data to discuss relationships between phylogeny and cytology in this genus comprehensively. However, the variations of the rDNA distribution pattern recognized in sections of *Aster* and *Teretiachaenium* may connect with polyphyly suggested by Ito *et al.* (1998).

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