

Chromosome numbers of some Asteraceae species from Universitas Indonesia Campus, Depok, Indonesia

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Manuscript received: 13 October 2018. Revision accepted: 24 October 2018.

Abstract. Salamah A, Oktarina R, Ambarwati EA, Putri DF, Dwiranti A, Andayani N. 2018. Chromosome numbers of some Asteraceae species from Universitas Indonesia Campus, Depok, Indonesia. *Biodiversitas* 19: 2079-2087. Asteraceae is the largest plant family, comprising of more than 1,600 genera and 23,000 species with a worldwide distribution. Many species of Asteraceae are found to be very significantly abundant in a range of habitats and are used as sources of medicines, food, forage, and other useful products. Many cytological studies have been carried out on Asteraceae. However, information about the chromosome numbers of Asteraceae in Indonesia is still very limited. In this study, the chromosome numbers of 15 species of Asteraceae found around the Universitas Indonesia campus were counted. For chromosomal preparations, the root/shoot tips were pretreated with 2 mM 8-hydroxyquinoline, stained with aceto-orcein, prepared using the squash method, and then observed with a microscope. Of the 15 species, 12 showed variation in chromosome numbers: *Cosmos caudatus*, *Elephantopus scaber*, *Tridax procumbens*, *Mikania micrantha*, *Sphagneticola trilobata*, *Ageratum conyzoides*, *Cyanthillium cinereum*, *Chromolaena odorata*, *Synedrella nodiflora*, *Youngia japonica*, *Eclipta prostrata*, and *Porophyllum ruderale*. The other three species showed no variation in chromosome number: *Emilia sonchifolia* (2n=10), *Sonchus arvensis* (2n=18), and *Cosmos sulphureus* (2n=24). We also found new variations in chromosome numbers that have not yet been listed in the Index to Plant Chromosome Numbers (IPCN).

Keywords: Asteraceae, chromosome number, Universitas Indonesia, squash method

INTRODUCTION

Asteraceae, or *Compositae*, is one of the largest and most widespread families of flowering plants, containing more than 1,600 genera and 23,000 annual and perennial herbs, shrubs, vines, trees, and epiphytes. They are found in every continent, except Antarctica, and in every environment, from forests to high-elevation grasslands. Asteraceae are less common in tropical wet forests and more common in open areas (Funk 2005). The plants are characterized by the arrangement of their flowers into an involucre pseudanthium called head or capitulum (Funk 2009). This family offers great potential benefits in many areas, such as food (Steenis et al. 1997), pharmaceuticals (Maryati and Suharmiyati 2003), agriculture (Pebriani et al. 2013), farms, and ecology (Malcolm 2007). These significant potential usages, together with the exceptionally large number of species, make this family an interesting object of research (Kumolo and Utami 2011). Cytological studies of this family have accordingly become an area of interest for many researchers (Raven et al. 1960; Moore and Frankton 1962; Solbrig et al. 1969; Stebbins 1971; Strother 1976; Watanabe et al. 1999). Heywood et al. (1977) reviewed various tribes of the family.

Many karyological and cytological studies have been carried out on Asteraceae. However, information about the chromosome numbers of species grown in Indonesia is still very limited. This paper reports the results of chromosome-

counting analysis of these species. The resulting information about the chromosome numbers of Asteraceae species in Indonesia will provide further insight into the taxonomy of Asteraceae and lay the foundations for further studies on apomixis in Asteraceae.

MATERIALS AND METHODS

Study area

Plant samples of 15 species of Asteraceae were collected from natural habitats around the Universitas Indonesia (UI) campus at Depok, Indonesia. Figure 1 showed the map of sampling locations.

Procedures

The analysis of chromosome numbers was conducted at the Laboratory of Plant Development at the Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Indonesia, the Center of Excellence for Indigenous Biological Resources-Genome Studies (CoE IBR-GS) FMIPA UI, and the UI-Olympus Bioimaging Center (UOBC). In this study, root/shoot tips from 15 species of Asteraceae, including two to six plants for each species, were examined. For chromosomal preparation, the root/shoot tips were pretreated with 2 mM 8-hydroxyquinoline for 6 hours, fixed in ethanol: acetic acid (3:1, v/v) for 24 hours, and preserved in 70% alcohol at 4°C until

used. The root/shoot tips were further washed in distilled water to remove the fixative solution. They were then hydrolyzed for 15 minutes in 1N HCl at room temperature, and stained in 2% aceto-orcein for 24 hours at 4°C. Slides were prepared using the squash method (Jong 1997).

The numbers of chromosomes were further observed and counted using a microscope trinocular Promo Star at CoE IBR-GS FMIPA UI and an Olympus IX73 inverted microscope and UPLSAPO 100X lens. The photographs were taken with an Olympus DP73 camera with C mount

adapter 0.5x. Data analysis was performed in the CoE IBR-GS laboratory and UOBC using Dimension cellSens software v1.11.

Data analysis

Descriptive analyses were performed based on the photographs obtained and processed from that software. The data collected were displayed in the form of pictures and tables showing the chromosome numbers of the Asteraceae species that were successfully counted.

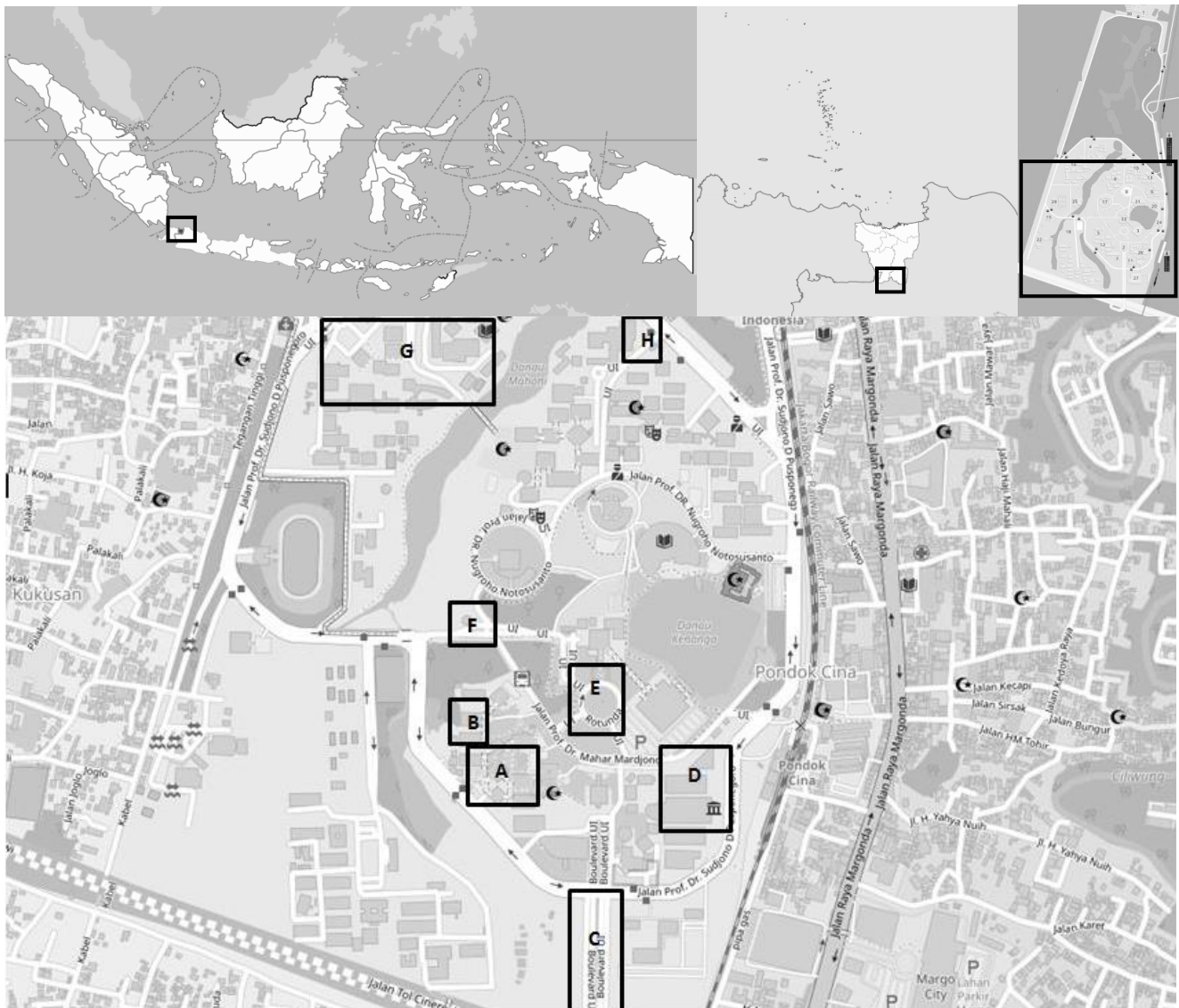


Figure 1. Asteraceae sampling locations in Universitas Indonesia. **A.** Faculty of Mathematics and Natural Sciences (*Cosmos caudatus*, *Ageratum conyzoides*, *Synedrella nodiflora*, *Eclipta prostrata*, *Sonchus arvensis*, *Cosmos sulphureus*, *Cosmos caudatus*, *Elephantopus scaber*, *Sphagneticola trilobata*), **B.** Faculty of Pharmacy (*Youngia japonica*, *Sonchus arvensis*, *Elephantopus scaber*, *Tridax procumbens*, *Sphagneticola trilobata*), **C.** Boulevard (*Cyanthillium cinereum*, *Chromolaena odorata*, *Clibadium surinamense*, *Porophyllum ruderale*, *Mikania micrantha*, *Sphagneticola trilobata*, *Tridax procumbens*), **D.** Health Sciences Cluster (*Porophyllum ruderale*, *Tridax procumbens*, *Eclipta prostrata*, *Cyanthillium cinereum*, *Emilia sonchifolia*, *Elephantopus scaber*, *Youngia japonica*, *Mikania micrantha*, *Synedrella nodiflora*, *Ageratum conyzoides*), **E.** Rotunda (*Tridax procumbens*, *Emilia sonchifolia*, *Elephantopus scaber*, *Synedrella nodiflora*, *Ageratum conyzoides*, *Cyanthillium cinereum*), **F.** Water tower (*Cosmos sulphureus*, *Eclipta prostrata*, *Emilia sonchifolia*), **G.** Faculty of Engineering (*Chromolaena odorata*, *Cyanthillium cinereum*, *Emilia sonchifolia*, *Synedrella nodiflora*, *Mikania micrantha*), **H.** Wisma Makara: *Youngia japonica*, *Mikania micrantha*, *Tridax procumbens*, *Synedrella nodiflora*

RESULTS AND DISCUSSION

The data from this experiment are presented in Table 1. Of the 15 species, 12 were found to vary in chromosome numbers, while the others did not (Table 1). Variation in chromosome numbers has previously been reported in many groups of Asteraceae (Ruas et al. 2000). In the present study, the variation in chromosome numbers occurred not only in a given individual plant (as, for example, in *Cosmos caudatus*, *Synedrella nodiflora*, *Cyanthillium cinereum*, *Chromolaena odorata*, *Elephantopus scaber*, *Mikania micrantha*, and *Sphagneticola trilobata*) but also in different individual plants as observed in *Ageratum conyzoides*. According to Kunakh et al. (2008), the condition of cells with varying numbers of chromosome found in one tissue is called mixoploidy (also known as polysomaty). The Asteraceae species plants found to be mixoploid within its individual plants were *Cosmos caudatus*, *Elephantopus scaber*, *Tridax procumbens*, *Mikania micrantha*, *Sphagneticola trilobata*, *Ageratum conyzoides*, *Cyanthillium cinereum*, *Chromolaena odorata*, *Synedrella nodiflora*, *Youngia japonica*, *Eclipta prostrata*, and *Porophyllum ruderale*. Kashin et al. (2011) explained that mixoploidy in Asteraceae is related to the phenomenon of apomixis.

The chromosome numbers of 15 species obtained in this experiment were compared to the chromosomal number data in the Index to Plant Chromosome Numbers (IPCN) (Missouri Botanical Garden 2017) and other appropriate reference sources (Table 1). Information about the chromosome numbers of *Emilia sonchifolia* and *Sonchus arvensis* was not found in the IPCN. However, Xie and Zheng (2003) reported that *E. sonchifolia* in China have a chromosome number of $2n=20$, although Baldwin (1964, in Mehra et al. 1965) found a different result ($2n=10$) which is similar to that found in UI campus (Figure 2.A). The chromosome number of *S. arvensis* in the Netherlands was $2n=54$ (Lemna and Messersmith 1990), while that in UI campus was $2n=18$ (Figure 2.B). *E. sonchifolia* ($n=10$) and *S. arvensis* ($2n=18$) in the UI campus showed no variations in chromosome number.

Cosmos sulphureus (Figure 2.C) from the UI campus showed no variation in chromosome number ($2n=24$). This datum has been reported to the IPCN and is similar to that of the same species grown in China (Chen et al. 2003). Different with *C. sulphureus*, *Cosmos caudatus* (Figure 2.D) showed that none of the chromosome numbers of the species observed ($2n=22$, $2n=30$, $2n=32$, $2n=36$, $2n=40$) have so far been reported to the IPCN, which states $2n=24$ and $2n=48$ (Jose and Mathew 1995).

Elephantopus scaber (Figure 2.E) at the UI campus showed variation in chromosome number ($2n=14$, $2n=18$, $2n=20$, $2n=22$). Of the chromosome number data, only one ($2n=22$) was also found in the IPCN. The chromosome number of $2n=22$ found in *E. scaber* was also observed in India (Mathew and Mathew 1988). Similar results were also found in *T. procumbens*. The chromosome numbers found in *T. procumbens* (Figure 2.F) in the UI campus were $n=9$, $2n=18$, $2n=27$, $2n=36$, and $2n=45$. Only one of those chromosome numbers ($2n=36$) has been registered at the IPCN (Jose and Mathew 1995).

Tridax procumbens from China has been found to have a similar chromosome number (Xie and Zheng 2003). The somatic chromosome number of *Mikania micrantha* (Figure 2.G) in the IPCN list is $2n=72$ (Ruas and Ruas 1987), while the numbers observed in the present study are $2n=24$ and $2n=32$. The two variations in chromosome numbers found here have not yet been reported to the IPCN.

According to the IPCN data, *Sphagneticola trilobata* (Figure 3.A) is known to have a chromosome number of $2n=56$ (Ren et al. 2012), while the chromosome numbers of the same species obtained from the present study were $2n=32$ and $2n=54$. These data are different from those found in Asia ($2n=56$, Ren et al. 2012) and have not yet been recorded in the IPCN. The chromosome number of *Ageratum conyzoides* (Figure 3.B) was not found in the IPCN. Razaq et al. (1994) found that *A. conyzoides* in Pakistan have a chromosome number of $n=10$ and $2n=20$, which has also been found in Nepal (Pushpa et al. 2013). Moreover, Morton (1993) and Dey (1979), in Pushpa et al. (2013), reported that they observed *A. conyzoides* with a chromosome number of $2n=40$. The chromosome numbers of *A. conyzoides* at the UI campus showed different results, namely $2n=37$. However, we also found *A. conyzoides* with the same chromosome number of that species observed by Morton (1993) and Dey (1979) ($2n=40$).

Similar to *A. conyzoides*, the chromosome number of *Cyanthillium cinereum* (Figure 3.C) is not yet found in the IPCN. Carr et al. (1999) found that *C. cinereum* has a chromosome number of $n=9$ but, apart from this, there is a lack of information about the chromosome numbers of that species. The chromosome numbers of *C. cinereum* observed in this study were $n=9$ and $2n=16$. We also found three variations in the chromosome numbers of *Chromolaena odorata* (Figure 3.D) ($2n=40$, $2n=46$, $2n=60$), and only one of them was similar to that reported in the IPCN ($2n=60$). Jose and Mathew (1995) showed that *C. odorata* in India has a chromosome number of $2n=48$. This means that the *C. odorata* observed in the present study have different chromosome numbers and higher variation than the species in India.

Synedrella nodiflora (Figure 3.E) in the UI campus varied in chromosome numbers ($2n=32$, $2n=34$, $2n=36$, $2n=38$, $2n=40$), and was more varied than that reported by Lorence (1995) in Marquesas Island, Hawaii ($2n=18$, $2n=32$, $2n=36$, $2n=38$, $2n=40$). The number of *S. nodiflora* chromosomes previously reported in the IPCN were $2n=18$ and $2n=36$ (Nirmala and Rao 1984), $2n=40$ (Xie and Zheng 2003), and $2n=34$, $2n=68$ (Jose and Mathew 1995). In the present study, we found new variations in the chromosome numbers of *S. nodiflora* that have not yet been reported in the IPCN.

The IPCN data of *Youngia japonica* (Figure 4.A) chromosome number reported by Nishikawa (1984) in Japan is in agreement with the chromosome number reported by Pak (1991) in Korea, which is $2n=16$. The chromosome number of *Y. japonica* found in UI campus were $2n=14$, $2n=18$, and $2n=26$. These three variations of chromosome numbers have not been indexed in IPCN. Similar results were also gained from the analysis of

Data in Table 1 clearly show that the variation in chromosome numbers of 12 species of Asteraceae is relatively high. Chromosome number of those 12 species listed in IPCN could be found in UI campus. Besides, other numbers also appeared. One of the probable mechanisms explaining this variation is the occurrence of euploidy and aneuploidy. According to Kashin et al. (2011), alterations in ploidy levels, such as aneuploidy and mixoploidy, are commonly found in the apical meristem cells of Asteraceae

of apomictic type. The variation in ploidy levels indicates that some Asteraceae species are able to reproduce sexually, while others reproduce by facultative apomixis. The sexually reproductive plants may generate diploid cells, while the facultative apomixis types generate more varied ploidy, such as haploid, aneuploid, and euploid cells. The connection between aneuploidy and apomixis is due to the inability of aneuploid plants to produce fertile pollen, which leads them to develop a mechanism of apomixis.

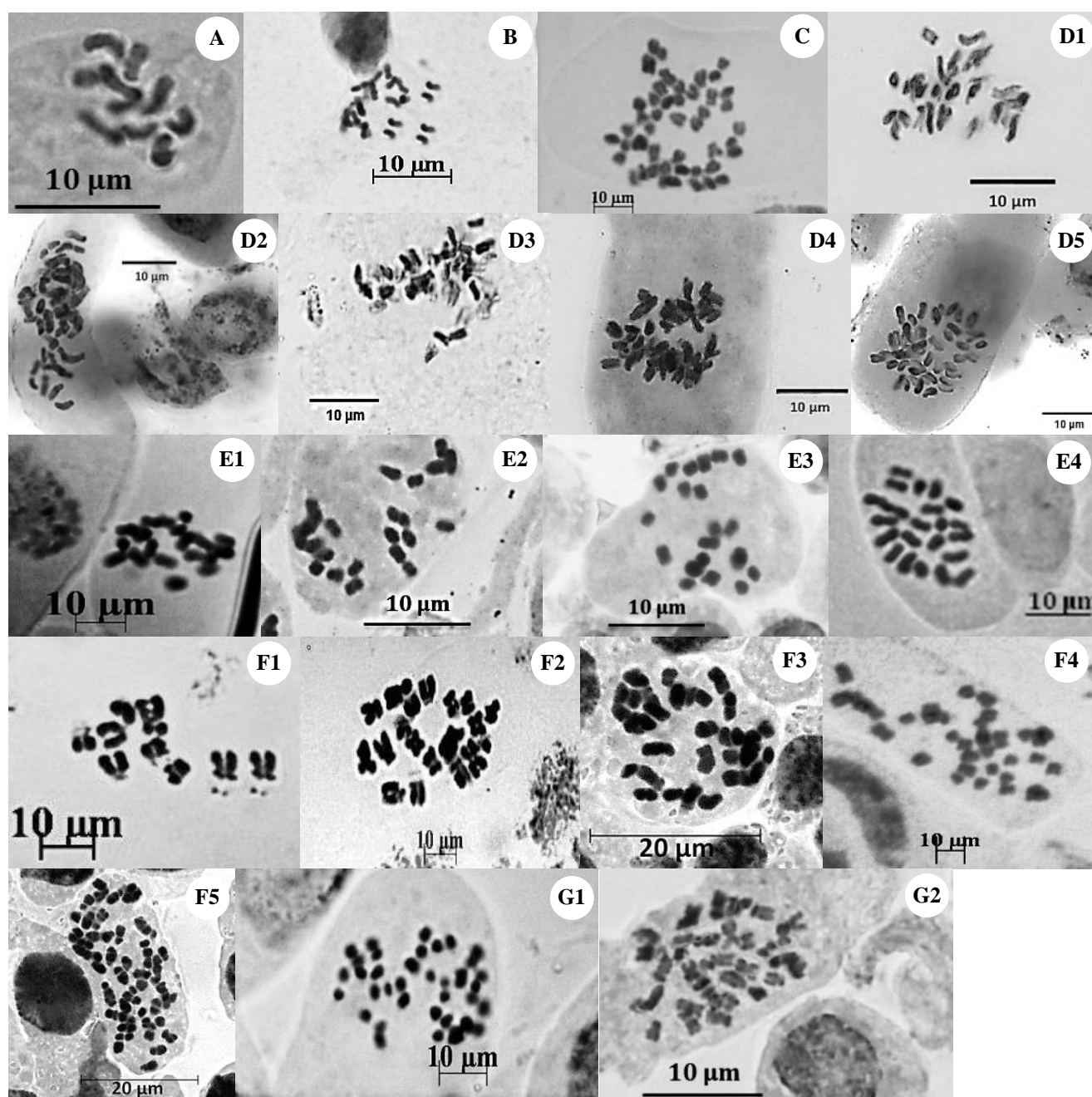


Figure 2. Chromosome number of **A.** *Emilia sonchifolia* $2n=10$ **B.** *Sonchus arvensis* $2n=18$ **C.** *Cosmos sulphureus* $2n=24$ **D.** *Cosmos caudatus* **D1.** $2n=22$ **D2.** $2n=30$ **D3.** $2n=32$ **D4.** $2n=36$ **D5.** $2n=40$ **E.** *Elephantopus scaber* **E1.** $2n=14$ **E2.** $2n=18$ **E3.** $2n=20$ **E4.** $2n=22$ **F.** *Tridax procumbens* **F1.** $n=9$ **F2.** $2n=18$ **F3.** $2n=27$ **F4.** $2n=36$ **F5.** $2n=45$ **G.** *Mikania micrantha* **G1.** $2n=24$ **G2.** $2n=32$

Apomixis is a method of embryo development that does not involve fertilization between ovum and pollen (Stebbins 1950). In some cases, pollen is still needed to fertilize the polar nucleus and establish endosperm. This kind of apomixis is known as pseudogamy apomixis. In other cases, such as autonomous apomixis (Richards 1986), the presence of pollen is not at all necessary for seed formation. Autonomous apomictic organisms tend to produce pollen with lower viability or even sterile pollen (Meirmans et al. 2006, Thompson and Whitton 2006, Thompson et al. 2008). The sterile pollen is formed by disturbances during meiotic division in aneuploid microspore mother cells. Apomixis is an asexual process in flowering plants which results in seeds that are of the same genotype as that of the female parent. It is achieved through processes that occur in the ovule and lead to the avoidance of meiosis, fertilization-independent embryo development and autonomous development of the

endosperm. In gametophytic apomixis, the maternal progeny is the product of an apomictical development of unreduced megagametophytes that differentiate either from a megaspore mother cell which failed to enter meiosis (diplospory) or a somatic nucellar cell which begins gametogenesis in the absence of sporogenesis (apospory). Endosperm development in gametophytic apomixes may follow pseudogamy or autonomous endosperm formation. Apomixis Gametophytic apomixis, irrespective of the mechanism used, are almost invariably polyploids, including mixoploid (Asker and Jerling 1992; Savidan 2000; Kantartzi and Roupakias 2010).

In the case of fertilization occurring between aneuploid gametes, or between diploid and aneuploid gametes, the resulting embryo is also very likely to be aneuploid. Aneuploid embryos grow and develop into plants that are also aneuploid, as are their sporogenous tissues. Nonetheless, problems arise in the pollen from aneuploid

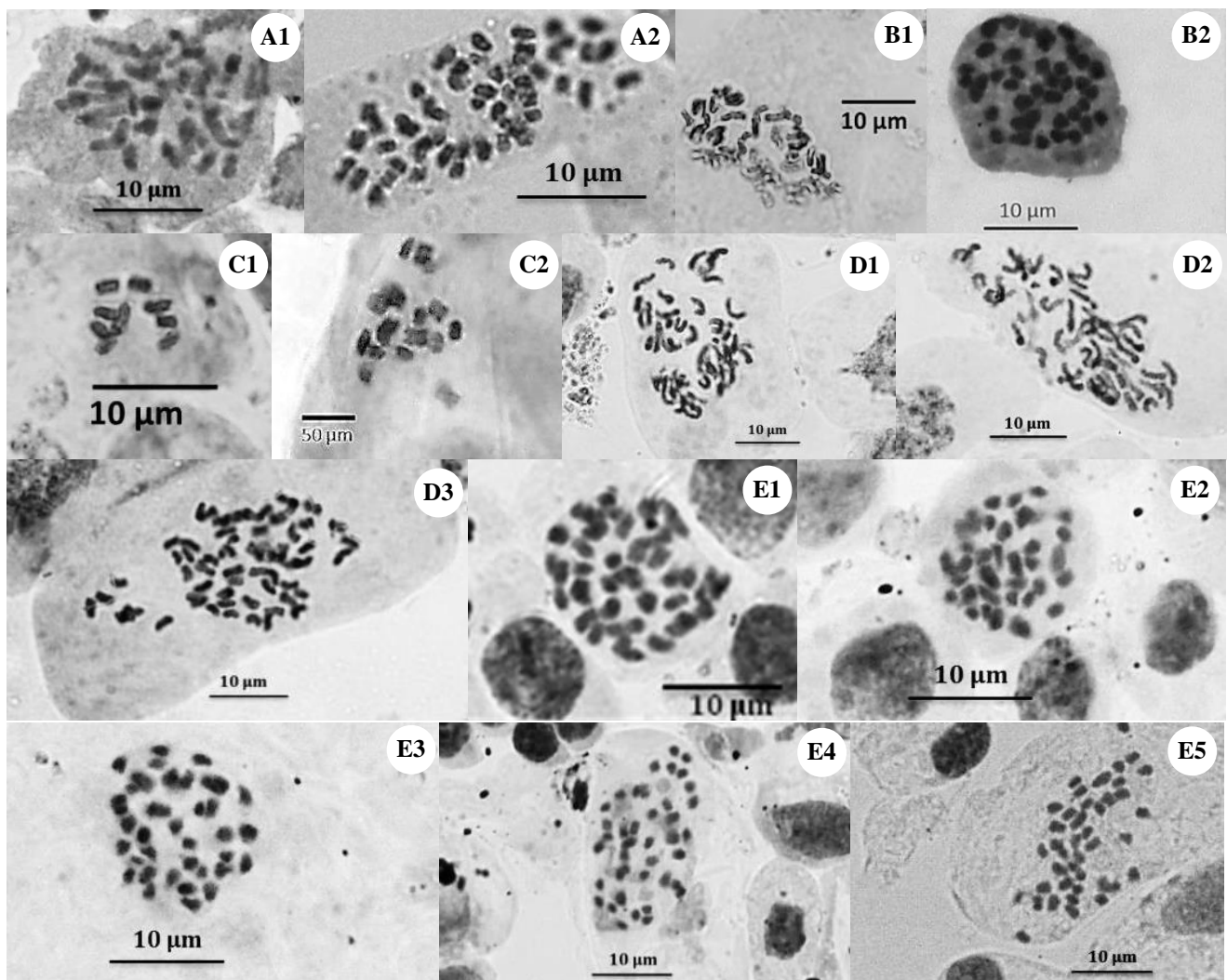


Figure 3. Chromosome number of **A.** *Sphagneticola trilobata* **A1.** $2n=32$ **A2.** $2n=54$, **B.** *Ageratum conyzoides* **B1.** $2n=37$ **B2.** $2n=40$, **C.** *Cyathillium cinereum* **C1.** $n=9$ **C2.** $2n=16$, **D.** *Chromolaena odorata* **D1.** $2n=40$ **D2.** $2n=46$ **D3.** $2n=60$, **E.** *Synedrella nodiflora* **E1.** $2n=32$ **E2.** $2n=34$ **E3.** $2n=36$ **E4.** $2n=38$ **E5.** $2n=40$

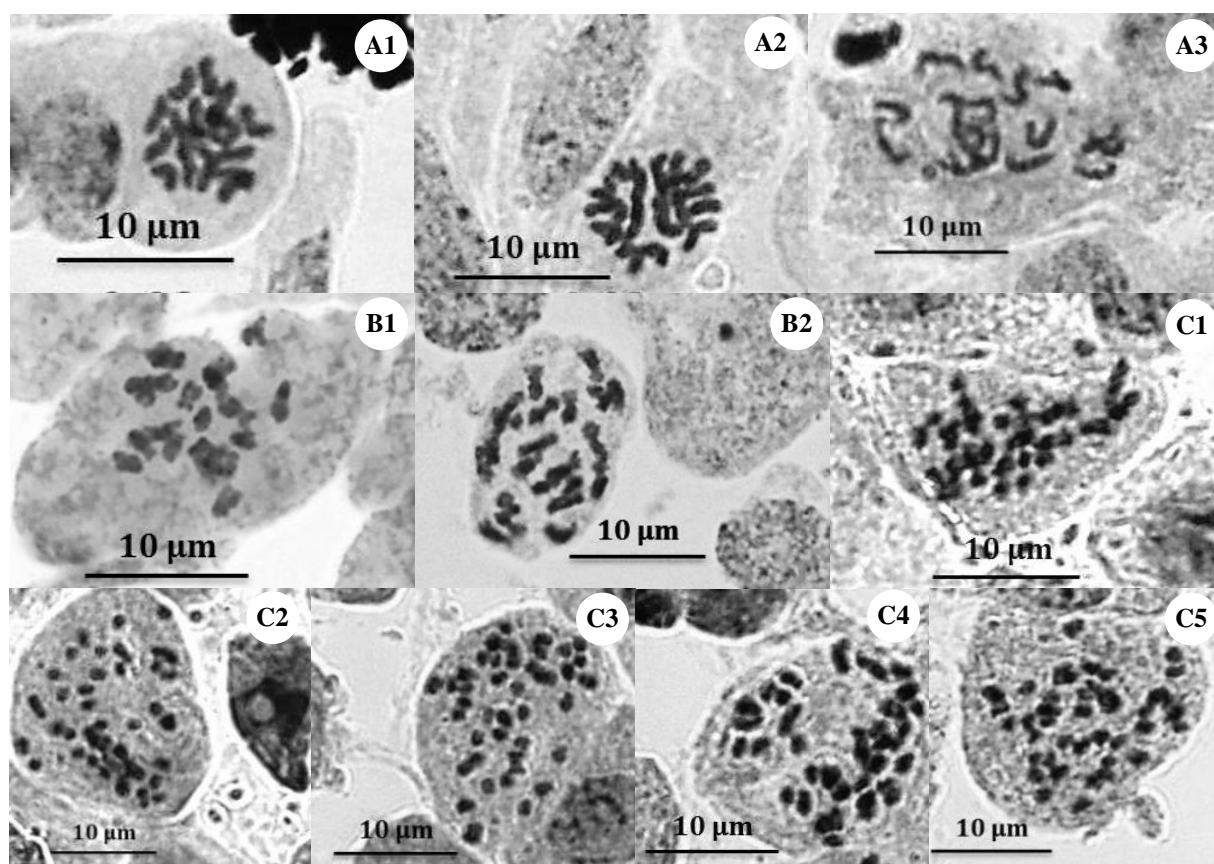


Figure 4. Chromosome number of **A.** *Youngia japonica* **A1.** $2n=14$ **A2.** $2n=18$ **A3.** $2n=26$, **B.** *Eclipta prostrata* **B1.** $2n=18$ **B2.** $2n=22$, **C.** *Porophyllum ruderale* **C1.** $2n=28$ **C2.** $2n=32$ **C3.** $2n=33$ **C4.** $2n=36$ **C5.** $2n=40$

sporogenous tissue. Aneuploid mother cells in sporogenous tissue suffer genomic instability, which can lead to the failure of the chromosome pairing process during prophase I of meiotic cell division. This situation interferes with the formation of pollen and leads to the formation of sterile pollen. The formation of sterile pollen in Asteraceae is most likely caused by an anomaly in spindle threads of microspore mother cells as discussed by Kashin et al (2011) in their research on the formation of sterile pollen in *Pilosella officinarum*, which is known as an example of Asteraceae experiencing autonomous facultative apomixis. The anomaly is a secondary effect of chromosomal failure in pairing and anomaly in prophase I (Kashin et al. 2011).

The formation of sterile pollen (in other words, the inability to produce fertile pollen) means that the reproductive process cannot proceed. This allows the plant to develop an alternative mechanism of adaptation to reproduce and produce offspring. Apomixis is one such alternative. By that mechanism, a process of reproduction forms an embryo in the absence of fertile pollen formation. The formation of aneuploidy in some plants can, therefore, be used as an indicator that the apomictic mechanism is present in the plant. More research is, however, required to determine the development of the microsporogenesis spores on the plants.

The evidence that apomixis is associated with mixoploidy have been reported in many plants (Hu et al. 1991; Mártonfi et al. 1996; Hojsgaard et al. 2008). In hemigamy, the nuclei of egg and sperm do not fuse but start independent mitotic divisions. The process was observed mostly in unreduced egg cells of apomictic plants. Hemigamic embryos may contain cells with nuclei of paternal and maternal origin separated or a mixoploid embryo is for cells of various eu- and aneuploid levels after fusion of mitotic groups of chromosomes or spindles in dividing binucleate cells (Bhojwani and Soh 2001).

Hu et al. (1991) assumed that the chromosomal instability contributed to the apomixis. Furthermore, environmental factors have also been reported to play significant roles in this chromosomal instability, and ploidy changed (Storme and Mason 2014). These factors may account for a great variety of the chromosome numbers of the same species investigated in this study. The chromosome number data is of great importance as the basic data for further chromosome analysis, such as the relationship between chromosome numbers with the great variety of Asteraceae. Further analysis such as karyotyping, Fluorescence In Situ Hybridization (FISH), and molecular markers will be beneficial to broaden the information gained from chromosome studies. To date, some karyotype

data of Asteraceae have been reported, for example, the karyotypes of *Barnadesia* and *Dasyphyllum* which are unimodal; *Mutisia campanulata* and *Trichocline catharinensis* which have one pair of the large chromosome (Watanabe et al. 2007). Furthermore, by using FISH, the distributional pattern of AT- and GC-rich regions, as well as the physical mapping of rDNA of *Artemisia*, have been reported (Torrell et al. 2003). Another karyotype analysis and FISH has been conducted on *Lactuca* spp. (Matoba et al. 2007).

More recently, the detailed GC-rich bands and rDNA loci of *Tanacetum* genomes were also disclosed (Olanj et al. 2015). However, there has been no report on karyotyping of the Asteraceae found in Indonesia. Thus, chromosome preparation, optimization and chromosome counting were performed in this study which may lead to further analysis in the near future. In conclusion, the chromosome numbers of 15 species of Asteraceae in the UI campus were successfully determined. Further work including karyotyping and FISH analysis is necessary to obtain more comprehensive information on the chromosome of Asteraceae found in UI campus.

ACKNOWLEDGEMENTS

The authors are grateful to Ministry of Research, Technology and Higher Education of the Republic of Indonesia for providing financial assistance through a PUPT grant, contract number 2710/UN2.R3.1/HKP05.00/2017, on behalf of Dr. Noviar Andayani. We thank the Laboratory UI-Olympus Bio-Imaging Center, the Plant Development Laboratory of Biology Department, FMNS, UI, Depok, Indonesia and the Center of Excellence for Indigenous Biological Resources-Genome Studies (CoE IBR-GS) FMNS, UI for the use of their facilities.

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