



## Chromosome number report of three *Carex* sect. *Mitratae* taxa (Cyperaceae) in Korea

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**ABSTRACT:** We report meiotic chromosome numbers of three taxa in *Carex* sect. *Mitratae* in Korea: *Carex breviculmis* R. Br. ( $n = 32_{II}, 33_{II}, 34_{II}$ ), *C. polyschoena* H. Lév. & Vaniot ( $n = 37_{II}, 38_{II}$ ), and *C. sabynensis* Less. ex Kunth ( $n = 27_{II}$ ). Section *Mitratae* is one of the most species-rich Asian groups in *Carex*, comprising approximately 45–80 taxa. Twenty-seven of these occur in Korea, and they are some of the most challenging taxa to identify due to their obscure and inconspicuous diagnostic characters. Including the counts reported here, half of the native Korean sect. *Mitratae* chromosome numbers have been documented. Their haploid chromosome numbers range from  $n = 10$  to  $n = 40$ , and many exhibit variations in the numbers counted within a taxon. These variations, along with the overall significant variation in sect. *Mitratae*, suggest that dynamic chromosome activity may be related to the high species diversity of *Carex*.

**Keywords:** *Carex*, chromosome, *Carex* sect. *Mitratae*, Cyperaceae

*Carex* sect. *Mitratae* Kükenthal (Cyperaceae) comprises 45–80 taxa, occurring in Asia, Australia, Europe, and New Zealand with high species diversity in East Asia (Tang et al., 2010; Hoshino et al., 2011). The section is characterized by a single, terminal spike inflorescence, tri- or di-stigmas, and membranous perigynia enclosing trigonous achenes with annulate or beak-like features at their apices (Tang et al., 2010; Nam, 2017). In Korea, there are about 180 *Carex* taxa in 43 sections, and sect. *Mitratae* is the largest one with 27 taxa, including one endemic, *C. sabynensis* Less. ex Kunth var. *leiosperma* Ohwi (Park et al., 2016; Nam, 2017). They grow sunny, wet places in forests and roadsides and bloom in early spring (Hoshino et al., 2011; Park et al., 2016).

Chromosome numbers dramatically vary in *Carex*, ranging from  $n = 6$  to  $n = 66$  (Tanaka, 1949; Roalson, 2008). Chromosomes in Cyperaceae are holocentric and lack localized centromeres. Holocentric chromosomes have spindle fibers attached along the entire length of the chromosome arms and viable fragments can be increased (fission, agmatoploidy) and/or decreased (fusion, symploidy) without DNA duplication/deletion events

(Malheiros-Gardé and Gardé, 1950; Luceño, 1994; Luceño and Guerra, 1996; Hipp et al., 2013). Due to these features, continuous chromosome number variation in *Carex* can result from either agmatoploidy/symploidy or aneuploidy (Luceño and Guerra, 1996; Hipp et al., 2009; Chung et al., 2011). Chromosome evolution plays an important role in the species richness of *Carex*, which is the most diverse flowering plant genus in temperate zones with more than 5,000 taxa worldwide (Hipp et al., 2009; Global *Carex* Group, 2015).

Many members of sect. *Mitratae* have variation in chromosome numbers within taxa, and univalent, trivalent, and/or quadrivalent chromosomes are observed in some species such as *Carex caryophyllea* Latourr. and *C. umbrosa* Host subsp. *huetiana* (Boiss.) Soó (Luceño, 1993; Chung et al., 2016). Recently, meiotic chromosome numbers from Korean populations in the section have been reported: *C. breviculmis* R. Br. ( $n = 33_{II}$ ) (Chung et al., 2017, 2018), *C. fernaldiana* H. Lév. & Vaniot ( $n = 33_{II}$ ) (Chung and Im, 2019), *C. polyschoena* H. Lév. & Vaniot ( $n = 26_{II}, 36_{II}, 37_{II}$ ) (Chung et al., 2016, 2018), *C. sabynensis* Less. ex Kunth ( $n = 27_{II}, 28_{II}, 38_{II}$ ) (Chung

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et al., 2016, 2017, 2018), and *C. tristachya* Thunb. ( $n = 21_{II}$ ) (Chung et al., 2016, 2017). *C. polyschoena* and *C. sabyneensis*, common early-spring bloomers, exhibit high variation in chromosome number.

In the present study, we report additional chromosome numbers of three common sect. *Mitratae* taxa in Korea, *Carex breviculmis*, *C. polyschoena*, and *C. sabyneensis*. These are the most common and widely distributed *Mitratae* species. Chromosome numbers reported for Korean taxa in the section are also documented.

## Materials and Methods

Immature male spikes of *Carex breviculmis*, *C. polyschoena*, and *C. sabyneensis* were fixed for meiotic chromosome number observation, following the methods of Rothrock and Reznicek (1996) and Chung et al. (2016). Spikes (terminal spikes in *Carex* sect. *Mitratae*) with immature anthers were preserved in a mixture of methanol, chloroform, and propionic acid (6:3:2) and then transferred to 70% ethanol. Fixed anthers were squashed in 1% acetic-orcein and observed at 1,000 $\times$  magnification (Nikon Eclipse 50i, Nikon, Tokyo, Japan). More than two meiotic division cells per individual were observed and photographed. Voucher specimens with mature perigynia were collected and identified following Hoshino et al. (2011) and Park et al. (2016). The vouchers were deposited at

Chonnam National University herbarium (CNU, department of division of biological science).

## Results and Discussion

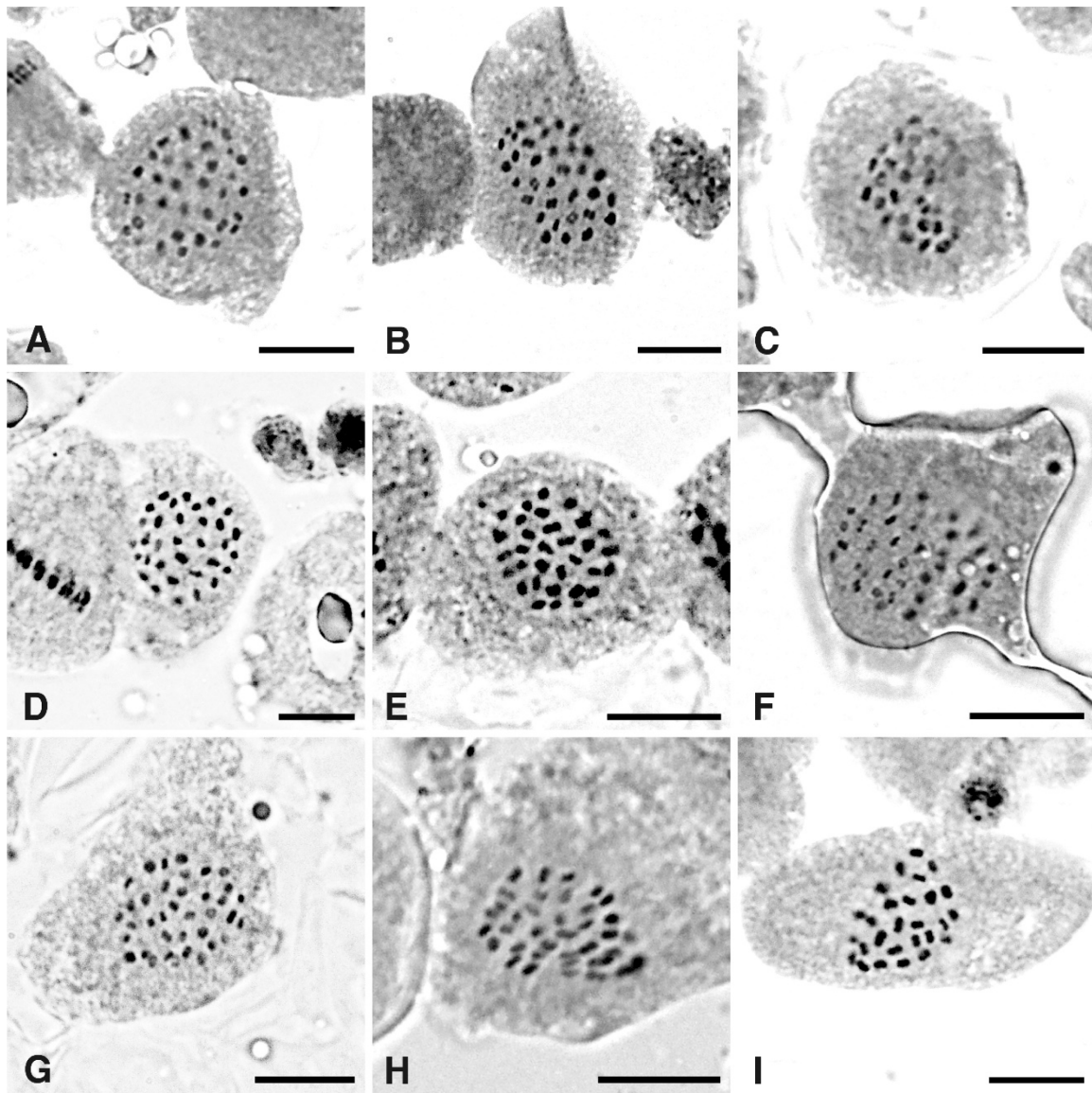
Meiotic chromosome numbers of three taxa in *Carex* sect. *Mitratae* were observed (Table 1). Both *C. breviculmis* and *C. polyschoena* exhibited variation in chromosome numbers within the taxon and/or individual. Four individuals of *C. breviculmis* exhibited continuous variation in chromosome numbers, from  $n = 32_{II}$  to  $n = 34_{II}$ . Two *C. polyschoena* individuals also had variation with  $n = 37_{II}$  and  $38_{II}$ . *C. sabyneensis* had the meiotic chromosome number of  $27_{II}$ . Their chromosomes were very small less than 2  $\mu\text{m}$  long, and constricted centromeres were not visible (Fig. 1).

### *Carex breviculmis* R. Br. ( $n = 32_{II}, 33_{II}, 34_{II}$ ) (Fig. 1A–E)

One count of  $n = 32_{II}$ , one count of  $n = 33_{II}$ , and two counts of  $n = 34_{II}$  were observed in *C. breviculmis* (Fig. 1A–E). The counts made in the present work were identical to those in Tanaka (1939), Hoshino (1981), Ohkawa and Yokota (1998), de Lange and Murray (2002), and Chung et al. (2017, 2018). Following the taxonomic treatment of *C. breviculmis* (Park et al., 2016; Nam, 2017), previous chromosome counts for *C. leucochlora* were included in the species (Table 1). All the individuals examined were collected in sunny and open

**Table 1.** *Carex* sect. *Mitratae* taxa investigated with voucher specimens and chromosome numbers.

Taxon (locality, voucher specimen)	Chromosome numbers counted, $n$	Previous counts, $2n$
<i>C. breviculmis</i> R. Br.		
Anseo-dong, Cheonan-si, Chungnam ( <i>Chung</i> 7013)	$34_{II}$	54 (Chung et al., 2016, reported as <i>C. leucochlora</i> )
Sinbu-dong, Cheonan-si, Chungnam ( <i>Chung</i> 7017)	$34_{II}$	c.64 (de Lange and Murray, 2002)
Chilseong-myeon, Goesan-gun, Chungbuk ( <i>Chung</i> 7029)	$33_{II}$	66 (Chung et al., 2017)
		66 (Chung et al., 2018)
		64, 68 (Tanaka, 1939 cited from Roalson, 2008)
Dongbu-ri, Goesan-eup, Goesan-gun, Chungbuk ( <i>Chung</i> 7035)	$32_{II}$	68 (Hoshino, 1981 reported as <i>C. leucochlora</i> )
		68 (Ohkawa and Yokota, 1998 reported as <i>C. leucochlora</i> )
		72 (Okuno, 1939 cited from Roalson, 2008)
<i>C. polyschoena</i> H. Lév. & Vaniot		
Chilseong-myeon, Goesan-gun, Chungbuk ( <i>Chung</i> 7019)	$37_{II}$	52 (Chung et al., 2016)
		72, 74 (Chung et al., 2018)
Dongbu-ri, Goesan-eup, Goesan-gun, Chungbuk ( <i>Chung</i> 7032-1)	$37_{II}, 38_{II}$	None
<i>C. sabyneensis</i> Less. ex Kunth		
Anseo-dong, Cheonan-si, Chungnam ( <i>Chung</i> 7009)	$27_{II}$	40 (Krogulevich, 1971)
		54 (Chung et al., 2016)
		54, 56 (Chung et al., 2018)
		60 (Yurtsev and Zhukova, 1982)
		76 (Chung et al., 2017)



**Fig. 1.** Photomicrographs of *Carex* meiotic chromosomes. **A–E.** *C. breviculmis*. **A, B.**  $n = 34_{II}$ , Chung 7013. **C.**  $n = 34_{II}$ , Chung 7017. **D.**  $n = 33_{II}$ , Chung 7029. **E.**  $n = 32_{II}$ , Chung 7035. **F–H.** *C. polyschoena*. **F.**  $n = 37_{II}$ , Chung 7019. **G.**  $n = 37_{II}$ , Chung 7032. **H.**  $n = 38_{II}$ , Chung 7032. **I.** *C. sabyensis*,  $n = 27_{II}$ , Chung 7009. Scale bars = 10  $\mu\text{m}$ .

habitats. The species commonly occurs throughout the country. By the long awns on pistillate scales, pubescent perigynia, and terminal, staminate spikes without peduncles, *C. breviculmis* is distinguished from morphologically similar species such as *C. mitrata* var. *aristata* Ohwi (Park et al., 2016; Nam, 2017).

***Carex polyschoena* H. Lév. & Vaniot ( $n = 37_{II}$ ,  $38_{II}$ ) (Fig. 1F–H)**

From two individuals two different meiotic chromosome numbers were observed,  $n = 37_{II}$  and the first report of  $n = 38_{II}$  in the species (Fig. 1F–H). This new count expands the

meiotic chromosome number variation range to  $n = 26_{II}$ ,  $36_{II}$ ,  $37_{II}$ ,  $38_{II}$  (Chung et al., 2016, 2018). The species occurs in Japan, Korea, and China (Park et al., 2016). Although the species very common in Korea growing through the county, in Japan only a few populations are found only in Tsushima Island, Nagasaki Prefecture, where the holotype of species was collected (Hoshino et al., 2011). Tang et al. (2010) and Govaerts (2020) treated *C. polyschoena* as a synonym of *C. pisiformis* Boott, but Hoshino et al. (2011) recognized them as two independent species and considered *C. pisiformis* as an endemic species in Japan. In addition, Hoshino (1981) reported

chromosome number of  $2n = 68$  for *C. pisiformis*. More recently, two taxa are distinguished by rhizome, pistillate inflorescence shape, and perigynium beak characters (Nam, 2017). Species delimitation of the species *C. polyschoena* and *C. pisiformis* should be reexamined covering entire distribution areas of the species.

### **Carex sabynensis Less. ex Kunth ( $n = 27_{II}$ ) (Fig. 1I)**

Multiple cells from one individual of *C. sabynensis* constantly had a meiotic chromosome number of  $n = 27_{II}$  (Fig. 1I) as in Chung et al. (2016, 2018), which was also observed from Korean populations. Counts from other Korean individuals show variation with numbers of  $n = 27_{II}, 28_{II}, 38_{II}$  (Table 1).

The species occurs broadly in East Asia, and variation in chromosome number is evident throughout its range (Hoshino et al., 2011). The species was considered as a subspecies of *C. umbrosa* Host – *C. umbrosa* subsp. *sabynensis* (Less. ex Kunth) Kük Govaerts (2020). However, Nam (2017) treated *C. sabynensis* as an independent species based on distinct pistillate inflorescence and perigynium characters.

### **Chromosome number variation in sect. *Mitratae***

The chromosome numbers of half of native Korean sect. *Mitratae* taxa have been documented (Table 2). The haploid numbers range from  $n = 10$  (*C. blepharicarpa* Franch.) to  $n = 40$  (*C. stenostachys* Franch. & Sav.), and most taxa exhibit

**Table 2.** Chromosome numbers reported in *Carex* sect. *Mitratae* in Korea.

Taxon	Chromosome number, $2n$	Distribution
<i>C. alterniflora</i> var. <i>rubrovaginata</i> J. Oda & Nagam.	None	Japan, Korea
<i>C. blepharicarpa</i> Franch.	30, 32 (Hoshino, 1981) 26-33, 41 (Hoshino and Okamura, 1993) 20 (Lee and Kim, 2008)	Japan, Korea
<i>C. breviculmis</i> R. Br.	72 (Okuno, 1939 cited from Roalson, 2008) 64, 68 (Tanaka, 1939 cited from Roalson, 2008) 68 (Hoshino, 1981 reported as <i>C. leucochlora</i> ) 68 (Ohkawa and Yokota, 1998 reported as <i>C. leucochlora</i> ) c.64 (de Lange and Murray, 2002) 54 (Chung et al., 2016, reported as <i>C. leucochlora</i> ) 66 (Chung et al., 2017) 66 (Chung et al., 2018) 64, 66, 68 (This study)	Japan, China, Russia, Nepal, India, Myanmar, Taiwan, Korea
<i>C. candolleana</i> H. Lév. & Vaniot	None	Japan, Korea
<i>C. conica</i> Boott	34, 35, 38 (Tanaka, 1938 cited from Roalson, 2008) 34, 38, 42 (Okuno, 1939 cited from Roalson, 2008) 34, 35, 38 (Tanaka, 1939 cited from Roalson, 2008) 34, 38 (Funabiki, 1958 cited from Roalson, 2008) 32, 36 (Hoshino and Okamoto, 1979) 32-38 (Hoshino, 1980) 32, 34, 36-38 (Hoshino, 1981) 32, 34, 36, 38 (Hoshino, 1989)	Japan, Korea
<i>C. fernaldiana</i> H. Lév. & Vaniot	66, 67, 68, 72 (Tanaka, 1940 cited in Roalson, 2008 as <i>C. sachalinensis</i> F. Schmidt var. <i>feraldiana</i> (H. Lév. & Vaniot) T. Koyama) 66 (Chung and Im, 2019)	Japan, Taiwan, Korea
<i>C. fibrillosa</i> Franch. & Sav.	None	Japan, China, Taiwan, Korea
<i>C. genkaiensis</i> Ohwi	None	Japan, Korea
<i>C. hypochlora</i> Freyn	None	China, Russia, Korea
<i>C. kamagariensis</i> K. Okamoto	None	Japan, Korea
<i>C. matsumurae</i> Franch.	66 (Okuno, 1939 cited in Roalson, 2008) 64 (Hoshino, 1981)	Japan, Korea
<i>C. meridiana</i> (Akiyama) Akiyama	None	Japan, Korea
<i>C. microtricha</i> Franch.	30-32 (Probatova et al., 2004)	Japan, Korea

Table 2. Continued.

Taxon	Chromosome number, $2n$	Distribution
<i>C. mitrata</i> Franch. var. <i>mitrata</i>	None	Japan, China, Taiwan, Korea
<i>C. mitrata</i> var. <i>aristate</i> Ohwi	None	Japan, China, Taiwan, Korea
<i>C. multifolia</i> Ohwi	30 (Okuno, 1939 cited in Roalson, 2008 as <i>C. dolichostachya</i> Hayata) 30, 60, 64–66 (Tanaka, 1940 cited in Roalson 2008 as <i>C. dolichostachya</i> Hayata) 30, 60, 64–66, 70 (Funabiki, 1958 cited in Roalson 2008 as <i>C. dolichostachya</i> Hayata) 70 (Hoshino, 1981)	Japan, Korea
<i>C. nervata</i> Franch. & Sav.	76 (Okuno, 1939 cited in Roalson, 2008) 76 (Tanaka, 1939b cited in Roalson, 2008)	Japan, Russia, Korea
<i>C. pocilliformis</i> Boott	38, 40 (Hoshino, 1981a cited in Roalson, 2008 as <i>C. tristachya</i> Thunb. var. <i>pocilliformis</i> (Boott) Kük.) 39 (Ohkawa and Yokota, 1998)	Japan, China, Taiwan, Korea
<i>C. polyschoena</i> H. Lév. & Vaniot	52 (Chung et al., 2016) 72, 74 (Chung et al., 2018) 74, 76 (this study)	Japan, Korea
<i>C. rugata</i> Ohwi	None	Japan, China, Taiwan, Korea
<i>C. sabyensis</i> var. <i>sabyensis</i>	40 (Krogulevich, 1971) 60 (Yurtsev and Zhukova, 1982) 54 (Chung et al., 2016) 76 (Chung et al., 2017) 54, 56 (Chung et al., 2018) 54 (this study)	Japan, China, Russia, Korea
<i>C. sabyensis</i> var. <i>leiosperma</i> Ohwi	None	Korea
<i>C. stenostachys</i> Franch. & Sav.	58 (Hoshino, 1981) 80 (Yurtsev and Zhukova, 1978 cited in Roalson, 2008) 58–60 (Hoshino et al., 1993) 58–61 (Hoshino et al., 1994 cited in Roalson, 2008)	Japan, Korea
<i>C. subbracteata</i> (Kük.) Ohwi	None	Japan, China, Russia, Korea
<i>C. toyoshimae</i> Tuyama	62 (Yano, 2006)	Japan, Korea
<i>C. tristachya</i> Thunb.	42 (Chung et al., 2016) 42 (Chung et al., 2017)	Japan, China, Korea
<i>C. tsuhimensis</i> (Ohwi) Ohwi	None	Japan, Korea

variation in their chromosome numbers. Among the fourteen taxa, only three have consistent chromosome numbers: *C. nervata* Franch. & Sav. ( $2n = 76$ ), *C. toyoshimae* Tuyama ( $2n = 62$ ), and *C. tristachya* Thunb. ( $2n = 42$ ). In contrast, *C. multifolia* Ohwi exhibits the broadest range of variation ( $2n = 30, 60, 64–66, 70$ ). Because chromosome variation in *Carex* provides important information on taxonomy as well as genetic diversity, it is encouraged to investigate additional individuals of every taxon (Hoshino et al., 1993; Hip et al., 2010).

Positive correlations between chromosome number and

genetic diversity and geographic distance have been found (Luceño and Castroviejo, 1991; Hipp et al., 2010). Varying chromosome numbers might reflect genetic diversity within and/or among individuals in a taxon but also might result from incongruent hypotheses on species delimitations among researchers. Previous taxonomic studies were limited to certain geographic areas, such as China (Tang et al., 2010) and Japan (Hoshino et al., 2011). Comprehensive systemic research targeting the section's entire geographic range are needed. In addition, laboratory and/or biological errors might present. For

instance, somatic metaphase chromosome number of *C. blepharicarpa* Franch. reported by Lee and Kim (2008) is  $2n = 20$ , but many chromosomes are overlapping each other in the image and seem to be considered as mono-centromere chromosomes by the authors. It could be  $2n =$  about 40. All the voucher specimens and raw chromosome data should be available to researchers, so that critical cytological data of *Carex*, taxonomically challenging taxa, are well-documented for further research on phylogeny and evolution.

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## Conflict of Interest

The authors declare that there are no conflicts of interest.

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