

Taxonomic reexamination of *Carex* subg. *Carex* (Cyperaceae)
from East and Southeast Asia

Kotaro T. Takahashi

2025

CONTENTS

Summary	1
General Introduction	2

Chapter I.

Phylogenetic analysis and infrageneric classification of the basal lineages of *Carex* subg. *Carex*

Abstract.....	4
Introduction	4
Materials and Methods	6
Results	9
Discussion.....	12
Acknowledgements	26
References	26
Tables	32
Figures	39
Appendix	45

Chapter II.

Phylogenetic Analyses of the *Carex filipes* complex (sect. *Paniceae*) in East Asia, with Reference to Morphology, Karyology and Taxonomy

Abstract.....	49
Introduction	49
Materials and Methods	52

Results	55
Discussion.....	59
Acknowledgements	73
References	74
Tables.....	81
Figures	87

Chapter III.

Molecular phylogenetic analysis of the *Carex macroglossa* complex with reference to variation in morphology, chromosomal features and species delimitation

Abstract.....	100
Introduction	100
Materials and Methods	102
Results	103
Discussion.....	105
Acknowledgements	108
References	109
Tables.....	112
Figures	115

Chapter IV.

Carex nasuensis, a new species from Honshu, Japan

Abstract.....	120
Introduction	120
Materials and Methods	121

Results & Discussion.....	123
Acknowledgements	127
References	127
Tables.....	130
Figures	132
Conclusion	138
Acknowledgement	139

Summary

Carex comprises approximately 2,150 species, representing a highly diversified group predominantly distributed in the temperate regions of the Northern Hemisphere. Its early diversification is considered to have occurred in East and Southeast Asia, an area recognized as a critical center of *Carex* diversity. Despite its significance, comprehensive sampling and molecular phylogenetic studies in this region remain inadequate, leaving its biodiversity and evolutionary history largely unexplored. To address this, this study aimed to elucidate the diversity and evolutionary history of *Carex* in East and Southeast Asia through two focused investigations.

In Chapter I, a reassessment of section delimitation within the basal lineages of subgenus *Carex* was conducted. This subgenus, containing approximately 1,500 species, is thought to have undergone its initial diversification in East and Southeast Asia. Comprehensive phylogenetic analyses, incorporating extensive plastid sequence data from specimens collected in Japan, China, Thailand, and the Himalayas, were carried out. This study clarified the phylogenetic positions of previously ambiguous taxa, such as *C. dissitiflora* and *C. satsumensis*, described a new section (sect. *Dissitiflorae*), and proposed revisions to the circumscription of three existing sections (sects. *Filicinae*, *Mundae*, and *Stramentitiae*).

In Chapter II to IV, a reexamination of species delimitation was performed for members of *Carex* sect. *Paniceae*, a highly diversified group in East Asia. To delineate species boundaries accurately, extensive sampling across their distribution ranges was conducted. Phylogenetic analyses based on chloroplast DNA (*atpB-rbcL*, *rpl16*, *rps16*, *trnK-matK-trnK*, *trnL-trnL-trnF*) and nuclear DNA (ITS, ETS), as well as chromosome counts and morphological measurements, were utilized to examine reproductive isolation. As a result, three new species (*C. curtispica*, *C. nasuensis*, *C. sohayakiensis*), two new taxonomic ranks (*C. kuzakaiensis*, *C. tsukudensis*), and one new hybrid (*C. ×kurogii*) were identified.

General Introduction

Carex L. (Cyperaceae) is the fifth-largest genus of flowering plants, comprising about 2,150 species (Roalson *et al.* 2021). It is morphologically distinct from other genera due to its unisexual flowers enclosed by a unique structure called a perigynium (Goetghebeur, 1998; Starr *et al.*, 2004). Since its formal description by Linnaeus in 1753, the genus has undergone minimal taxonomic reorganization. Although recent molecular phylogenetic studies have incorporated several minor genera (*Cymophyllus*, *Kobresia*, *Schoenoxiphium*, and *Uncinia*), they share the trait of unisexual flowers enclosed by a perigynium (Global Carex Group, 2015). Remarkably, *Carex* exhibits high diversity in temperate to cold-temperate regions while showing an inverse latitudinal gradient in species richness in tropical areas (Kindlmann *et al.* 2007, Escudero *et al.* 2012, Martín-Bravo *et al.* 2019). This distribution is attributed to its early diversification within East and Southeast Asia during the first 10 million years of its evolutionary history, followed by dispersal into the cold-temperate zones of the Northern Hemisphere (Martín-Bravo *et al.* 2019). Consequently, East Asia is considered both the cradle of *Carex* diversity and a "museum" of its existing species diversity.

Among the six subgenera of *Carex*, the subgenus *Carex* stands out with approximately 1,500 species, representing the majority of the genus' diversity. This subgenus is particularly diverse in East Asia compared to other subgenera (Martín-Bravo *et al.* 2019, Roalson *et al.* 2021). However, taxonomic studies in this region have predominantly relied on morphological data, leading to significant issues in both section and species delimitation. Many sections of *Carex* from East and Southeast Asia have been revealed to be polyphyletic (Jiménez-Mejías *et al.* 2016, Martín-Bravo *et al.* 2019), indicating problems with traditional morphology-based classifications. Furthermore, species delimitation is problematic due to substantial intraspecific morphological variation, leaving the boundaries of many species unclear (Simpson & Koyama 1998, Katsuyama 2015). The root of these issues lies in the severe lack of sampling in this region compared to other regions (Martín-Bravo *et al.* 2019), resulting in insufficient molecular data to support taxonomic revisions.

To accurately understand the diversity of *Carex* in East Asia, a region critical to the genus' evolutionary history, the following steps are essential: (i) Comprehensive sampling covering entire sections or species, (ii) Construction of robust molecular phylogenies using extensive DNA sequence data, (iii) Reevaluation of diagnostic traits to facilitate taxonomic revisions. This study addresses these challenges by investigating the evolutionary history and diversity of *Carex* in East and Southeast Asia through two main approaches. Chapter I revisits section delimitation in the basal lineages of subgenus *Carex* using plastid DNA-based phylogenies and morphological observations. Chapter II–IV focuses on species delimitation within sect. *Paniceae* G. Don by employing molecular phylogenetic analyses, chromosome observations, and morphological assessments to evaluate reproductive isolation and refine species boundaries.

Chapter I

Phylogenetic analysis and infrageneric classification of the basal lineages of *Carex* subg. *Carex*

Abstract

Carex (Cyperaceae) is a diverse genus with approximately 2,150 species. Recent molecular studies showed that, although the genus has a nearly cosmopolitan distribution, East and Southeast Asia is the cradle of its diversification. They further showed that *Carex* comprises six subgenera, among which subg. *Carex* is the largest in number of species. Despite these studies, the relationships and classification of basal lineages within subg. *Carex* remain unresolved, owing to their considerable morphological diversity and limited sampling of Asian plants in previous molecular studies. This report is based on a comprehensive phylogenetic analysis of the basal lineages within subg. *Carex* that used extensive chloroplast CDS data (70 loci, 58,601 bp) obtained from samples collected in Japan, China, Thailand, and the Himalaya. Based on the phylogenetic results as well as an examination of eleven morphological characters, seven sections, *Albae*, *Decorae*, *Dissitiflorae* (a new section for *C. dissitiflora* and *C. bostrychostigma*), *Filicinae*, *Graciles*, *Indicae* and *Mundae* are recognized as basal monophyletic lineages in subg. *Carex*. In the phylogeny of subg. *Carex*, *Dissitiflorae* occupied the most basal position with 100% bootstrap support. A clade (100%) comprising *Albae*-*Graciles* subclade (100%) and *Indicae*-*Mundae* subclade (100%) diverged as the next branch, followed by the *Decorae*-*Filicinae* clade (100%) and the Core subg. *Carex* clade (84%).

Introduction

Carex L. (Cyperaceae), the fifth largest genus of angiosperms (Frodin 2004), comprises approximately 2,150 species (Roalson *et al.* 2021). *Carex* is distinguished from other genera of Cyperaceae Juss. by its unisexual flowers and presence of the perigynium that corresponds to a

prophyll enclosing the achene (Goetghebeur 1998, Starr *et al.* 2004). Based on recent molecular phylogenetic analyses, six subgenera are recognized within *Carex*: *Carex*, *Euthyceras* Peterm., *Psyllophorae* (Degland) Peterm., *Siderosticta* M. J. Waterway, *Uncinia* (Pers.) Peterm. and *Vigneae* (P. Beauv. ex T. Lestib.) Heer (Villaverde *et al.* 2020). Another molecular analysis (Martín-Bravo *et al.* 2019) focusing on biogeography and diversification showed that, although *Carex* has a nearly cosmopolitan distribution, East and Southeast Asia is the cradle of *Carex* diversification. Therefore, the species of East and Southeast Asia are crucial for understanding early and subsequent evolutionary history within *Carex*.

Among the six subgenera, *C. subg. Carex* is the most derived group in the genus (Villaverde *et al.* 2020) and is by far the largest in number of species and morphologically the most variable within *Carex*. It comprises around 1,500 species, which is approximately 70% of all species in the genus (Roalson *et al.* 2021). Figure 1 is a phylogenetic tree modified from Roalson *et al.* (2021) showing the new framework of the infrasubgeneric classification of *C. subg. Carex* based on the latest molecular phylogenetic studies utilizing ITS, ETS and *matK* markers (Jiménez-Mejías *et al.* 2016, Martín-Bravo *et al.* 2019). Even though they showed that *C. subg. Carex* comprises twelve clades in five major lineages, the relationships between them were unclear, but they did provide comparisons with sections proposed in previous treatments (Kükenthal 1909, Ohwi 1936, Nelves 1951, 1955, Dai *et al.* 2010). As for the twelve clades, Roalson *et al.* (2021) recognized six of them as sections (*C. sects. Albae* (Asch. & Graebn.) Kük., *Alliiformes* Akiyama, *Cryptostachyae* Franch., *Graciles* Kük., *Grallatoriae* Kük. and *Secalinae* (O. Lang) O. Lang ex Kük.), and left the remaining six as taxonomically informal clades (Decora Clade, Dissitiflora Clade, Indica Clade, Tristachya Clade), *Incertae Sedis* and a large clade shown as ‘Fig. 1c,’ which we show as ‘Core subg. *Carex*’ in Fig. 1. According to Roalson *et al.* (2021, p. 727), the “informal clade names are used to represent clades that either have the potential to be described as formal sections eventually, but that are not currently recognized due to uncertainty about nomenclature or morphological diagnosability of the clade, or are strongly supported by molecular data.”

Despite the importance of the species of East and Southeast Asian as stated above, the previous phylogenetic studies included relatively few species from East and Southeast Asia. Martín-Bravo et al. (2019), who analyzed the largest number of East and Southeast Asian species, included 51% of the species from East and Southeast Asia and more than 97% of the species from circumboreal regions. The unresolved relationships among the aforementioned eleven basal lineages of *C.* subg. *Carex* (Roalson et al. 2021) are likely due to the limited samples from East and Southeast Asia that have been phylogenetically analyzed in previous studies and also the short DNA sequences (only ETS, ITS and *matK*) that were used in previous phylogenetic reconstructions. This study aimed to extensively sample the species of East and Southeast Asia, to construct a robust phylogenetic tree using a broad set of chloroplast CDS regions, to assess the circumscription of the eleven basal lineages, and to clarify their relationships. We further analyzed morphological characteristics supporting the individual clades through observations of herbarium specimens and literature surveys in an attempt to resolve the taxonomic uncertainty surrounding the Dissitiflora, Indica and Decora clades in Roalson et al. (2021).

Materials and Methods

Sources of materials for chloroplast CDS analysis

All samples used in this study, along with voucher data, are listed in Table 1. The targeted taxa consisted of 13 sections (*Carex* sects. *Albae*, *Chlorostachyae*, *Debiles* (J. Carey) Ohwi, *Decorae* (Kük.) Ohwi, *Euprepes* Nelmes & Airy Shaw, *Graciles*, *Indicae* (L. H. Bailly) C. B. Clarke, *Lageniformes* (Ohwi) Nelmes, *Mapaniifoliae* Nelmes & Airy Shaw, *Mitratae* Kük., *Mundae* Kük. and *Scabrellae* Kük.) specified as early-diverging lineages of *Carex* subg. *Carex* by Roalson et al. (2021) (Fig. 1). Three additional sections (*Pacificae* Ohwi, *Grallatoriae* and *Alliiformes*) and ‘Incertae Sedis’ (*C. finitima* Boott) treated by Roalson et al. (2021), whose phylogenetic positions were not determined by Roalson et al. (2021), were also included in this analysis.

Forty four samples of 43 species were collected from Japan, China, Thailand and the Himalaya.

Identification of the species followed Hoshino *et al.* (2020) for samples collected in Japan, Dai *et al.* (2010) for those from China, Simpson & Koyama (1998) for those from Thailand, and Koyama (1978) for those from the Himalaya. One sample collected in Tak, Thailand (*M. N. Tamura et al.* 69917, KYO), was similar to *C. nodiflora* Boeckeler, but differed by having narrower leaves (2–4 mm wide vs. 5–10 mm wide in *C. nodiflora*) and a longer spike (10–15 mm long vs. 6–8 mm long in *C. nodiflora*), was identifiable based on Simpson & Koyama (1998) and previous taxonomic treatments; it was treated as *C. aff. nodiflora* (Fig. 2C).

DNA extraction and sequencing

Total DNA was extracted from leaves dried in silica gel or from herbarium specimens using the CTAB method described in Takahashi *et al.* (2021). The DNA was fragmented to approximately 300–800 bp using the xGen DNA Library Prep Kit (Coralville, IA, USA). A DNA library was prepared and sequenced to produce 150-bp paired-end (PE) reads using the DNBSEQ-G400 platform at BGI (Shenzhen, China).

Reconstruction of chloroplast CDS sequence data

In addition to the plastome sequences obtained from the 44 samples used in this study, plastome sequences (including sequencing reads) of 34 species covering all of the six subgenera of *Carex* were retrieved from NCBI for this analysis (Table 1). Due to significant variation in the chloroplast genome of *Carex*, which resulted in a high rate of sequence rearrangements and an increase in repetitive sequences, assembling the chloroplast genome from short-read data is challenging (Xu *et al.* 2023). Therefore, only the CDS regions were extracted for the phylogenetic analysis in this study. Sequence reads were mapped to the plastome of *C. siderosticta* (GenBank accession number NC_027250.1; unpublished by J. Jung, J. Park, and S. Kim) using the ‘Map to reference’ function in Geneious Prime 2024.7.16 (<http://www.geneious.com>); the ‘transfer annotations’ function was used to recover CDS

regions.

Phylogenetic analysis

The concatenated CDS sequences were aligned in MAFFT to produce 78 sequence matrices. A maximum-likelihood (ML) phylogenetic tree was constructed using RAxML-NG. Model selection was conducted with Modeltest-NG, resulting in the selection of the TIM+G+I model. One thousand bootstrap replicates were performed. Three species from the *C.* subg. *Siderosticta* were used as outgroups, because species of *C.* subg. *Siderosticta* were consistently placed as the most basal lineages in *Carex* in previous phylogenetic analyses (Starr *et al.* 2015, Jiménez-Mejías *et al.* 2016, Villaverde *et al.* 2020). Raw reads obtained in this study are available in the NCBI Sequence Read Archive, BioProject PRJDB19586.

Morphology of basal lineages of Carex subg. Carex

Sources of materials used for morphological analysis are listed in Appendix 1. The taxa examined morphologically in this study were included in the basal lineages of *Carex* subg. *Carex* illustrated in Roalson *et al.* (2021), namely sects. *Albae*, *Debiles*, *Decorae*, *Euprepes*, *Graciles*, *Hymenochlaenae*, *Indicae*, *Mapaniifoliae*, *Molliculae* and *Mundae*, all recognized by Dai *et al.* (2010).

The eleven morphological characters were analyzed using herbarium specimens preserved in the herbaria BKF, KYO, OKAY, and TI. The characters were color of basal sheath, type of inflorescence (spike unit), sexuality of terminal spike, color of pistillate scales, direction of perigynia in relation to spike axis, shape of perigynia in lateral view, shape of perigynia in front view, venation of perigynia, number of style branches, shape of style base, and shape of cladophyll.

Additional morphological observations utilized digital images of specimens in the herbaria A, BM, BOT, F, FLAS, GB, GH, K, KUN, L, LE, M, MEL, MICH, MIN, MO, MOR, MT, NL, NY, P, PE, V, VT, WU, Z, and ZT via the Global Biodiversity Information Facility (GBIF) (<https://www.gbif.org/>),

and the Chinese Virtual Herbarium (<https://www.cvh.ac.cn/>). Data from characters that poorly observable in digital images, such as shape of perigynia in front view and shape of style base, were supplemented with data from monographs and regional floras (Kükenthal 1909, Mackenzie 1931–1935, Chermezon 1937, Nelves 1951, Akiyama 1955, Nelves 1955, Raymond 1959, Simpson & Koyama 1998, Kukkonen 2001, Ball & Reznicek 2002, Hoshino *et al.* 2020) or from descriptions in the protologues of new species (Clarke 1908, Lévillé 1918, Kükenthal 1926, Urban 1929, Chevalier 1931, Guillaumin 1937, Blake 1947, Steyermark 1952, Hess 1953, Asplund 1954, Nelves 1954, Podlech 1961, Koyama 1980, Camelbeke & Reznicek 1996, Ball 1998, Wheeler 2002, Katsuyama 2010, Jiménez-Mejías & Roalson 2017).

Results

Phylogenetic analysis

Total length of aligned chloroplast CDS from 70 exons of sequences of the 78 taxa (76 species) of *Carex* analyzed in this study were 58,601 bp of 70 exons, containing 4,832 variable sites and 3,442 parsimony-informative sites.

The ML tree (Fig. 3) showed that the present phylogenetic relationships of the six subgenera of *Carex* recognized by Villaverde *et al.* (2020) agreed with those of Villaverde *et al.* (2020), which had been also based on chloroplast CDS sequences, and confirmed the coherence of *C.* subg. *Carex* with 100% bootstrap support (BS). We analyzed more than two species of each of six basal lineages of *C.* subg. *Carex* (proposed by Roalson *et al.* 2021), i.e. *C.* sect. *Graciles*, *C.* sect. *Grallatoriae*, plus the *Decora*, *Dissitiflora*, *Indica* and *Tristachya* clades, and confirmed that they were monophyletic with 100% BS, respectively, although we could not confirm monophyly of each of the remaining five basal lineages of *C.* subg. *Carex* (Roalson *et al.* 2021): *C.* sect. *Albae*, *C.* sect. *Alliiformes*, *C.* sect. *Cryptostachyae*, *Incertae Sedis* and *C.* sect. *Secalinae*, because we analyzed only one species from each of the former four lineages, and did not analyze any species from the one latter lineage (Fig. 3).

The relationships among the basal lineages of *C. subg. Carex* were revealed with high bootstrap support in this study (Fig. 3) and differed from those of Roalson *et al.* (2021). Here, the Dissitiflora Clade, which had been phylogenetically unplaced by Roalson *et al.* (2021), was sister to the rest of *C. subg. Carex*, which received 100% BS. A clade (100% BS) comprising *C. sect. Albae*, *C. sect. Graciles* and Indica Clade was sister to a clade (100% BS) of the Decora Clade and a clade (84% BS) of the remaining *C. subg. Carex* (Core subg. *Carex* Clade) (100% BS). Further, *C. sect. Graciles*, which had been placed as a sister to the Indica Clade by Roalson *et al.* (2021), was revealed to be sister to *C. sect. Albae* (100% BS). *Carex sect. Alliiformes*, *C. sect. Cryptostachyae*, *C. sect. Grallatoriae*, Tristachya Clade and Incertae Sedis proved to be included in the Core subg. *Carex* Clade, although Roalson *et al.* (2021) had phylogenetically placed *C. sect. Cryptostachyae* and Tristachya Clade outside the Core subg. *Carex* Clade, and had suspended phylogenetic placement of *C. sect. Alliiformes*, *C. sect. Grallatoriae* and Incertae Sedis.

In our study, the Indica Clade, which included several different sections recognized by Dai *et al.* (2010) (Appendix 1), consisted of two distinct subclades, Indica Clade I (100% BS) and Indica Clade II (100% BS) (Fig. 3). The Indica Clade I comprised species from *C. sect. Mundae* (*C. munda* Boott) and *C. sect. Chlorostachyae* (*C. handelii* Kük.), while the Indica Clade II included species from *C. sects. Decora* (*C. perakensis* C. B. Clarke), *Euprepes* (*C. atrivaginata* Nelm), *Graciles* (*C. longipes* D. Don), *Indicae* (*C. indica* L., *C. stramentitia* Boott ex Boeckeler), *Mapaniifoliae* (*C. helferi* Boeckeler, *C. bavicola* Raymond) and *Scabrellae* (*C. tricephala* Boeckeler, *C. aff. nodiflora*). The Decora Clade also consisted of two distinct subclades, Decora Clade I (100% BS) and Decora Clade II (100% BS) (Fig. 3). The Decora Clades I and II included species of *C. sects. Indicae* and *Decora* recognized by Dai *et al.* (2010), respectively (Appendix 1).

Additionally, *C. satsumensis* Franch. & Sav., whose phylogenetic placement had been uncertain, was found to occupy the most basal position in *C. subg. Vigneae* with 100% BS.

Morphological characteristics of basal lineages of Carex subg. Carex

The morphological characteristics determined in this study are shown in Table 2. The specimens examined and the literature cited are listed in Appendix 1.

[Color of basal sheath] — The basal sheath of members of the Dissitiflora Clade, *C. sect. Albae*, *C. sect. Graciles* and Indica Clades I & II was yellowish brown or dark brown. In contrast, the sheaths of the Decora Clades I & II were reddish brown or reddish purple.

[Type of inflorescence] — Inflorescences were paniculate in Decora Clade I, and racemose in *C. sect. Albae* and Indica Clade I. In the other clades, they were primarily racemose, but partially branched.

[Sexuality of terminal spike] — In most species in the basal lineages of *C. subg. Carex*, the terminal spikes were androgynous. However, in *C. sect. Albae* and some species of *C. sect. Graciles* and Decora Clade II, they were only staminate.

[Color of pistillate scales] — The pistillate scales corresponded in color to the basal sheaths. In the Dissitiflora Clade, *C. sect. Albae*, *C. sect. Graciles* and Indica Clades I & II, the pistillate scales ranged from white to yellowish brown, while in Decora Clades I & II, they were reddish brown or reddish purple.

[Direction of perigynia in relation to spike axis] — In the Dissitiflora Clade, the perigynia were oriented almost parallel to the spike axis ($<15^\circ$), while they were arranged obliquely to the spike axis ($>30^\circ$) in the other clades.

[Shape of perigynia in lateral view] — The perigynia in the Dissitiflora Clade were narrowly lanceolate, while those of the other clades were ellipsoid or ovoid.

[Shape of perigynia in front view] — The perigynia were biconvex or plano-convex in all species of *C. sect. Graciles* in addition to *C. longipes* within Indica Clade II. The perigynia of other species were primarily trigonous, but those of *C. sect. Albae* were strongly swollen, resulting in indistinctly edged trigonous perigynia.

[Venation of perigynia] —The perigynia of the Indica Clade I were characterized by 2 or 3 prominent veins (Fig. 4). In the other clades, the veins were usually several to many and not prominent.

[Number of style branches] — The number of style branches corresponded to the front shape of the perigynia. The styles were bifurcate in *sect. Graciles* and *C. longipes* of the Indica Clade II and trifurcate in the other species.

[Shape of style base] — The style base in *C. sect. Albae* and Indica Clade II was strongly thickened. It was globosely thickened in *C. sect. Albae* (Kükenthal 1909, Dai *et al.* 2010), while it was pyramidal with a discoid-annulate base in the Indica Clade II (Fig. 5A–D). In contrast, the style base in the Dissitiflora Clade and in the Decora clades I & II was only slightly thickened (Fig. 5E–H).

[Shape of cladophyll] — The cladophyll at the base of the lateral branches was tubular in *C. sect. Albae*, *C. sect. Graciles*, and Decora Clade II in the same manner as in the Core subg. *Carex* Clade. The cladophyll of the Decora Clade I was utriculiform, while in Indica Clade II, it was more reduced in size and often glume-like or vestigial. In the Dissitiflora Clade and in *C. munda* of the Indica Clade I, a fertile utriculiform perigynia, producing lateral branches was occasionally present (Fig. 6).

Discussion

Relationships among the basal lineages of Carex subg. Carex

The Dissitiflora Clade (*C. dissitiflora* and *C. bostrychostigma*), whose phylogenetic placement was previously unclear (Roalson *et al.* 2021), was resolved as the most basal lineage within *Carex* subg. *Carex* with 100% bootstrap support, although the same phylogenetic placement of *C. dissitiflora* alone had already been revealed by Starr *et al.* (2015), Lévillé-Bourret *et al.* (2018) and Lévillé-Bourret & Starr (2019). *Carex* sects. *Graciles* and *Albae* as well as the Indica Clades I & II formed a clade (100% BS), which was sister to all the remaining members of *C. subg. Carex* (100% BS). This topology agreed with previous findings (Starr *et al.* 2015, Martín-Bravo *et al.* 2019, Villaverde *et al.*

2020). The Decora Clades I & II (100% BS) were sister to the Core subg. *Carex* Clade (84% BS). Those relationships agreed with those of Starr et al. (2015) and Lévillé-Bourret & Starr (2019).

Roalson *et al.* (2021) placed *C. sect. Cryptostachyae* and Tristachya Clade outside the clade of Core subg. *Carex* and left the phylogenetic placements of *C. sects. Alliiiformes* and *Grallatoriae* pending. However, based on our study, *C. sects. Alliiiformes*, *Cryptostachyae* and *Grallatoriae* as well as the Tristachya Clade were revealed to be inside the clade of Core subg. *Carex* (84% BS).

Recognition of a new section corresponding to the Dissitiflora Clade

Our study demonstrated for the first time that *Carex dissitiflora* and *C. bostrychostigma* form a strongly supported monophyletic group and represent the earliest diverging lineage within *Carex* subg. *Carex*. The monophyly of *C. dissitiflora* and *C. bostrychostigma* is also morphologically supported; both species have cylindrical spikes (Fig. 2B). Ohwi (1936) and Akiyama (1955) considered these two species to be closely related and included them in the same section (*C. sect. Debiles*). However, the other species of *C. sect. Debiles* have been shown by Martín-Bravo *et al.* (2019) and this study to be included in the Core subg. *Carex* Clade.

Both *C. dissitiflora* and *C. bostrychostigma* have the perigynia sparsely arranged in erect, short-pedunculate spike and with the perigynia attached nearly parallel to the spike axis (at an angle of less than 15°) at maturity (Fig. 2B). In contrast, other species of *C. sect. Debiles* have drooping, long-pedunculate spikes with densely packed perigynia that are attached obliquely to the spike axis (at an angle of 30° or more). Thus, the morphology provides evidence that *C. dissitiflora* and *C. bostrychostigma* are phylogenetically distinct from other members of *C. sect. Debiles*.

Several researchers (Kükenthal 1909, Katsuyama 2015, Hoshino *et al.* 2020) have placed *C. dissitiflora* and *C. bostrychostigma* in different sections due to differences such as sexuality of the inflorescence (androgynous terminal spike in *C. dissitiflora* vs. staminate terminal spike in *C. bostrychostigma*) and lateral branches arising from fertile perigynia (present in *C. dissitiflora* vs.

absent in *C. bostrychostigma*). However, we noticed that *C. bostrychostigma* occasionally produces pistillate flowers at the base of the terminal spike and confirmed Akiyama's (1955) finding that *C. bostrychostigma* occasionally produces lateral branches from fertile perigynia (Fig. 6). The morphological differences in the sexuality of the inflorescence and lateral branches arising from fertile perigynia are thus insufficient for placing the two species in different sections.

Based on our new findings from both phylogenetic analysis (monophyly of *C. dissitiflora* and *C. bostrychostigma*) and morphological observations (shared traits, i.e. cylindrical spikes and sparse perigynia attached nearly parallel to the spike axis), we establish a new section, *C. sect. Dissitiflorae*, for *C. dissitiflora* and *C. bostrychostigma*.

Recognition of sections within the Indica Clade

The Indica Clade I consists of *C. munda* (*C. sect. Mundae*) and *C. handelii* (*C. sect. Chlorostachyae*). Although these two species have traditionally been placed in different sections due to the presence or absence of fertile prophylls, they share several common morphological features: an androgynous terminal spike and sparsely arranged perigynia with 2 or 3 prominent veins (Table 2). The shared morphological traits support the coherence of Indica Clade I, which we therefore recognize in this study as *C. sect. Mundae*.

The Indica Clade II includes *C. perakensis* (*C. sect. Decorae*) (Fig. 2D), *C. atrivaginata* (*C. sect. Euprepes*), *C. longipes* (*C. sect. Graciles*), *C. balanzae*, *C. bavicola*, *C. helferi* (Fig. 2F), *C. indica* (Fig. 2G), *C. stramentitia* (*C. sect. Indicae*), *C. tricephala* (Fig. 2E) and *C. aff. nodiflora* (*C. sect. Scabrellae*) (Fig. 2C) (Appendix 1). Among these, eight species from *C. sects. Decorae, Indicae*, and *Scabrellae* were classified in *C. sect. Stramentitiae* by Nelmès (1951), who characterized section *Stramentitiae* by its whitish or yellowish-brown pistillate scales and conspicuously basally swollen style with a most basal disc-shaped portion (Fig. 5A–C). This feature was also observed in species of *C. sect. Euprepes*, including *C. atrivaginata* (Nelmès 1943a), and in species of *C. sect. Mapaniifoliae*,

including *C. helferi* (Nelmes 1943b).

Among the twelve species of *C. sect. Graciles* analyzed in this study, *C. longipes* was the only species that was not included in the *C. sect. Graciles* clade but was instead placed in the Indica Clade II. Although *C. longipes* has traditionally been classified in *C. sect. Graciles* due to its bifid style, it shares the conspicuously swollen style base (Fig. 5D) with species of the Indica Clade II. Thus, Indica Clade II, including *C. longipes*, was morphologically supported by the conspicuous swollen style base and is recognized in this study as *C. sect. Indicae*.

Reevaluation of sectional delimitation in the Decora Clade

The Decora Clade consisted of two robust subclades, i.e. the Decora Clades I & II, which were morphologically supported, respectively. The Decora Clade I shares utriculiform cladophylls, while the Decora Clade II has tubular cladophylls. Although the Decora Clade I encompassed most species of *C. sect. Indicae*, it did not include the type species of *C. sect. Indicae* (*C. indica*), and thus, we used the name *C. sect. Filicinae* (C. B. Clarke) Nelmes for the taxon corresponding to the Decora Clade I. The species included in Decora Clade I were assigned to *C. sects. Cruciatae* (C. B. Clarke) Nelmes and *Filicinae* recognized by Nelmes (1951). *Carex* sects. *Cruciatae* and *Filicinae* can be morphologically distinguished from each other because the former shares pale brown pistillate scales with reddish brown flecks (Fig. 2I), while the latter has reddish brown or dark reddish brown pistillate scales (Fig. 2H). However, as our analysis showed *C. sect. Cruciatae* to not be monophyletic, we united *C. sects. Cruciatae* and *Filicinae* into a single section under the name of *C. sect. Filicinae*. The Decora Clade II comprises *C. sect. Decorae*, which we recognized for this clade.

Sectional placement of Carex malaccensis and C. elatior

Based on our morphological examination, Roalson *et al.*'s (2021) sectional classification of *C. malaccensis* C. B. Clarke and *C. elatior* Boeckeler (Appendix 1) might be changed.

Roalson *et al.* (2021) included *C. malaccensis* in *C. sect. Rhomboidales* Kük. based on a phylogenetic tree of Martín-Bravo *et al.* (2019), in which the DNA sequence data of *C. malaccensis* consisted only of ETS. However, BLAST searches for samples of *C. malaccensis* used in Martín-Bravo *et al.* (2019) (*B. L. Burt & P. J. B. Woods 1780*, E, digital image!) indicated resemblance to the species of *C. sect. Rhomboidales* based on ETS DNA sequences, but to species of *C. sect. Indicae* based on *matK* DNA sequences. Based on our morphological observations, the globose spikes and milky white pistillate scales indicate the close resemblance of *C. malaccensis* to *C. tricephala* of *C. sect. Indicae*. Thus, although we do not know the reason *C. malaccensis* and species of *C. sect. Rhomboidales* resemble each other in their ETS DNA sequences, we tentatively consider *C. malaccensis* to be included in *C. sect. Indicae* based on the resemblance of the *matK* DNA sequences, which as one of the DNA barcode regions are often used for species identification (CBOL Plant Working Group 2009), and morphology. Further study is needed to determine the exact taxonomic position of *C. malaccensis*.

Martín-Bravo *et al.* (2019) analyzed two samples of *C. elatior* phylogenetically, but their phylogenetic positions conflicted with each other. One sample (*Gautier, L. LG4745*, P, digital image!) was placed in a clade corresponding to the Roalson *et al.*'s (2021) Indica Clade, while the other (*Razafindraibe, H. & P. Antilahimena 83*, P, digital image!) was in a clade corresponding to Roalson *et al.*'s (2021) Decora Clade. Roalson *et al.* (2021) used the results only from the former sample without explanation in the treatment of *C. elatior* and positioned *C. elatior* in the Indica Clade (Appendix 1). However, we consider the former sample to have been misidentified because it differs significantly from the descriptions of Boeckeler (1880) and Kükenthal (1909) for the type specimen of *C. elatior* in having pale brown pistillate glumes (vs. reddish-brown in *C. elatior*) and the staminate part of the spikes equal to or longer than the pistillate part (vs. staminate part much shorter than pistillate part in *C. elatior*). We identified the latter sample as *C. elatior* based on Boeckeler (1880), Kükenthal (1909) and Chermezon (1937). Thus, we consider *C. elatior* to be phylogenetically positioned in Roalson *et al.*'s (2021) Decora Clade, which consists of our Decora Clades I (for *C. sect.*

Filicinae) and II (for *C. sect. Decorae*) (Appendix 1). Based on morphology (Table 2), we include *C. elatior* in *C. sect. Filicinae* rather than in *C. sect. Decorae*.

Phylogenetic position of Carex satsumensis

The phylogenetic position of *C. satsumensis* has been controversial. It was traditionally classified in *C. subg. Vigneastra* (Tuck.) Kük., whose species were mostly embedded in *C. subg. Carex* in this study. However, *C. satsumensis* was placed as sister to *C. subg. Vignea* by Yano *et al.* (2014) and Starr *et al.* (2015) using nuclear ITS sequences and by Lévillé-Bourret & Starr (2019) using *matK+ndhF+rps16+ETS-1f+ITS+indels+morphology*, while to all subgenera of *Carex* except *C. subg. Siderosticta* by Lévillé-Bourret *et al.* (2018) using *matK+ndhF+rps16+ETS-1f+ITS+indels*. However, the bootstrap support was low in all of those studies. Our study revealed that *C. satsumensis* is sister to *C. subg. Vignea* with 100% bootstrap support.

As *Carex satsumensis* and the species of *C. subg. Vignea* share sessile, multispicate inflorescences with unsheathed bracts (Fig. 2A), we consider *C. satsumensis* to be included in *C. subg. Vignea*. *Carex satsumensis* differs from the species of *C. subg. Vignea* in having utriculiform cladophylls, which can be considered to be a plesiomorphic character state of *C. subg. Vignea*, as inferred by Starr *et al.* (2015).

Taxonomic treatment

Key to sections of *Carex* subg. *Carex*

- 1a. Perigynia narrowly lanceolate, parallel ($<15^\circ$) to inflorescence axis at maturity
 **1.** *C. sect. Dissitiflorae*
- 1b. Perigynia ellipsoid or ovoid, obliquely ($>30^\circ$) oriented to the inflorescence axis at maturity **2**
- 2a. Cladophyll glume-like or utriculiform **3**
- 2b. Cladophyll tubular **5**
- 3a. Spikes cylindrical Core subg. *Carex* (sect. *Polystachyae*)
- 3b. Spikes oblong to ovoid..... **4**
- 4a. Pistillate scales white or yellowish brown, without spots; basal sheath pale brown to dark brown;
 style base strongly thickened, discoid-annulate in basal portion
 **5.** *C. sect. Stramentitiae*
- 4b. Pistillate scales wholly reddish (or castaneous) brown or pale brown with reddish brown spots;
 basal sheath reddish brown or reddish purple; style base not or slightly thickened..... **6.** *C. sect.*
Filicinae
- 5a. Inflorescence paniculate or racemose, at least partly branched; spike(s) 1–12 in a fascicle within
 each sheath **6**
- 5b. Inflorescence racemose, not branched; spike always single within each sheath **7**
- 6a. Stigmas 2; achene biconvex or plano-convex **3.** *C. sect. Graciles*
- 6b. Stigmas 3; achene trigonous **7.** *C. sect. Decorae*
- 7a. Terminal spike androgynous..... **8**

- 7b. Terminal spike staminate or gynecandrous 9
- 8a. Veins of perigynia 2 or 3, elevated 4. *C. sect. Mundae*
- 8a. Veins of perigynia many, not elevated.....
Core subg. *Carex* (sects. *Cryptostachyae*, *Radicales*)
- 9a. Leaves involute on margins; bract sheath-like, bladeless 2. *C. sect. Albae*
- 9b. Leaves flat; bract leaf-like or scale-like
Core subg. *Carex* (excl. sects. *Polystachyae*, *Cryptostachyae*, *Radicales*)

1. *Carex* sect. *Dissitiflorae* K. T. Takah. & M. N. Tamura, *sect. nov.*

Rhizome short, or short stoloniferous; clothed with dark brown fibers of disintegrating sheaths. Culms cespitose, densely tufted, 20–80 cm tall, trigonous, smooth. Leaves shorter than or equaling culm, blade 15–50 cm long, 1–7 mm wide, flat, thin, midrib slightly scabrous abaxially, 2 main lateral veins slightly scabrous adaxially, basally sheathing. Involucral bracts leaf-like, basally sheathing. Spikes 2–8, each arising from sheath of involucral bract; terminal spike staminate or androgynous, linear-cylindric, 2–5 cm long, peduncle slender; lateral spikes pistillate or androgynous, spaced less than 6 cm apart, narrowly cylindric, 2–4 cm long, loosely flowered; peduncle rather short, slender, sometimes with several-flowered lateral branches emerging from fertile perigynia. Pistillate scales pale brown to yellowish brown, ovate-lanceolate, 4.5–5 mm long, membranous, several veined, margin broadly hyaline, apex acute. Perigynia pale green, parallel ($<15^\circ$) to inflorescence axis, longer than glume, narrowly lanceolate, trigonous, 5–12 mm long, membranous, base attenuate, apex tapering to long beak, orifice obliquely truncate. Achenes yellowish brown, narrowly oblong or elliptic, trigonous, 3–4 mm long, enveloped by perigynium, base short stipitate; stigmas 3.

Type species. Carex dissitiflora Franch. in Bull. Soc. Philom. Paris 7: 32 (1895).

Distribution. Endemic to East Asia (Japan, Korea, Northeast China and Far East Russia).

Representative species. *C. bostrychostigma* and *C. dissitiflora*.

Notes. Sect. *Dissitiflorae* differs from *C. sect. Mundae* in having long-beaked, narrowly lanceolate perigynia and from *C. sect. Debiles* in having erect spikes with a short peduncle and sparse perigynia attached nearly parallel ($<15^\circ$) to the spike axis at maturity.

2. *Carex* sect. *Albae* (Ascherson & Graebner) Kük. in Pflanzenr. (Engler) IV. 20 (Heft 38): 499 (1909).

Basionym: *Carex* [unranked] *Albae* Ascherson & Graebner in Synopsis der Mitteleuropäischen Flora 2(2): 156 (1903).

Type species. *Carex alba* Scop., Fl. Carniol., ed. 2. 2: 216 (1772).

Distribution. East to Central Asia and North America.

Representative species. *C. alba*, *C. eburnea*, *C. mckittrickensis* and *C. ussuriensis*.

Notes. Sect. *Albae* is unique among the early diverging lineages of *C. subg. Carex* in its wide distribution across high latitude regions of the Northern Hemisphere (*C. alba* and *C. eburnea*). It differs from other sections in having involute leaf margins, sheath-like bracts without a blade and strongly swollen perigynia.

3. *Carex* sect. **Graciles** Kük. in Engler, Bot. Jahrb. Syst. 27: 516 (1899).

Type species. *Carex gracilis* R. Br., Prodr. Fl. Nov. Holl.: 242 (1810).

Distribution. East Africa, West, South, and Southeast Asia, Australia and the Pacific islands.

Representative species. *C. autumnalis*, *C. bilateralis*, *C. bodinieri*, *C. brunnea* (= *C. gracilis*), *C. chichijimensis*, *C. chuii*, *C. ereica*, *C. gentilis*, *C. gongshanensis*, *C. hattoriana*, *C. henryi*, *C. lenta*, *C. lushanensis*, *C. mosoynensis*, *C. nachiana*, *C. omeiensis*, *C. orbicularinucis*, *C. pergracilis*, *C. sacrosancta*, *C. sendaica*, *C. sinodissitiflora*, *C. stipitinux*, *C. tabatae*, *C. tamakii* and *C. teinogyna*.

Notes. Many species of sect. *Graciles* are endemic to East Asia, where it is highly diverse. It differs from other early diverging sections of *C.* subg. *Carex* (except for *C. longipes* of sect. *Stramentitiae*) in its bifid style.

4. *Carex* sect. **Mundae** Kük. in Pflanzenr. (Engler) IV. 20 (Heft 38): 254 (1909).

Type species. *Carex munda* Boott, Illstr. Gen. *Carex* 1: 7 (1858).

Distribution. South China, Bhutan and Nepal.

Representative species. *C. barbayaki*, *C. handelii*, *C. harrysmithii* and *C. munda*.

Notes. In Kükenthal (1909), the fertile cladophylls were emphasized as a key characteristic for recognizing *C. sect. Munda*. However, based on our study, we characterize it by the androgynous terminal spike and sparse perigynia with 2 or 3 prominent veins. Based on the phylogenetic analysis using ITS sequences by Lu *et al.* (2021), *C. barbayaki* (sect. *Decorae*) and *C. harrismithii* (sect. *Mollicurae*) formed a clade with *C. munda* and *C. handelii*. Those two species also have sparse perigynia with 2 prominent veins (Kükenthal 1929, Jiménez-Mejías & Roalson 2017), supporting the recognition of all four species (*C. barbayaki*, *C. handelii*, *C. harrismithii* and *C. munda*) as members of sect. *Munda*. These four species are distributed exclusively at high elevations (> 2000 m alt.) in the Himalaya and Hengduan mountains.

5. *Carex* sect. *Indicae* (Tuck. ex L. H. Bailey) C. B. Clarke in Hook. f., Fl. Brit. India 6: 713 (1894).

Basionym: *Carex* [unranked] *Indicae* Tuck. [Enum. Meth.: 10 (1843), nom. nud.] ex L. H. Bailey in Proc. Amer. Acad. Arts 22: 89 (1886).

= *Carex* sect. *Stramentitiae* (C. B. Clarke) Nelmes in Reinwardtia 1(3): 250 (1951).

= *Carex* sect. *Euprepes* Nelmes & Airy Shaw in Hooker's Icon. Pl.: 35, t. 3433 (1943).

= *Carex* sect. *Mapaniifoliae* Nelmes & Airy Shaw in Hooker's Icon. Pl.: 35, t. 3434 (1943).

Type species. *Carex indica* L., Mant. Pl. Altera: 574 (1771).

Distribution. Southeast and South Asia, Pacific Islands and Australia.

Representative species. *C. atrivaginata*, *C. balansae*, *C. bavicola*, *C. callista*, *C. cataphyllodes*, *C. cirrhulosa*, *C. cochinchinensis*, *C. commixta*, *C. euprepes*, *C. helferi*, *C. horsfieldii*, *C. indica*, *C. indiciformis*, *C. jeanpertii*, *C. laosensis*, *C. longipes*, *C. malaccensis*, *C. meeboldiana*, *C. nodiflora*, *C.*

pandanophylla, *C. perakensis*, *C. poilanei*, *C. repanda*, *C. stramentitia*, *C. taprobanensis*, *C. tavoyensis*, *C. thailandica*, *C. tricephala*, *C. trichophylla*, *C. vietnamica*, *C. wightiana* and *C. zizaniifolia*.

Notes. Sect. Indicae comprises *C.* sects. *Stramentitiae*, *Euprepes* and *Mapaniifoliae* recognized by Nelmès / Nelmès & Airy Shaw and *C. longipes*. It is characterized by glume-like or utriculiform cladophylls, white to straw-colored pistillate scales, and the style base strongly thickened into basal-most discoid-annulate portion (Fig. 5A–D).

6. *Carex* sect. *Filicinae* (C. B. Clarke) Nelmès in Reinwardtia 1(3): 286 (1951).

Basionym: *Carex* [unranked] *Filicinae* C. B. Clarke in J. Linn. Soc. Bot. 37: 4 (1904).

= *Carex* sect. *Cruciatae* (C. B. Clarke) Nelmès in Reinwardtia 1(3): 275 (1951).

= *Carex* sect. *Scabrellae* Kük. in Engler, Pflanzenr. 38(IV. 20): 286 (1909).

Type species. *Carex filicina* Nees in Wight, Contrib. Bot. Ind.: 123 (1834).

Distribution. Pantropical regions.

Representative species. *C. albobiridis*, *C. amicta*, *C. andringitrensis*, *C. angolensis*, *C. aztecica*, *C. bathiei*, *C. beckii*, *C. brassii*, *C. burttii*, *C. castanostachya*, *C. ceylanica*, *C. chlorosaccus*, *C. condensata*, *C. continua*, *C. cruciata*, *C. cubensis*, *C. deciduisquama*, *C. distracta*, *C. doisutepensis*, *C. echinochloe*, *C. ekmanii*, *C. elatior*, *C. euryphylla*, *C. filicina*, *C. graminifolia*, *C. guffroyi*, *C. haematosaccus*, *C. heterodoxa*, *C. hildebrandtiana*, *C. hirtigluma*, *C. hultenii*, *C. humboldtiana*, *C.*

humpatensis, *C. lamprochlamys*, *C. leptocladus*, *C. lindleyana*, *C. macrophyllidion*, *C. madrensis*, *C. merxmuelieri*, *C. neochevalieri*, *C. neoguinensis*, *C. neohebridensis*, *C. novogaliciana*, *C. parvigluma*, *C. polystachya*, *C. porrecta*, *C. proxima*, *C. pyramidalis*, *C. rafflesiana*, *C. ramosa*, *C. raphidocarpa*, *C. renschiana*, *C. rutenbergiana*, *C. sambiranensis*, *C. sanguinea*, *C. sarawaketensis*, *C. scabrella*, *C. scabripes*, *C. schliebenii*, *C. sodiroi*, *C. spicatopaniculata*, *C. spicigera*, *C. standleyana*, *C. steudneri*, *C. subfilicinoides*, *C. subscabrella*, *C. tenuipaniculata*, *C. tricholepis*, *C. tsaratananensis*, *C. valbrayi*, *C. vesiculosa*, *C. wahlenbergiana* and *C. zuluensis*.

Notes. Encompassing the majority of species diversity of *C. sect. Indicae* recognized by Dai *et al.* (2010), *sect. Filicinae* is widely distributed in tropical regions. It can be distinguished from *C. sect. Stramentitiae* by its reddish brown pistillate scales, reddish brown basal sheaths, and slightly thickened style base (Fig. 5E–H).

7. *Carex* sect. *Decorae* (Kük.) Ohwi in Mem. Coll. Sci. Kyoto Imp. Univ., Ser. B, Biol. 11: 338 (1936).

Basionym: *Carex* subsect. *Decorae* Kük. in Das Pflanzenreich IV. 20 (Heft 38): 541. (1909).

Type species. *Carex decora* Boott, Illustr. Gen. *Carex* 1: 5 (1858).

Distribution. South, Southeast and East Asia.

Representative species. *C. anomoea*, *C. baiposhanensis*, *C. celebica*, *C. daltonii*, *C. decora*, *C. dielsiana*, *C. gandakiensis*, *C. inclinis*, *C. insignis*, *C. jizhuangensis*, *C. kumaonensis*, *C. morii*, *C. munipoorensis*, *C. neopolycephala*, *C. phyllocaula*, *C. polycephala*, *C. pomiensis*, *C. pontica*, *C. pulchra*, *C. scitula*, *C. shuangbaiensis*, *C. stracheyi*, *C. subperakensis*, *C. turrita*, *C. urelytra*, *C.*

verticillata, *C. walkeri*, *C. winterbottomii* and *C. zhenkangensis*.

Notes. Sect. *Decorae* has paniculate inflorescences with lateral branches, but lacks utriculiform cladophylls, which distinguishes it from *C. sect. Filicinae*. Based on the results of the phylogenetic analysis of Martín-Bravo *et al.* (2019), some species of *C. sect. Hymenochlaenae* (*C. polycephala*, *C. neopolycephala*, *C. munipoorensis* and *C. shuangbaiensis*), that exhibit androgynous spikes and paniculate inflorescences, were included in this section.

Amendments to Nomenclature

Carex sect. ***Pacifica*** Ohwi in Mem. Coll. Sci. Kyoto Imp. Univ., Ser. B, Biol. 11(5): 458 (1936). —
Lectotype species (**hic designatus**): *Carex satsumensis* Franch. & Sav.

≡ *Carex* sect. *Japonicae* Kük. in Pflanzenr. (Engler) IV. 20 (Heft 38): 252 (1909), nom. illegit. non Franch (1898).

Notes. In many recent studies (e.g. Dai *et al.* 2010, Hoshino *et al.* 2020, Roalson *et al.* 2021), *C. satsumensis* was treated as the only species of *C. sect. Japonicae* Kük. Kükenthal (1909) designated *C. nikoensis* Franch. et Sav. (= *C. satsumensis* based on our determination) as the type species of *C. sect. Japonicae* Kük. However, as Franchet (1898) included *C. japonica* Thunb. as one of the members of *C. [unranked] Japonicae* Franch., *C. japonica* therefore becomes the type of *C. [unranked] Japonicae*. Consequently, *C. sect. Japonicae* Kük. is an illegitimate later homonym, and *C. sect. Pacificae* Ohwi, which was established in 1936 for *C. satsumensis* (= *C. nikoensis*), takes priority.

Although *C. satsumensis* and *C. nikoensis* were originally described simultaneously by Franchet & Savatier (1879), *C. nikoensis* was reduced to *C. satsumensis* (Franchet 1898) before *C. satsumensis*

was reduced to *C. nikoensis*. Accordingly, we here designate *C. satsumensis* as the lectotype of *C. sect. Pacificae*.

Acknowledement

We extend our cordial gratitude to the staff members of Forest Herbarium (BKF), Department of National Parks, Wildlife and Plant Conservation, Thailand, for their help during the expeditions. We express our sincere thanks to Prof. Hidetoshi Nagamasu (Kyoto University Museum) for providing valuable comments on the nomenclature. Our thanks are due to Dr. Hiroshi Tobe and Dr. David E. Boufford for critically reading the manuscript. Thanks are also due to the directors and staff members of BKF, KYO, OKAY and TI for allowing us to use their facilities and collections. This work was supported in part by JSPS KAKENHI (Grant Number JP21KK0132 to MT, JP22K05697 to OY) and JSPS-NRCT Joint Research Program (Grant number JPJSBP120229202 to MT).

References

- Akiyama, S. 1955. Carices of the Far Eastern Region of Asia. Hokkaido Univ. Press, Sapporo (in Japanese).
- Asplund, E. 1954. *Carex hultenii* n. sp. Sven. Bot. Tidskr. 48: 95–98.
- Ball, P. W. 1998. *Carex mckittrickensis* (Cyperaceae), a new species from western Texas. Novon 8(3): 220–224.
- Ball, P. W. & A. A. Reznicek. 2002. *Carex*. In: Flora of North America Editorial Committee (ed.), Flora of North America North of Mexico, vol. 23. Magnoliophyta: Commelinidae (in part): Cyperaceae, pp. 254–272. Oxford University Press, New York.
- Blake, S. T. 1947. The Cyperaceae collected in New Guinea by L. J. Brass, I. J. Arnold Arboretum 28(1): 99–116.
- Boeckeler, O. 1880. Cyperaceen. In: Buchenau, F. (ed.), Reliquiae Rutenbergianae I. Abh. Naturwiss.

- Ver. Bremen, vol. VII, pp. 36–41. C. Ed. Müller, Bremen.
- Camelbeke, K. & A. A. Reznicek. 1996. *Carex porrecta* (Cyperaceae), a distinctive new species from northern South America and Costa Rica. *Novon* 6: 423–425.
- CBOL Plant Working Group. 2009. A DNA barcode for land plants. *PNAS* 106: 12794–12797.
- Chermezon, H. 1937. Cypéracées. *In*: Humbert, H. & J. F. Leroy (eds.), *Flore de Madagascar et des Comores : plantes vasculaires* (Issue 29), pp. 273–299. Tananarive, Imprimerie Officielle, Madagascar.
- Chevalier, A. 1931. A propos d'un *Carex* nouveau de la Guinée française. *Bull. Mus. Natl. d'Hist. Nat.* 3(5): 466–468.
- Clarke, C. B. 1908. New genera and species of Cyperaceae. *Bull. Misc. Inform. Add. Ser.* 8: 1–196.
- Dai, L. K., S. Y. Liang, S. R. Zhang, Y. C. Tang, T. Koyama & G. C. Tucker. 2010. *Carex* L. *In*: Wu, Z. Y., P. H. Raven & D. Y. Hong (eds.), *Flora of China*, vol. 23, pp. 285–461. Science Press, Beijing, and Missouri Botanical Garden Press, St. Louis.
- Franchet, A. 1898. Les *Carex* de l'Asie orientale III. *Nouv. Arch. Mus. Hist. Nat., Sér.* 3, 10: 39–120.
- Franchet, A. & L. Savatier. 1879. *Enumeratio Plantarum in Japonia Sponte Crescentium*, vol. 2, p. 132. Apud F. Savy Bibliopolam, Paris.
- Frodin, D. G. 2004. History and concepts of big plant genera. *Taxon* 53: 753–776.
- Goetghebeur, P. 1998. Cyperaceae. *In*: Kubitzki, K. (ed.), *The Families and Genera of Vascular Plants*, vol. 4. Flowering Plants: Monocotyledons, pp. 141–190. Springer, Berlin.
- Guillaumin, A. 1937. Contribution à la flore des Nouvelles-Hébrides (Plantes recueillies par M. et Mme. Aubert de la Rue dans leur 2e voyage [1935-1936]) *In*: Summerhayes (ed.), *Phanérogames*. *Bull. Mus. Natnl. d'Hist. Nat.* 9(4): 283–306.
- Hess, H. E. 1953. Über die Gattungen *Heleocharis* und *Carex* aus Angola und dem unteren Kongo. *Ber. Schweiz. Bot. Ges.* 63: 318–359 (in Germany).

- Hoshino, T., T. Katsuyama, T. Masaki & M. Michikawa. 2020. *Carex* L. *In*: Iwatsuki, K., D. E. Boufford & H. Ohba (eds.), Flora of Japan, vol. 4a. Angiospermae: Monocotyledoneae (a), pp. 226–344. Kodansha, Tokyo.
- Jiménez-Mejías, P., M. Hahn, K. Lueders, J. R. Starr, B. H. Brown, B. N. Chouinard, K. S. Chung, M. Escudero, B. A. Ford, K. A. Ford, S. Gebauer, B. Gehrke, M. H. Hoffmann, X. F. Jin, J. Jung, S. Kim, M. Luceño, E. Maguilla, S. Martín-Bravo, M. Míguez, A. Molina, R. F. C. Naczi, J. E. Pender, A. A. Reznicek, T. Villaverde, M. J. Waterway, K. L. Wilson, J. C. Yang, S. R. Zhang, A. L. Hipp & E. H. Roalson. 2016. Megaphylogenetic specimen-level approaches to the *Carex* (Cyperaceae) phylogeny using ITS, ETS, and *matK* sequences: implications for classification. *Syst. Bot.* 41: 500–518.
- Jiménez-Mejías, P. & E. H. Roalson. 2017. Two new Asian species of *Carex* (Cyperaceae). *Phytotaxa* 298(3): 283–288.
- Katsuyama, T. 2010. Cyperaceae *In*: Ohba, H., Y. Iokawa & L. R. Sharma (eds.), Flora of Mustang, Nepal, pp. 431–471. Kodansha, Tokyo.
- Katsuyama, T. 2015. *Carex* of Japan, enlarged and revised ed. Bun'ichi Sogo Shuppan, Tokyo (in Japanese).
- Koyama, T. 1978. Cyperaceae. *In*: Hara, H., W. T. Stearn & L. H. J. Williams (eds.), An Enumeration of the Flowering Plants of Nepal, vol. 1, pp. 96–120. British Museum (National History), London.
- Koyama, T. 1980. Cyperaceae of Ceylon. *Bot. Mag.* 93: 342.
- Kükenthal, G. 1909. Cyperaceae-Caricoideae. *In*: Engler, H. G. A. (ed.), Das Pflanzenreich IV, 20 (Heft 38). W. Engelmann, Leipzig.
- Kükenthal, G. 1926. Cyperaceae novae vel criticae imprimis antillanae. *Feddes Repert. Spec. Nov. Regni Veg.* 23: 183–222.
- Kükenthal, G. 1929. Plantae Sinensis XXII. Cyperaceae. *In*: Skottsberg, C. (ed), Acta Horti Gothoburg.

5: 36–49.

Kukkonen, I. 2001. Cyperaceae. *In*: Ali, S. I. & M. Qater (eds.), Flora of Pakistan. No. 206. Missouri Botanical Garden Press, Sr. Louis.

Léveillé, H. 1918. Revue Internationale Paraissant tous les deux mois. Monde Pl. 19: 13–16.

Léveillé-Bourret, É. & J. R. Starr. 2019. Molecular and morphological data reveal three new tribes within the Scirpo-Caricoid Clade (Cyperoideae, Cyperaceae). *Taxon* 68: 218–245.

Léveillé-Bourret, É., J. R. Starr & B. A. Ford. 2018. Why are there so many sedges? Sumatroscirpeae, a missing piece in the evolutionary puzzle of the giant genus *Carex* (Cyperaceae). *Mol. Phylogenet. Evol.* 119: 93–104.

Lu, Y.-F., X.-F. Jin, H. Ikeda, O. Yano, C. Benítez-Benítez, W.-J. Chen, Y.-D. Liu, P. Jiménez-Mejías & M.-J. Yu. 2021. Revisiting of *Carex* sect. *Confertiflorae* s.l. (Cyperaceae): new data from molecular and morphological evidence and first insights on *Carex* biogeography in East Asia. *J. Syst. Evol.* 59: 668–686.

Mackenzie, K. K. 1931–1935. Cyperaceae-Cariceae. North American Flora, vol 18, pp. 1–478. New York Botanical Garden, New York.

Martín-Bravo, S., P. Jiménez Mejías, T. Villaverde, M. Escudero, M. Hahn, D. Spalink, E. H. Roalson, A. L. Hipp & the Global *Carex* Group (C. Benítez, L. P. Bruederle, E. Fitzek, B. A. Ford, K. A. Ford, M. Garner, S. Gebauer, M. H. Hoffmann, X. F. Jin, I. Larridon, É. Léveillé Bourret, Y. F. Lu, M. Luceño, E. Maguilla, J. I. Márquez Corro, M. Míguez, R. Naczi, A. A. Reznicek & J. R. Starr). 2019. A tale of worldwide success: behind the scenes of *Carex* (Cyperaceae) biogeography and diversification. *J. Syst. Evol.* 57: 695–718.

Nelmes, E. 1940. Notes on *Carex* XIV. –New African species of the subgenus *Indocarex*. *Bull. Misc. Inform. Kew* 4: 160–162.

Nelmes, E. 1943a. *Carex euprepes* Nelmes. Cyperaceae. Tribus Cariceae. *In*: Cotton, A. D. (ed.),

- Hooker's Icones Plantrum: 35. t. 3433. Royal Botanic Gardens, Kew.
- Nelmes, E. 1943b. *Carex adrienii* E. G. Camus. Cyperaceae. Tribus Cariceae. In: Cotton, A. D. (ed.), Hooker's Icones Plantrum: 35. t. 3434. Royal Botanic Gardens, Kew.
- Nelmes, E. 1951. The genus *Carex* in Malaysia. *Reinwardtia* 1(3): 221–450.
- Nelmes, E. 1954. *Carex brasii*. Mem. New York Bot. Gard. 9: 100.
- Nelmes, E. 1955. The genus *Carex* in Indo-China, including Thailand and Lower Burma. Mém. Mus. Natnl. d'Hist. Nat., Sér. B, Botanique 4(2): 83–182.
- Ohwi, J. 1936. Cyperaceae Japonicae I. Mem. Coll. Sci. Kyoto Imp. Univ., Ser. B, Biol. 11: 417–428.
- Podlech, D. 1961. Cyperaceae Afriganae. Mitt. Bot. Staatssamml. München 4: 107–124.
- Raymond, M. 1959. Carices Indochinenses necnon Siamenses. Mém. Jardin Bot. Montréal 53: 1–125.
- Reznicek, A. A. 1990. Four new species of *Carex* (Cyperaceae) from Mexico, with notes on the Mexican *Carex* flora. Contr. Univ. Michigan Herb. 17: 279–293.
- Roalson, E. H., P. Jiménez-Mejías, A. L. Hipp, C. Benítez-Benítez, L. P. Bruederle, K.-S. Chung, M. Escudero, B. A. Ford, K. Ford, S. Gebauer, B. Gehrke, M. Hahn, M. Q. Hayat, M. H. Hoffmann, X.-F. Jin, S. Kim, I. Larridon, É. Lévillé-Bourret, Y.-F. Lu, M. Luceño, E. Maguilla, J. I. Márquez-Corro, S. Martín-Bravo, T. Masaki, M. Míguez, R. F. C. Naczi, A. A. Reznicek, D. Spalink, J. R. Starr, Uzma, T. Villaverde, M. J. Waterway, K. L. Wilson & S.-R. Zhang. 2021. A framework infrageneric classification of *Carex* (Cyperaceae) and its organizing principles. *J. Syst. Evol.* 59: 726–762.
- Simpson, D. A. & T. Koyama. 1998. Cyperaceae. In: Santisuk T. & K. Larsen (eds.), *Flora of Thailand* 6(4): 247–485.
- Starr, J. R., S. A. Harris & D. A. Simpson. 2004. Phylogeny of the unispicate taxa in Cyperaceae tribe Cariceae I: genetic relationship and evolutionary scenarios. *Syst. Bot.* 29: 528–544.
- Starr, J. R., F. H. Janzen & B. A. Ford. 2015. Three new, early diverging *Carex* (Cariceae, Cyperaceae)

- lineages from East and Southeast Asia with important evolutionary and biogeographic implications. *Mol. Phylogenet. Evol.* 88: 105–120.
- Steyermark, J. A. 1952. A new *Carex* from Guatemala and Honduras. *Ceiba* 3(1): 23.
- Takahashi, K. T., J. Oda, S. Fuse & M. N. Tamura. 2021. Biosystematic studies of *Carex* (Cyperaceae)
- I. Molecular phylogenetic analysis of the *C. macroglossa* complex with reference to variation in morphology, chromosomal features and species delimitation. *Acta Phytotax. Geobot.* 72: 81–91.
- Urban, I. 1929. *Plantae Haitienses et Dominees novae vel rariores VII. a cl. E. L. Ekman 1924–1928 lectae.* *Arkiv för Botanik* 22A(17): 8.
- Villaverde, T., P. Jiménez-Mejías, M. Luceño, M. J. Waterway, S. Kim, B. Lee, M. Rincón-Barrado, M. Hahn, E. Maguilla, E. H. Roalson, A. L. Hipp & the Global *Carex* Group. 2020. A new classification of *Carex* (Cyperaceae) subgenera supported by a HybSeq backbone phylogenetic tree. *Bot. J. Linn. Soc.* 194: 141–163.
- Wheeler, G. A. 2002. *Carex* (Cyperaceae) from South America: three new species and some name changes. *Darwiniana* 40(1–4): 201–203
- Xu, S., K. Teng, H. Zhang, K. Gao, J. Wu, L. Duan, Y. Yue & X. Fan. 2023. Chloroplast genomes of four *Carex* species: Long repetitive sequences trigger dramatic changes in chloroplast genome structure. *Front Plant Sci.* 14:1100876.
- Yano, O., H. Ikeda, X. F. Jin & T. Hoshino. 2014. Phylogeny and chromosomal variations in East Asian *Carex*, *Siderostictae* group (Cyperaceae), based on DNA sequences and cytological data. *J. Plant Res.* 127: 99–107.

Tables

Table 1. Classification, voucher data and Genbank numbers for samples of *Carex* used in the present phylogenetic analysis based on chloroplast CDS regions. Infrageneric recognition follow Dai *et al.* (2010). Samples with asterisk in the voucher column were obtained from NCBI.

Subgenus	Section	Species	Voucher	Accession number
<i>Carex</i> L.	<i>Albae</i> (Asch. & Graebn.) Kük.	<i>Carex eburnea</i> Boott	*	SRR10527493
	<i>Alliiformes</i> Akiyama	<i>C. alliiformis</i> C. B. Clarke	Japan. Kagoshima: Ibusuki-shi, <i>Hoshino, T. et al. s.n.</i> (OKAY-20585)	DRR620980
	<i>Chlorostachyae</i> Tuck. ex Meinsh.	<i>C. handelii</i> Kük.	China. Yunnan: Dali, <i>Ikeda, H. et al. 1414013</i> (OKAY)	DRR620996
	<i>Carex</i> L.	<i>C. hirta</i> L.	*	SRR10527483
	<i>Ceratocystis</i> Dumort.	<i>C. viridula</i> Michx.	*	SRR10527454
	<i>Clandestinae</i> G. Don	<i>C. humilis</i> Leyss.	*	SRR10527482
	<i>Cryptostachyae</i> Franch	<i>C. cryptostachys</i> Brongn.	China. Guangdong: Qingyuan, <i>Chen, W.-J. et al. 14042460</i> (OKAY)	DRR620988
	<i>Decorae</i> (Kük.) Ohwi	<i>C. decora</i> Boott	Nepal. Jaljale Himal, <i>Ikeda, H. et al. 1225016</i> (OKAY)	DRR620989
		<i>C. inclinis</i> C.B. Clarke	Nepal. Lho: Sama Gaon, <i>Ikeda H. 1225066</i> (OKAY)	DRR621000
		<i>C. perakensis</i> C.B. Clarke	Thailand. Loei: Phu Luang, <i>Tamura, M.N. et al. 68816</i> (KYO)	DRR621007
		<i>C. perakensis</i> C.B. Clarke	Thailand. Loei: Phu Luang, <i>Tamura, M.N. et al. 68842</i> (KYO)	DRR621008
		<i>C. stracheyi</i> Boott ex C.B. Clarke	Nepal. Simar Kharka: Dhaulo Odar, <i>Ikeda H. et al. 1215029</i> (OKAY)	DRR621014
	<i>Debiles</i> (J. Carey) Ohwi	<i>C. bostrychostigma</i> Maxim.	*	PP790556
		<i>C. finitima</i> Boott	Nepal. Simar Kharka: Dhaulo Odar, <i>Ikeda H. et al. 1215031</i> (OKAY)	DRR620993
	<i>Euprepes</i> Nelves & Airy Shaw <i>Graciles</i> Kük.	<i>C. atrivaginata</i> Nelves	*	SRR5314643
		<i>C. autumnalis</i> Ohwi	Japan. Okayama: <i>Takahashi, H. & H. Katayama 28218</i> (OKAY)	DRR620981
		<i>C. brunnea</i> Thunb.	Japan. Wakayama: Arita-shi, <i>Takahashi, K.T. 2043</i> (KYO)	DRR620983
		<i>C. chichijimensis</i> Katsuy.	Japan. Tokyo: Chichijima Isl., <i>Katsuyama, T. et al. s.n.</i> (OKAY-19868)	DRR620984
		<i>C. hattoriana</i> Nakai ex Tuyama	Japan. Tokyo: Hahajima Isl., <i>Katsuyama, T. s.n.</i> (OKAY-19913)	DRR620997
		<i>C. henryi</i> C.B. Clarke ex Franch.	Nepal. Manag: Geo, <i>Ikeda, H. et al. 20814116</i> (TI)	DRR620999
		<i>C. lenta</i> D. Don	Japan. Kyoto: Kyoto-shi, <i>Takahashi, K.T. 2005</i> (KYO)	DRR621002
		<i>C. longipes</i> D. Don	China. Yunnan: Dali, <i>Ikeda H. et al. 1414108</i> (OKAY)	DRR621003
		<i>C. nachiana</i> Ohwi	Japan. Kagoshima: Soo-shi, <i>Yoshino, Y. et al. s.n.</i> (OKAY-23167)	DRR621006
		<i>C. sacrosancta</i> Honda	Japan. Shizuoka: Hamamatsu-shi, <i>Katsuyama, T. s.n.</i> (OKAY-21660)	DRR621011
		<i>C. sendaica</i> Franch.	Japan. Shizuoka: Shimoda-shi, <i>Naito, U. s.n.</i> (OKAY-23878)	DRR621013
		<i>C. tabatae</i> Katsuy.	Japan. Kagoshima: Amami Isl., <i>Katsuyama, T. s.n.</i> (OKAY-23380)	DRR621017
		<i>C. tamakii</i> T. Koyama	Japan. Okinawa: Yona-field, <i>Yano, O. & M. Amano s.n.</i> (OKAY)	DRR621018
		<i>C. teinogyna</i> Boott	Japan. Okayama: Takahashi-shi, <i>Katayama, H. 50793</i> (OKAY)	DRR621019
	<i>Grallatoriae</i> Kük.	<i>C. grallatoria</i> Maxim. var. <i>grallatoria</i>	Japan. Shizuoka: Shizuoka-shi, <i>Oda, J. & C. Hasekura 8481</i> (KYO)	DRR620994
		<i>C. grallatoria</i> Maxim. var. <i>heteroclita</i> (Franch.) Kük. ex Matsum.	Japan. Nara: Yoshino-gun, <i>Oda, J. 8906</i> (KYO)	DRR620995

Table 1. (Continued)

Subgenus	Section	Species	Voucher	Accession number
<i>Carex</i> L.				
	<i>Griseae</i> (L.H. Bailey) Kük.	<i>C. grisea</i> Wahlenb.	*	SRR10527487
		<i>C. plantaginea</i> Lam.	*	SRR5314669
	<i>Hymenochlaenae</i> (Drejer) L.H. Bailey	<i>C. prasina</i> Wahlenb.	*	SRR10527467
	<i>Indicae</i> (L.H. Bailey) C.B. Clarke	<i>C. condensata</i> Nees	Thailand. Tak: Tha Song Yang, <i>Tamura, M.N. et al. 68542</i> (KYO)	DRR620985
		<i>C. continua</i> C.B. Clarke	Thailand. Mae Hong Son: Mae Sarian, <i>Tamura, M.N. et al. 68526</i> (KYO)	DRR620986
		<i>C. cruciata</i> Wahlenb.	Thailand. Mae Hong Son: Pai, <i>Tamura, M.N. et al. 68508</i> (KYO)	DRR620987
		<i>C. doisutepensis</i> T. Koyama	Thailand. Loei: Phu Luang, <i>Tamura, M.N. et al. 68839</i> (KYO)	DRR620991
		<i>C. filicina</i> Nees	Thailand. Mae Hong Son: Pai, <i>Tamura, M.N. et al. 68509</i> (KYO)	DRR620992
		<i>C. indica</i> L.	Thailand. Loei: Phu Rua, <i>Tamura, M.N. et al. 68842</i> (KYO)	DRR621001
		<i>C. stramentitia</i> Boott ex Boeck	Thailand. Tak: Umphang, <i>Tamura, M.N. et al. 69935</i> (KYO)	DRR621015
		<i>C. subfilicinoides</i> Kük.	China. Yunnan: Nujiang Lisu Autonomous, <i>Ikeda, H. et al. 1414095</i> (OKAY)	DRR621016
		<i>C. vesiculosa</i> Boott	Nepal. Lamjung: Tal, <i>Ikeda, H. et al. 20814118</i> (TI)	DRR621022
	<i>Lageniformes</i> (Ohwi) Nelmes	<i>C. breviscapa</i> C.B. Clarke	Thailand. Loei: Phu Luang, <i>Tamura, M.N. et al. 68837</i> (KYO)	DRR620982
		<i>C. rhynchachaenium</i> C.B. Clarke	Thailand. Loei: Phu Luang, <i>Tamura, M.N. et al. 68838</i> (KYO)	DRR621010
	<i>Limosae</i> (Heuff.) Meinsh.	<i>C. magellanica</i> Lam.	*	SRR10527479
	<i>Mapaniifoliae</i> Nelmes & Airy Shaw	<i>C. bavicola</i> Raymond	*	SRR5314645
		<i>C. helferi</i> Boeckeler	Thailand. Yala: Betong, <i>Tamura, M.N. 9230</i> (KYO)	DRR620998
	<i>Mitratae</i> Kük.	<i>C. pisiformis</i> Boott	*	SRR10527469
		<i>C. pocilliformis</i> Boott	Japan. Nara: Kashiba-shi, <i>Oda, J. 8276</i> (KYO)	DRR621009
		<i>C. tristachya</i> Thunb.	Japan. Ehime: Toon-shi, <i>Oda, J. 8686</i> (KYO)	DRR621021
	<i>Mundae</i> Kük.	<i>C. dissitiflora</i> Franch.	Japan. Gifu: Takayama, <i>Takahashi K.T. & S. Takahashi 2011</i> (KYO)	DRR620990
		<i>C. munda</i> Boott	China. Yunnan: Dali, <i>Ikeda, H. et al. 1414006</i> (OKAY)	DRR621004
		<i>C. munda</i> Boott	*	SRR10527476
	<i>Paludosae</i> G. Don	<i>C. amplifolia</i> Boott	*	SRR10527508
		<i>C. pellita</i> Muhl. ex Willd.	*	SRR10527472
	<i>Polystachyae</i> Tuck.	<i>C. myosurus</i> Nees	Nepal. Khayekot: Lithi, <i>Ikeda, H. et al. 1215064</i> (OKAY)	DRR621005
	<i>Praelongae</i> (Kük.) Nelmes	<i>C. dimorpholepis</i> Steud.	*	SRR5314646
	<i>Racemosae</i> G. Don	<i>C. atropicta</i> Steud.	*	SRR10527506
		<i>C. mertensii</i> J.D. Prescott ex Bong.	*	SRR10527478
	<i>Radicales</i> (Kük.) Nelmes	<i>C. speciosa</i> Kunth	*	SRR5314644
	<i>Rhynchocystis</i> Dumort.	<i>C. pendula</i> Huds.	*	SRR10527473
	<i>Scabrellae</i> Kük.	<i>C. aff. nodiflora</i>	Thailand. Tak: Umphang, <i>Tamura, M.N. et al. 69917</i> (KYO)	DRR620979
		<i>C. tricephala</i> Boeckeler	Thailand. Loei: Phu Rua, <i>Tamura, M.N. et al. 68843</i> (KYO)	DRR621020

Table 1. (Continued)

Subgenus	Section	Species	Voucher	Accession number
<i>Carex</i> L.	<i>Spirostachyae</i> (Drejer) L.H. Bailey	<i>C. punctata</i> Gaudin	*	SRR10527466
	<i>Squarrosae</i> J. Carey	<i>C. frankii</i> Kunth	*	SRR10527490
	<i>Sylvaticae</i> Rouy	<i>C. sylvatica</i> Huds.	*	SRR10527458
<i>Euthyceras</i> Petern.	<i>Inflatae</i> Kük.	<i>C. breweri</i> Boott	*	SRR10527514
	<i>Leptocephalae</i> L.H. Bailey	<i>C. leptalea</i> Wahlenb.	*	SRR10527437
<i>Psyllophorae</i> (Degland) Petern.	<i>Junciformes</i> (Boeckeler) Kük.	<i>C. patagonica</i> Wahlenb.	*	SRR10527513
<i>Siderosticta</i> M.J. Waterway	<i>Hypolytroides</i> Nelmes	<i>C. hypolytroides</i> Ridl.	*	SRR5314667
	<i>Siderostictae</i> Franch. ex Ohwi	<i>C. kucyniakii</i> Raymond	*	SRR5314657
		<i>C. siderosticta</i> Hance	*	SRR30791880
<i>Uncinia</i> (Pers.) Petern.	<i>Uncinia</i> (Pers.) Baill.	<i>C. hamata</i> Sw.	*	SRR10437498
<i>Viginea</i> (P. Beauv. ex T. Lestib.) Heer	<i>Divisae</i> Christ ex Kük.	<i>C. gayana</i> E. Desv.	*	SRR10527442
	<i>Foetidae</i> (Tuck. ex L.H. Bailey) Kük.	<i>C. maritima</i> Gunnerus	*	SRR10527440
	<i>Gibbae</i> Kük.	<i>C. gibba</i> Wahlenb.	*	NC_061300
	<i>Pacificae</i> Ohwi	<i>C. satsumensis</i> Franch. & Sav.	Japan, Osaka: Mt. Kongo-san, <i>Takahashi, K.T. 2014</i> (KYO)	DRR621012
	<i>Phaestoglochin</i> Dumort.	<i>C. divulsa</i> Stokes	*	SRR10527445

Table 2. Morphological characters and character states of species and sections of basal lineages of *Carex* subg. *Carex*. Character states with an asterisk were quoted from the literatures listed in Appendix 1. “-” indicates no morphological information.

Classification		Morphological characters										
Section of the present study	Species	Color of basal sheath	Type of inflorescence (spike unit)	Sexuality of terminal spike	Color of pistillate scales	Direction of perigynia to spike axis	Shape of perigynia in lateral view	Shape of perigynia in front view	Venation of perigynia	Number of style branching	Shape of style base	Shape of cladophyll
Dissitiflorae	<i>C. bostrychostigma</i> Maxim.	yellowish brown	racemose, partly paniculate	male, rarely androgynous	yellowish brown	parallel	narrowly lanceolate	trigonus	several veined	3	not thickened	tubular or fertile utriculiform
	<i>Carex dissitiflora</i> Franch.	dark brown	racemose, partly paniculate	androgynous	pale green	parallel	narrowly lanceolate	trigonus	several veined	3	not thickened	tubular or fertile utriculiform
Albae	<i>C. alba</i> Scop.	pale brown	racemose	male	yellowish brown	oblique	broadly elliptic	swollen trigonous*	several veined*	3*	globosely thickened*	tubular*
	<i>C. eburnea</i> Boott	brown	racemose	male	yellowish brown	oblique	elliptic	swollen trigonous*	several veined*	3*	globosely thickened*	tubular*
	<i>C. mckittrickensis</i> P.W. Ball	light brown	racemose	male	light brown	oblique	elliptic	swollen trigonous*	many veined*	3*	enlarged*	-
	<i>C. ussuriensis</i> Kom.	yellowish brown	racemose	male	yellowish green	oblique	broadly elliptic	swollen trigonous*	several veined*	3*	globosely thickened*	tubular*
Graciles	<i>C. autumnalis</i> Ohwi	dark brown	racemose	male	yellowish brown	oblique	elliptic	plano-convex	several veined	2	thickened*	tubular
	<i>C. bilateralis</i> Hayata	dark brown	racemose, partly paniculate	androgynous	yellowish brown	oblique	elliptic	plano-convex*	several veined*	2*	thickened*	tubular*
	<i>C. bodinieri</i> Franch.	dark brown	racemose	androgynous	brown	oblique	broadly elliptic	plano-convex*	many veined*	2*	slightly thickened*	tubular*
	<i>C. brunnea</i> Murray	brown	racemose, partly paniculate	androgynous	yellowish brown	oblique	elliptic	plano-convex	many veined	2	slightly thickened	tubular
	<i>C. chichijimensis</i> Katsuy.	dark brown	racemose	androgynous	margin brown	oblique	ovate	biconvex	many veined	2	slightly thickened*	-
	<i>C. chuii</i> Nelm.	brown	racemose	male	yellowish brown	oblique	broadly elliptic	compressed plano-convex*	many veined*	2*	slightly thickened*	tubular*
	<i>C. erica</i> Tang & F.T. Wang ex L.K. Dai	brown	racemose	androgynous	stramineous	oblique	oblong-elliptic	plano-convex*	several veined*	2*	slightly thickened*	tubular*
	<i>C. gentilis</i> Franch.	brown	racemose, partly paniculate	androgynous	brown	oblique	elliptic	plano-convex*	many veined*	2*	slightly thickened*	tubular*
	<i>C. gongshanensis</i> Tang & F.T. Wang ex Y.C. Yang	dark brown	racemose, partly paniculate	androgynous	brown	oblique	elliptic	plano-convex*	many veined*	2*	slightly thickened*	tubular*
	<i>C. hattoriana</i> Nakai ex Tuyama	dark brown	racemose	androgynous	reddish brown	oblique	elliptic	biconvex	many veined	2	thickened	tubular
	<i>C. henryi</i> (C.B. Clarke) T. Koyama	black brown	paniculate	androgynous	yellowish brown	oblique	elliptic	plano-convex	many veined	2	slightly thickened	tubular
	<i>C. lenta</i> D. Don	dark brown	racemose	androgynous	brown	oblique	elliptic	biconvex	many veined	2	thickened	-
	<i>C. lushanensis</i> Kük.	dark brown	racemose, partly paniculate	androgynous	pale brown	oblique	elliptic	plano-convex*	many veined*	2*	slightly thickened*	tubular
	<i>C. mossyensis</i> Franch.	black brown	racemose, partly paniculate	androgynous	yellowish brown	oblique	elliptic-ovate	compressed plano-convex*	many veined*	2*	slightly thickened*	tubular*
	<i>C. nachiana</i> Ohwi	dark brown	racemose, partly paniculate	androgynous	brown	oblique	elliptic-ovate	compressed biconvex	many veined	2	thickened	tubular
	<i>C. omeiensis</i> Tang	dark brown	racemose	male	yellowish brown	oblique	elliptic	plano-convex*	many veined*	2	slightly thickened*	tubular*
	<i>C. orbicularinucis</i> L.K. Dai	dark brown	racemose, partly paniculate*	androgynous	pale brown	oblique	suborbicular*	plano-convex*	many veined*	2*	thickened*	tubular*
	<i>C. pergracilis</i> Nelm.	brown	racemose	terminal male, lateral female	yellowish brown	oblique	elliptic	plano-convex*	many veined	2*	slightly thickened*	tubular
	<i>C. sacrosancta</i> Honda	blackish brown	racemose	androgynous	brown	oblique	elliptic	biconvex	many veined	2	thickened	-
	<i>C. sendica</i> Franch.	dark brown	racemose	androgynous	reddish brown	oblique	elliptic	plano-convex	several veined	2	slightly thickened	tubular
	<i>C. sindosistiflora</i> Tang & F.T. Wang ex L.K. Dai	brown	racemose, partly paniculate	androgynous	pale stramineous brown	oblique	oblong-lanceolate	plano-convex*	many veined	2*	slightly thickened*	tubular*
	<i>C. stipitimus</i> C.B. Clarke ex Franch.	dark brown	racemose, partly paniculate	male	yellowish brown	oblique	elliptic	plano-convex*	many veined	2*	slightly thickened*	tubular*
	<i>C. tabatake</i> Katsuy.	dark brown	racemose	androgynous	brown	oblique	elliptic	biconvex	several veined	2	thickened	-
	<i>C. tamakii</i> T. Koyama	dark brown	racemose	androgynous	brown	oblique	elliptic	biconvex	several veined	2	thickened	-
	<i>C. teinogyna</i> Boott	dark brown	racemose, partly paniculate	androgynous	yellowish brown	oblique	oblong-elliptic	plano-convex	several veined	2	slightly thickened	-
Mundae	<i>C. barhayaki</i> Jim. Mejias & Roalson	pale brown*	racemose	androgynous	brown*	oblique	elliptic	trigonus*	2 veins prominent	3*	cylindrical*	-
	<i>C. handelii</i> Kük.	pale brown	racemose	androgynous	yellowish brown	oblique	elliptic	trigonus	2 veins prominent	3*	thickened*	tubular
	<i>C. harrysmithii</i> Kük.	brown	racemose	androgynous	yellowish brown	oblique	narrowly elliptic	trigonus	2 veins prominent*	3*	slightly thickened*	-
	<i>C. munda</i> Boott	pale brown	racemose	androgynous	yellowish brown	oblique	elliptic	trigonus	2-3 veins prominent	3*	slightly thickened*	tubular or fertile utriculiform
Indicae	<i>C. atrivaginata</i> Nelm.	dark brown	paniculate	androgynous	dirty white or light brown	oblique	long elliptic	trigonus*	-	3*	-	-
	<i>C. balansae</i> Franch.	pale brown	paniculate	androgynous	pale light brown	oblique	elliptic	trigonus*	many veined*	3*	strongly thickened*	utriculiform*
	<i>C. bavicola</i> Raymond	yellowish brown	paniculate	androgynous	yellowish brown	oblique	long rhomboid	trigonus*	-	3*	-	-
	<i>C. callista</i> Nelm.	yellowish brown	paniculate	androgynous	white	oblique	-	trigonus*	-	3*	-	utriculiform*
	<i>C. cataphyllodes</i> Nelm.	pale brown	paniculate	androgynous	pale brown	oblique	elliptic	trigonus*	many veined*	3*	-	utriculiform*
	<i>C. cirrhulosa</i> Nees	dark brown*	paniculate	androgynous	pale brown	oblique	rhomboid-elliptic	trigonus*	many veined*	3*	pyramidally thickened above discoid base*	utriculiform or glumiform*
	<i>C. cochinchinensis</i> Raymond	pale grayish brown	paniculate	androgynous	-	oblique	ovate-elliptic	trigonus*	many veined*	3*	thickened*	humped utriculiform*
	<i>C. commixta</i> Steud.	pale brown	paniculate	androgynous	pale brown	oblique	elliptic	trigonus*	several veined	3*	pyramidally thickened*	-
	<i>C. deciduigrama</i> F.T. Wang & Tang ex P.C. Li	dark brown	paniculate	androgynous	pale brown	oblique	narrowly-elliptic	trigonus*	many veined*	3*	thickened and conic*	utriculiform*
	<i>C. distracta</i> C.B. Clarke	yellowish brown	paniculate	androgynous	yellowish brown	oblique	-	trigonus*	-	3*	-	utriculiform*
	<i>C. eugrepes</i> Nelm.	pale brown	paniculate	androgynous	whitish stramineous	oblique	elliptic	trigonus*	many veined*	3*	thickened above discoid base*	vestigial or glumiform*

Table 2. (Continued)

Classification		Morphological characters										
Section of the present study	Species	Color of basal sheath	Type of inflorescence (spike unit)	Sexuality of terminal spike	Color of pistillate scales	Direction of perigynia to spike axis	Shape of perigynia in lateral view	Shape of perigynia in front view	Venation of perigynia	Number of style branching	Shape of style base	Shape of cladophyll
<i>Indicae</i>	<i>C. helferi</i> Boeckeler	pale brown	paniculate	androgynous	pale brown to whitish	oblique	elliptic	trigonus*	many veined	3	pyramidally thickened forming disc*	humped glumiform*
	<i>C. horsfieldii</i> Nelmex	grayish brown	paniculate	androgynous	pale yellowish brown	oblique	elliptic	trigonus*	many veined*	3*	strongly inflated*	utriculiform*
	<i>C. indica</i> L.	dark brown	paniculate	androgynous	pale yellowish brown	oblique	ovate	trigonus	many veined*	3	thickened above discoid base	utriculiform*
	<i>C. indiciformis</i> F.T. Wang & Tang ex P.C. Li	yellowish brown	paniculate	androgynous	pale yellowish white	oblique	ovate	trigonus*	many veined*	3*	strongly thickened*	utriculiform*
	<i>C. jeanpertii</i> E.G. Camus	yellowish brown	paniculate	androgynous	yellowish brown	oblique	broadly elliptic	flattened-trigonus*	many veined*	3*	pyramidally thickened above discoid base*	vestigial utriculiform*
	<i>C. laosensis</i> Nelmex	-	paniculate	androgynous	whitish or brownish	oblique	elliptic	trigonus*	many veined*	3*	pyramidally thickened*	-
	<i>C. longipes</i> D.Don	dark brown	racemose, partly paniculate	androgynous	yellowish brown	oblique	elliptic	plano-convex	many veined	2	thickened above discoid base	sometimes utriculiform
	<i>C. malaccensis</i> C.B. Clarke	pale brown	spike or paniculate	androgynous	milky white to light brown	oblique	elliptic	trigonus*	many veined*	3*	pyramidally thickened*	subutriculiform or glumiform*
	<i>C. meeboldiana</i> Kük.	dark brown	paniculate	androgynous	pale staminateous or light brown	oblique	rhomboid-elliptic	trigonus*	many veined*	3*	-	-
	<i>C. nodiflora</i> Boeckeler	dark brown*	paniculate	androgynous	pale green	oblique	elliptic-rhomboid	trigonus*	many veined*	3*	pyramidally thickened above discoid base*	subutriculiform*
	<i>C. aff. nodiflora</i>	dark brown	paniculate	androgynous	pale green, partly yellowish brown	oblique	elliptic-rhomboid	trigonus	many veined	3	pyramidally thickened above discoid base	subutriculiform or glumiform
	<i>C. pandanophylla</i> C.B. Clarke	pale grayish brown	paniculate	androgynous	pale brown	oblique	-	-	-	3*	-	-
	<i>C. perakensis</i> C.B. Clarke	dark brown	paniculate	androgynous	yellowish brown	oblique	obovate-elliptic	trigonus	many veined	3	thickened and curved	utriculi-glumiform
	<i>C. poilanei</i> Raymond	-	paniculate	androgynous	whitish brown	oblique	narrowly elliptic	trigonus*	many veined*	3*	-	humped glumiform*
	<i>C. repanda</i> C.B. Clarke	dark brown	paniculate	androgynous	milky white with reddish streak	oblique	elliptic	trigonus*	many veined*	3*	slightly thickened*	utriculiform*
	<i>C. stramentitila</i> Boott ex Boeckeler	pale brown	paniculate	androgynous	whitish, stramineous	oblique	ovate	trigonus	several veined	3	strongly thickened*	utriculiform
	<i>C. taprobanensis</i> T. Koyama	dark brown	paniculate	androgynous	yellowish brown	oblique	rhomboid-ovoid	trigonus*	many veined*	3*	strongly thickened*	utriculiform*
	<i>C. tavyensis</i> Nelmex	grayish brown	paniculate	androgynous	pale brown	oblique	elliptic	-	many veined*	3*	-	-
	<i>C. thailandica</i> T. Koyama	pale brown	paniculate	androgynous	pale brown	oblique	elliptic	trigonus	many veined	3	-	utriculiform
	<i>C. tricephala</i> Boeckeler	yellowish brown	spike or paniculate	androgynous	milky white to brown	oblique	elliptic	trigonus	many veined	3	pyramidally thickened	subutriculiform or glumiform
	<i>C. trichophylla</i> Nelmex	dark brown	paniculate	androgynous	pale brown	oblique	elliptic	trigonus*	many veined*	3*	pyramidally thickened above discoid base*	-
	<i>C. vietnamica</i> Raymond	dark brown*	paniculate*	androgynous*	-	oblique	-	trigonus*	many veined*	3*	thickened*	-
	<i>C. wightiana</i> Nees	dark brown	paniculate	androgynous	light brown	oblique	elliptic	subinflated-trigonus*	many veined*	3*	pyramidally thickened*	utriculi-glumiform*
	<i>C. zizaniifolia</i> Raymond	dark brown	paniculate	androgynous	pale brown	oblique	oblong-elliptic	trigonus*	many veined*	3	-	utriculiform*
<i>Filicinae</i>	<i>C. alboviridis</i> C.B. Clarke	pale reddish purple	paniculate	androgynous	pale brown	oblique	subinflated-elliptic	subinflated-trigonus*	many veined*	3	-	utriculiform*
	<i>C. amicta</i> Boott	reddish brown	paniculate	androgynous	reddish brown	oblique	ovate	subinflated-trigonus*	-	3*	thickened*	utriculiform*
	<i>C. andringitrensis</i> Cherm.	pale, partly reddish brown	paniculate	androgynous	reddish or dark purple	oblique	elliptic	trigonus*	many veined*	3*	-	utriculiform*
	<i>C. angolensis</i> Nelmex	reddish brown	paniculate	androgynous	pale brown	oblique	elliptic	trigonus*	many veined*	3*	-	utriculiform*
	<i>C. azteca</i> Mack.	dark purplish brown	paniculate	androgynous	dark reddish brown	oblique	elliptic-ovate	trigonus*	few veined	3*	slightly thickened*	utriculiform*
	<i>C. bathiei</i> H. Lév	reddish brown	paniculate	androgynous	pale brown partly reddish brown	oblique	elliptic	trigonus*	many veined*	3*	-	utriculiform*
	<i>C. beckii</i> G.A. Wheeler	reddish brown	paniculate	androgynous	reddish brown	oblique	subinflated-elliptic	trigonus*	many veined*	3*	-	utriculiform*
	<i>C. brassii</i> Nelmex	reddish purple	paniculate	androgynous	chestnut brown	oblique	elliptic	trigonus*	many veined*	3*	not thickened*	utriculiform*
	<i>C. burttii</i> Noltie	reddish purple	paniculate	androgynous	pale brown	oblique	narrowly-elliptic	trigonus*	-	3*	-	utriculiform*
	<i>C. castanostachya</i> K.Schum. ex Kük.	reddish purple	paniculate	androgynous	brown	oblique	oblong-lanceolate	trigonus*	weakly veined*	3*	-	utriculiform*
	<i>C. ceylanica</i> Boeckeler	reddish brown	paniculate	androgynous	shiny brown	oblique	lanceolate-elliptic	trigonus*	many veined*	3*	not thickened*	utriculiform*
	<i>C. chlorosaccus</i> C.B. Clarke	reddish brown	paniculate	androgynous	pale green	oblique	ovate	trigonus*	many veined*	3*	not thickened*	utriculiform*
	<i>C. condensata</i> Nees	reddish brown	paniculate	androgynous	brown	oblique	inflated-telliptic	trigonus	several veined	3	bent, slightly thickened	utriculiform
	<i>C. continua</i> C.B. Clarke	pale brown, partly reddish brown	paniculate	androgynous	reddish brown	oblique	ovate-elliptic	trigonus	many veined	3	not thickened	utriculiform
	<i>C. cruciata</i> Wahlenb.	reddish brown	paniculate	androgynous	pale brown with reddish patches	oblique	inflated-elliptic	inflated-trigonus	several veined	3	slightly thickened	utriculiform
	<i>C. cubensis</i> Kük.	pale, partly reddish brown	paniculate	androgynous	reddish brown	oblique	elliptic	trigonus*	many veined	3*	not thickened	utriculiform*
	<i>C. doisutepensis</i> T. Koyama	reddish brown	paniculate	androgynous	reddish brown	oblique	elliptic	inflated-trigonus*	many veined	3	not thickened	utriculiform
	<i>C. echinochloe</i> Kunze	reddish brown	paniculate	androgynous	pale brown partly reddish brown	oblique	ovate	trigonus*	many veined*	3*	slightly thickened*	utriculiform*
<i>C. ekmanii</i> Kük.	brown partly reddish brown	paniculate	androgynous	reddish brown	oblique	elliptic	trigonus*	several veined*	3*	not thickened*	utriculiform*	
<i>C. elatior</i> Boeckeler	dark reddish brown	paniculate	androgynous	reddish brown	oblique	elliptic	subinflated-trigonus*	many veined*	3*	-	utriculiform*	
<i>C. eurphylla</i> Cherm.	reddish brown	paniculate	androgynous	reddish purple	oblique	elliptic	trigonus*	many veined*	3*	-	utriculiform*	

Table 2. (Continued)

Classification		Morphological characters										
Section of the present study	Species	Color of basal sheath	Type of inflorescence (spike unit)	Sexuality of terminal spike	Color of pistillate scales	Direction of perigynia to spike axis	Shape of perigynia in lateral view	Shape of perigynia in front view	Venation of perigynia	Number of style branching	Shape of style base	Shape of cladophyll
<i>Filicinae</i>	<i>C. graminifolia</i> Cherm.	reddish brown	paniculate	androgynous	whitish green	oblique	elliptic	trigonous*	many veined*	3*	-	utriculiform*
	<i>C. guffroyi</i> H. Lév. & H. Perrier	reddish brown	paniculate	androgynous	reddish brown	oblique	ovate-elliptic	trigonous*	several veined*	3*	-	utriculiform*
	<i>C. haematosaccus</i> C.B. Clarke	reddish brown	paniculate	androgynous	reddish brown	oblique	elliptic	trigonous*	many veined*	3*	not thickened*	utriculiform*
	<i>C. heterodoxa</i> Cherm.	pale reddish purple	paniculate	androgynous	reddish brown	oblique	elliptic	trigonous*	many veined*	3*	-	utriculiform*
	<i>C. hildebrandtiana</i> Boeckeler	reddish purple	paniculate	androgynous	reddish brown	oblique	narrowly elliptic	trigonous*	many veined*	3*	slightly thickened*	utriculiform*
	<i>C. hirtigluma</i> C.B. Clarke	reddish purple	paniculate	androgynous	reddish brown	oblique	ovate	trigonous*	many veined*	3*	-	utriculiform*
	<i>C. hulthenii</i> Aspl.	pale brown	paniculate	androgynous	reddish brown	oblique	elliptic	inflated-trigonous*	many veined*	3*	slightly thickened*	utriculiform*
	<i>C. humboldtiana</i> Steud.	reddish brown	paniculate	androgynous	dark reddish brown	oblique	elliptic	trigonous*	many veined*	3*	slightly thickened*	utriculiform*
	<i>C. humpatensis</i> H.E. Hess	reddish brown	paniculate	androgynous	reddish brown	oblique	elliptic	trigonous*	many veined*	3*	not thickened*	utriculiform*
	<i>C. lamprochlamys</i> S.T.Blake	reddish brown	paniculate	androgynous	reddish brown	oblique	elliptic	trigonous*	many veined*	3*	slightly thickened*	utriculiform*
	<i>C. leptocladus</i> C.B. Clarke	pale reddish brown	paniculate	androgynous	dark brown	oblique	elliptic	trigonous*	-	3*	-	utriculiform*
	<i>C. lindleyana</i> Nees	reddish brown	paniculate	androgynous	reddish brown	oblique	elliptic	trigonous*	many veined*	3*	slightly thickened*	utriculiform*
	<i>C. macrophyllidion</i> Nelmes	pale reddish brown	paniculate	androgynous	pale reddish brown	oblique	elliptic	trigonous*	many veined*	3*	-	utriculiform*
	<i>C. madrensis</i> L.H. Bailey	light brown	paniculate	androgynous	reddish brown	oblique	elliptic	trigonous*	several veined*	3*	not thickened*	utriculiform*
	<i>C. merxmulleri</i> Podlech	reddish brown	paniculate	androgynous	reddish brown	oblique	elliptic	trigonous*	many veined*	3*	-	-
	<i>C. neochevalieri</i> Kük. ex A. Chev.	bright brown	paniculate	androgynous	reddish brown	oblique	elliptic	trigonous*	many veined*	3*	-	utriculiform*
	<i>C. neoguinensis</i> C.B. Clarke	dark reddish brown	paniculate	androgynous	reddish brown	oblique	elliptic	trigonous*	many veined	3*	-	utriculiform*
	<i>C. neohybridensis</i> Guillaumin & Kük.	reddish brown	paniculate	androgynous	reddish brown	oblique	narrowly-elliptic	trigonous*	many veined*	3*	slightly thickened*	utriculiform*
	<i>C. novogaliciana</i> Reznicek	dark brown partly reddish brown	paniculate	androgynous	pale brown partly reddish brown	oblique	elliptic	trigonous*	many veined*	3*	not thickened*	utriculiform*
	<i>C. parvigluma</i> C.B. Clarke	pale brown, partly reddish brown	paniculate	androgynous	reddish brown	oblique	elliptic	subinflated-trigonous*	many veined*	3*	-	utriculiform*
	<i>C. polystachya</i> Sw. ex Wahlenb.	pale reddish brown	paniculate	androgynous	pale reddish brown	oblique	elliptic	trigonous*	many veined*	3*	not thickened*	utriculiform*
	<i>C. porrecta</i> Reznicek & Camelb.	reddish brown	paniculate	androgynous	reddish brown to dark brown	oblique	ovate	trigonous*	many veined*	3*	-	utriculiform*
	<i>C. proxima</i> Cherm.	reddish brown	paniculate	androgynous	reddish brown	oblique	elliptic	trigonous*	many veined*	3*	-	utriculiform*
	<i>C. pyramidalis</i> Kük.	reddish brown	paniculate	androgynous	reddish brown	oblique	ovate	trigonous*	many veined*	3*	-	utriculiform*
	<i>C. rafflesiana</i> Boott	reddish brown	paniculate	androgynous	reddish brown	oblique	elliptic	trigonous*	many veined*	3*	not thickened*	utriculiform*
	<i>C. ramosa</i> Wild.	reddish brown	paniculate	androgynous	reddish brown	oblique	elliptic	trigonous*	several veined*	3*	not thickened*	utriculiform*
	<i>C. raphidocarpa</i> Nees	reddish brown	paniculate	androgynous	reddish brown	oblique	elliptic	trigonous*	many veined*	3*	not thickened*	utriculiform*
	<i>C. renschiana</i> Boeckeler	pale reddish purple	paniculate	androgynous	reddish brown	oblique	elliptic	trigonous*	many veined*	3*	not thickened*	utriculiform*
	<i>C. rutenbergiana</i> Cherm.	pale brown partly reddish brown	paniculate	androgynous	dark reddish purple	oblique	elliptic	trigonous*	-	3*	-	utriculiform*
	<i>C. sambiranensis</i> (H. Lév.) Cherm.	pale brown partly reddish brown	paniculate	androgynous	pale reddish brown	oblique	elliptic	trigonous*	-	3*	-	utriculiform*
	<i>C. sanguinea</i> Boott	reddish brown	paniculate	androgynous	reddish purple	oblique	elliptic	trigonous*	many veined	3*	slightly thickened*	utriculiform*
	<i>C. sarawakensis</i> Kük.	dark reddish brown	paniculate	androgynous	dark reddish brown	oblique	elliptic	trigonous*	many veined*	3*	not thickened*	utriculiform*
	<i>C. scabrella</i> Wahlenb.	brown partly reddish brown	paniculate	androgynous	reddish brown	oblique	elliptic	trigonous*	many veined*	3*	not thickened*	utriculiform*
	<i>C. scabripes</i> Cherm.	reddish brown to purple	paniculate	androgynous	reddish purple	oblique	ovate	trigonous*	many veined*	3*	-	utriculiform*
	<i>C. schliebenii</i> Podlech	reddish purple	paniculate	androgynous	pale reddish brown	oblique	elliptic	trigonous*	many veined*	3*	-	utriculiform*
	<i>C. sodiroi</i> Kük.	reddish brown	paniculate	androgynous	reddish brown	oblique	elliptic	subinflated-trigonous*	many veined*	3*	-	utriculiform*
	<i>C. spicatopaniculata</i> Boeckeler ex C.B. Clarke	reddish brown	paniculate	androgynous	reddish brown	oblique	elliptic	subinflated-trigonous*	many veined*	3*	-	utriculiform*
	<i>C. spicigera</i> Nees	reddish brown	paniculate	androgynous	reddish brown	oblique	ovoid-elliptic	trigonous*	many veined*	3*	thickened*	utriculiform*
	<i>C. standleyana</i> Steyerm.	pale reddish brown	paniculate	androgynous	dark reddish brown	oblique	ovate	trigonous*	many veined*	3*	-	utriculiform*
	<i>C. steudneri</i> Boeckeler	dark reddish brown	paniculate	androgynous	dark reddish brown	oblique	ovate	trigonous*	several veined	3*	not thickened*	utriculiform*
	<i>C. subfilicinoides</i> Kük.	reddish purple brown*	paniculate	androgynous	reddish brown	oblique	elliptic	trigonous*	several veined	3	slightly thickened*	utriculiform
	<i>C. subscabrella</i> Kük.	reddish purple brown*	paniculate*	androgynous*	reddish*	oblique	elliptic*	trigonous*	many veined*	3*	-	utriculiform*
	<i>C. tenuipaniculata</i> P.C. Li	reddish brown	paniculate	androgynous	density brown spot*	oblique	elliptic	trigonous*	several veined*	3*	slightly thickened*	utriculiform*
	<i>C. tricholepis</i> Nelmes	pale brown partly reddish brown	paniculate	androgynous	pale green partly reddish brown	oblique	elliptic-ovate	trigonous*	many veined*	3*	-	utriculiform*
	<i>C. tsaratananensis</i> Cherm.	reddish brown	paniculate	androgynous	reddish brown	oblique	elliptic	trigonous*	many veined*	3*	-	utriculiform*
	<i>C. valbrayi</i> H. Lév.	reddish brown	paniculate	androgynous	reddish or green*	oblique	elliptic	trigonous*	many veined*	3*	-	utriculiform*
	<i>C. vesiculosa</i> Boott	reddish brown	paniculate	androgynous	reddish brown	oblique	elliptic	trigonous*	few veined	3	slightly thickened	utriculiform
	<i>C. wahlenbergiana</i> Boott	reddish brown	paniculate	androgynous	reddish brown	oblique	elliptic	trigonous*	many veined*	3*	not thickened*	utriculiform*
	<i>C. zuluensis</i> C.B. Clarke	dark reddish brown*	paniculate	androgynous	shiny coppery	oblique	ovate	trigonous*	several veined*	3*	-	utriculiform*

Table 2. (Continued)

Classification		Morphological characters										
Section of the present study	Species	Color of basal sheath	Type of inflorescence (spike unit)	Sexuality of terminal spike	Color of pistillate scales	Direction of perigynia to spike axis	Shape of perigynia in lateral view	Shape of perigynia in front view	Venation of perigynia	Number of style branching	Shape of style base	Shape of cladophyll
Decorae	<i>C. anomoea</i> Hand.-Mazz.	reddish brown	spike or paniculate	androgynous	reddish brown	oblique	narrowly elliptic	obtusely trigonous*	weakly veined*	3	slightly thickened*	tubular
	<i>C. baiposhanensis</i> P.C. Li	reddish brown	paniculate	male	reddish brown	oblique	elliptic	trigonous*	many veined*	3*	slightly thickened*	tubular*
	<i>C. celebica</i> Kük.	reddish brown	racemose, partly paniculate	male	reddish brown	oblique	ovoid-elliptic	trigonous*	weakly veined	3*	somewhat thickened*	-
	<i>C. daltoni</i> Boott	-	racemose, partly paniculate	androgynous	reddish brown	oblique	elliptic	trigonous*	-	3*	slightly thickened*	tubular*
	<i>C. decora</i> Boott	reddish purple	racemose, partly paniculate	androgynous	reddish brown	oblique	elliptic	trigonous	weakly veined*	3	thickened*	tubular
	<i>C. dielsiana</i> Kük.	reddish brown	paniculate	androgynous	reddish brown	oblique	elliptic	obtusely trigonous*	many veined*	3*	slightly thickened*	-
	<i>C. gandakiensis</i> Katsuy.	reddish brown	racemose	androgynous	pale brown	oblique	ovate	compressed trigonous*	many veined*	3*	-	-
	<i>C. inclinix</i> Boott ex C.B. Clarke	reddish purple	paniculate	androgynous	reddish brown	oblique	elliptic	trigonous	many veined*	3*	-	tubular
	<i>C. insignis</i> Boott	reddish purple	paniculate	androgynous or male	reddish brown	oblique	elliptic	trigonous*	several veined*	3*	slightly thickened*	tubular*
	<i>C. jizhuangensis</i> S.Yun Liang	reddish purple*	paniculate*	androgynous*	reddish purple*	oblique	elliptic*	trigonous*	many veined*	3*	curved*	tubular*
	<i>C. kumaonensis</i> Kük.	reddish brown	paniculate	androgynous	pale green	oblique	elliptic	trigonous*	many veined*	3*	-	-
	<i>C. morii</i> Hayata	reddish purple	paniculate	androgynous	light brown	oblique	elliptic	compressed trigonous*	weakly veined*	3*	slightly thickened*	tubular*
	<i>C. muniporensis</i> C.B. Clarke	reddish brown	racemose	androgynous	reddish brown	oblique	ovate	trigonous*	several veined*	3*	-	tubular*
	<i>C. neopolycephala</i> Tang & F.T. Wang ex L.K. Dai	reddish brown	racemose, partly paniculate	androgynous	pale brown	oblique	elliptic	trigonous*	2-veined and the others inconspicuous*	3*	-	tubular*
	<i>C. phyllocaula</i> Nelves	reddish brown	paniculate	androgynous	reddish brown	oblique	elliptic	trigonous*	-	3*	thickened*	tubular below, glumiform above*
	<i>C. polycephala</i> Boott	reddish brown	paniculate	androgynous	pale brown, partly reddish brown	oblique	oblong	trigonous*	2-veined and the others inconspicuous*	3*	not thickened*	tubular*
	<i>C. pomiensis</i> Y.C. Yang	reddish brown	paniculate	androgynous	reddish brown	oblique	elliptic	trigonous*	-	3*	-	tubular*
	<i>C. pontica</i> Albov	reddish brown	racemose, partly paniculate	androgynous	reddish brown	oblique	elliptic	trigonous*	many veined*	3*	not thickened*	tubular*
	<i>C. pulchra</i> Boott	reddish brown	racemose, partly paniculate	androgynous	reddish brown	oblique	elliptic	trigonous*	-	3*	-	tubular*
	<i>C. scitula</i> Boott	reddish brown	paniculate	androgynous	reddish brown	oblique	oblique	trigonous*	many veined*	3*	not thickened*	tubular*
	<i>C. shuangbaiensis</i> L.K. Dai	reddish brown	paniculate	androgynous	reddish brown	oblique	elliptic	trigonous*	3-veined*	3*	-	tubular*
	<i>C. stracheyi</i> Boott ex C.B. Clarke	dark reddish brown	racemose	androgynous	reddish brown	oblique	elliptic	trigonous*	several veined*	3*	-	tubular*
	<i>C. subperakensis</i> L.K. Ling & Y.Z. Huang	purplish brown*	paniculate*	androgynous*	-	oblique	*	flattened trigonous*	many veined*	3*	slightly thickened*	tubular*
	<i>C. turrita</i> Kük.	reddish brown	racemose	androgynous	reddish brown	oblique	elliptic	trigonous*	several veined*	3*	-	tubular*
	<i>C. urelytra</i> Ohwi	dark brown, partly reddish brown	paniculate	androgynous	dark brown	oblique	elliptic	flattened trigonous	many veined	3	-	tubular
	<i>C. verticillata</i> Zoll. & Moritzi	reddish purple	racemose	male	reddish brown	oblique	elliptic	trigonous*	-	3*	slightly thickened*	tubular*
	<i>C. walkeri</i> Am. ex Boott	reddish purple	paniculate	androgynous	reddish brown	oblique	elliptic	trigonous*	several veined	3*	not thickened*	tubular*
	<i>C. winterbottomii</i> C.B. Clarke	reddish purple	racemose	androgynous	reddish brown	oblique	elliptic	trigonous*	several veined	3*	-	tubular*
	<i>C. zhenkangensis</i> Tang & F.T. Wang ex S.Yun Liang	dark reddish brown	paniculate	male	brown*	oblique	ovate	-	2-veined near ridge*	3*	curved*	tubular*

Figures

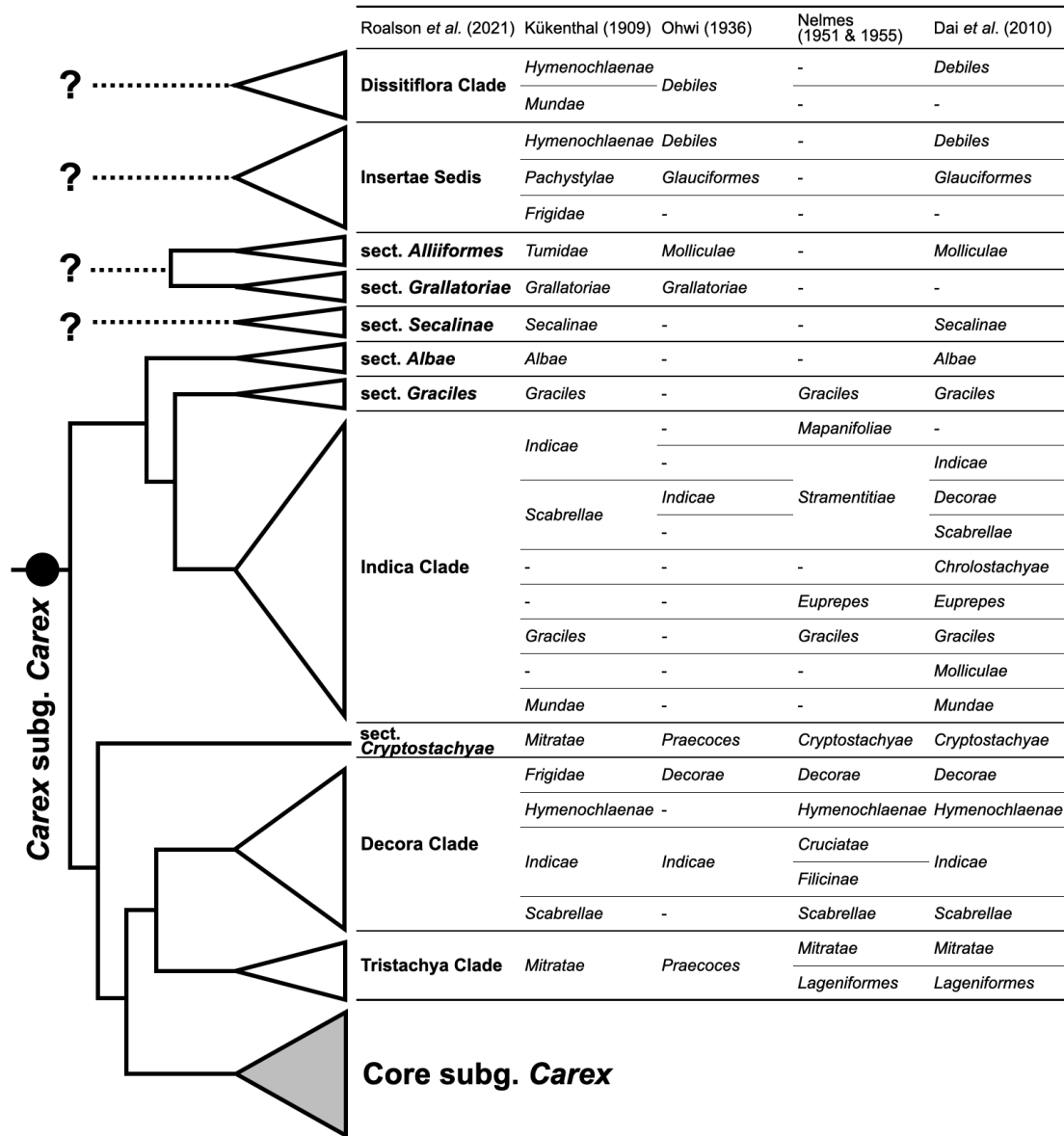


Fig. 1. Left, a diagram shows that the phylogenetic relationships of *Carex* subg. *Carex*, based on the phylogenetic analyses of Jiménez-Mejías *et al.* (2016), Martín-Bravo *et al.* (2019) and Villaverde *et al.* (2020), summarized by Roalson *et al.* (2021). Right, a table shows that the comparison of sectional circumscription within the early-diverging lineages of *Carex* subg. *Carex*, proposed by Roalson *et al.* (2021), Kükenthal (1909), Ohwi (1936), Nelmes (1951, 1955) and Dai *et al.* (2010). “-” indicates that there is no description of the corresponding species and that the section recognition is unknown. Nelmes (1951) documented species from Malaysia, while Nelmes (1955) focused on those from the

Indochina Peninsula. As there are no contradiction between Nelmes (1951) and Nelmes (1955) in their sectional circumscription, they are summarized in a single column in this table. Roalson *et al.* (2021) included *C. nitidiutriculata* (*C. sect. Hymenochlaenae* (Drejer) L. H. Bailey) in the Indica Clade. However, the specimen of *C. nitidiutriculata* (Heng Li *et al.* 20253, E) used in the phylogenetic analyses should be correctly identified as *C. perakensis* C.B. Clarke (*C. sect. Decorae*), and therefore, *C. sect. Hymenochlaenae* is not included in the Indica Clade. As Roalson *et al.* (2021) withheld the placements of Dissitiflora Clade, Insertae Sedis, a clade of sects. *Alliiformes* and *Grallatoriae*, and sect. *Secalinae* on their phylogenetic tree, we marked them with "?".

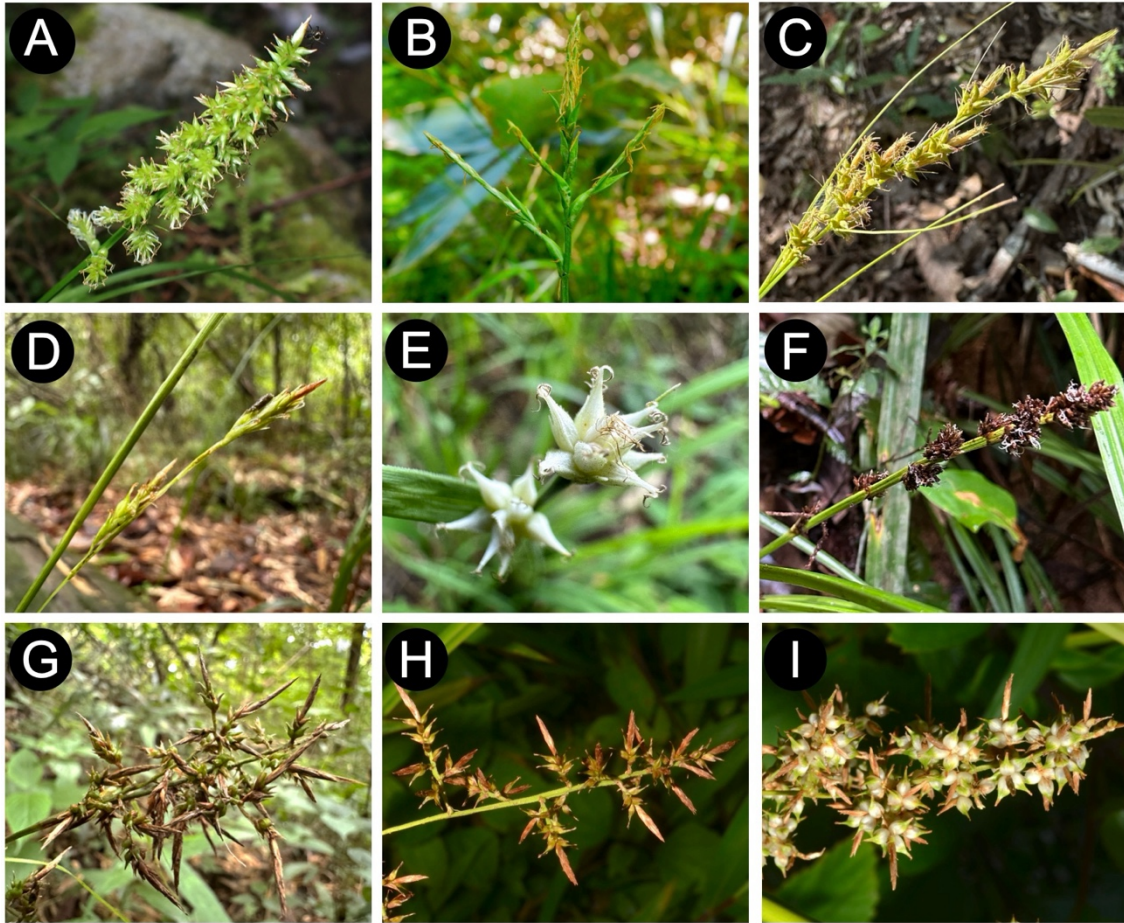


Fig. 2. Inflorescences of *Carex* subg. *Carex*. **A**, *Carex satsumensis* (sect. *Pacificae*) from Osaka, Japan; **B**, *C. dissitiflora*; (sect. *Mundae*) from Hiroshima, Japan; **C**, *C. aff. nodiflora* (sect. *Scabrellae*) from Tak, Thailand; **D**, *C. perakensis* (sect. *Decorae*) from Loei, Thailand; **E**, *C. tricephala* (sect. *Scabrellae*) from Loei, Thailand; **F**, *C. helferi* (sect. *Mapanifoliae*) from Yala, Thailand; **G**, *C. indica* (sect. *Indicae*) from Loei, Thailand; **H**, *C. filicina* (sect. *Indicae*) from Mae Hong Son, Thailand; **I**, *C. cruciata* (sect. *Indicae*) from Mae Hong Son, Thailand. Section names in parentheses follow Dai *et al.* (2010).

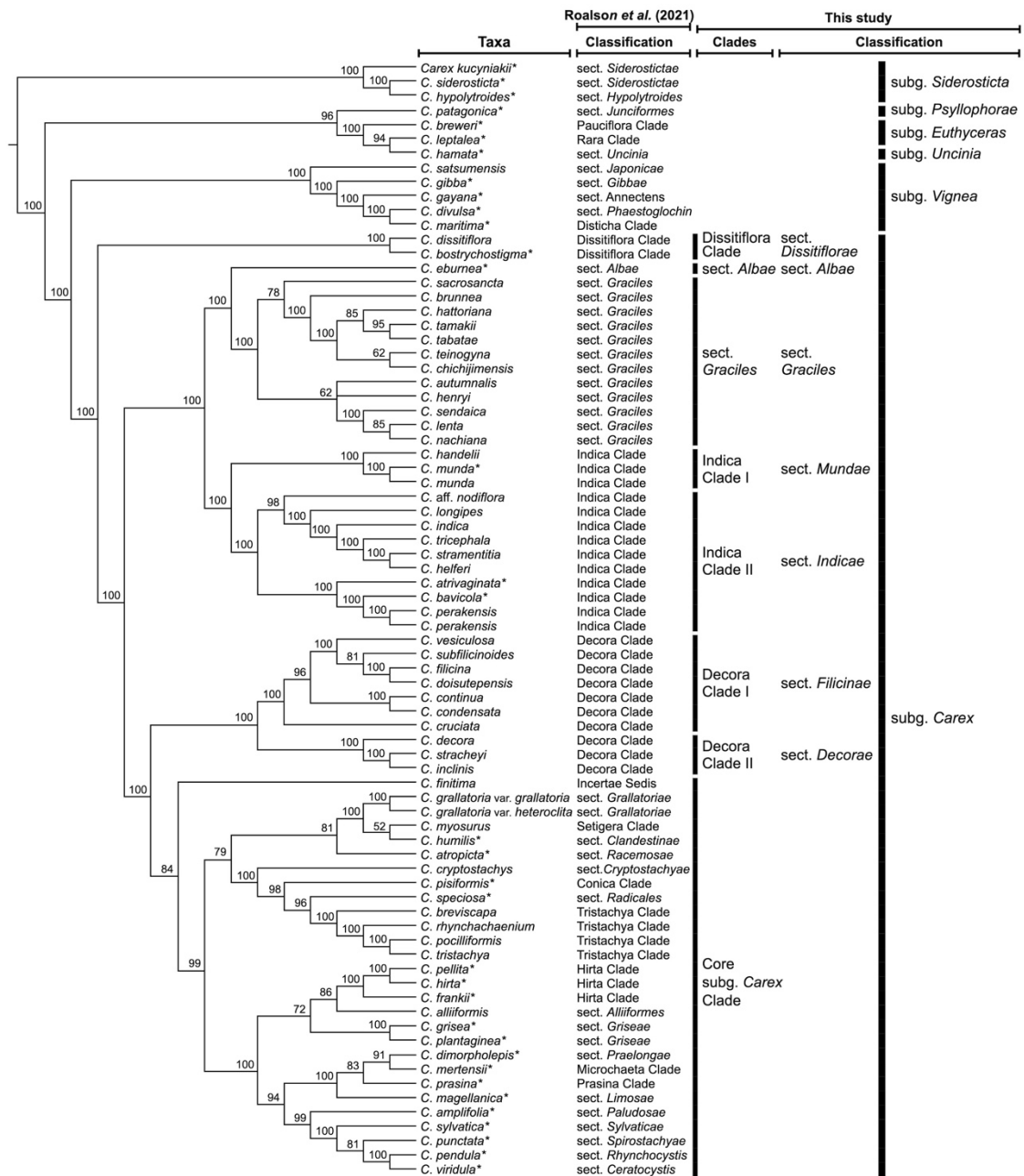


Fig. 3. Phylogenetic reconstruction of *Carex* based on the chloroplast CDS sequences (78 species, 70 loci, 58,601bp) under the concatenation approach (ML analysis performed with RAXML-NG). Numbers above branches indicate bootstrap values (%). Sequence data of taxa with "*" were quoted from NCBI. *Carex* subg. *Siderosticta* was used as an outgroup for this analysis.



Fig. 4. Perigynia on lateral spikes. **A**, *Carex handelii* (Ikeda, H. et al. 1414013, OKAY); **B**, *C. munda* (Ikeda, H. et al. 1414006, OKAY). Bars = 1 mm.

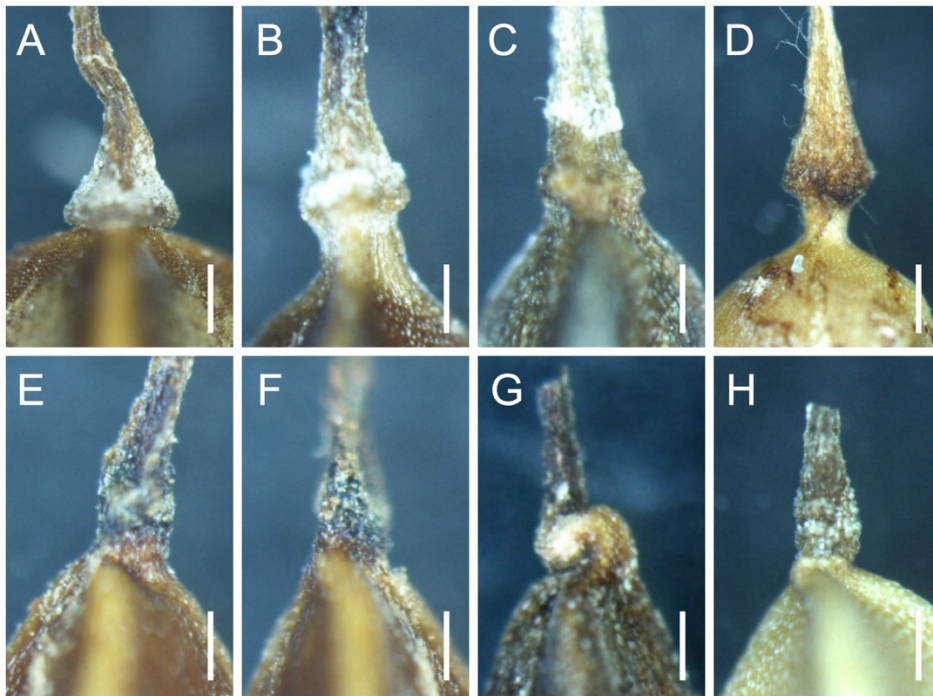


Fig. 5. Upper part of achenes and basal part of styles. **A**, *Carex tricephala* (Tamura, M. N. et al. 68843, KYO); **B**, *C. aff. nodiflora* (Tamura, M. N. et al. 69916, KYO); **C**, *C. indica* (Tamura, M. N. et al. 68842, KYO); **D**, *C. longipes* (Ikeda, H. et al. 1225006, OKAY); **E**, *C. filicina* (Tamura, M. N. et al. 68509, KYO); **F**, *C. continua* (Tamura, M. N. et al. 68526, KYO); **G**, *C. condensata* (Tamura, M. N. et al. 68542, KYO); **H**, *C. cruciata* (Tamura, M. N. et al. 68508, KYO). Bars = 400 µm.

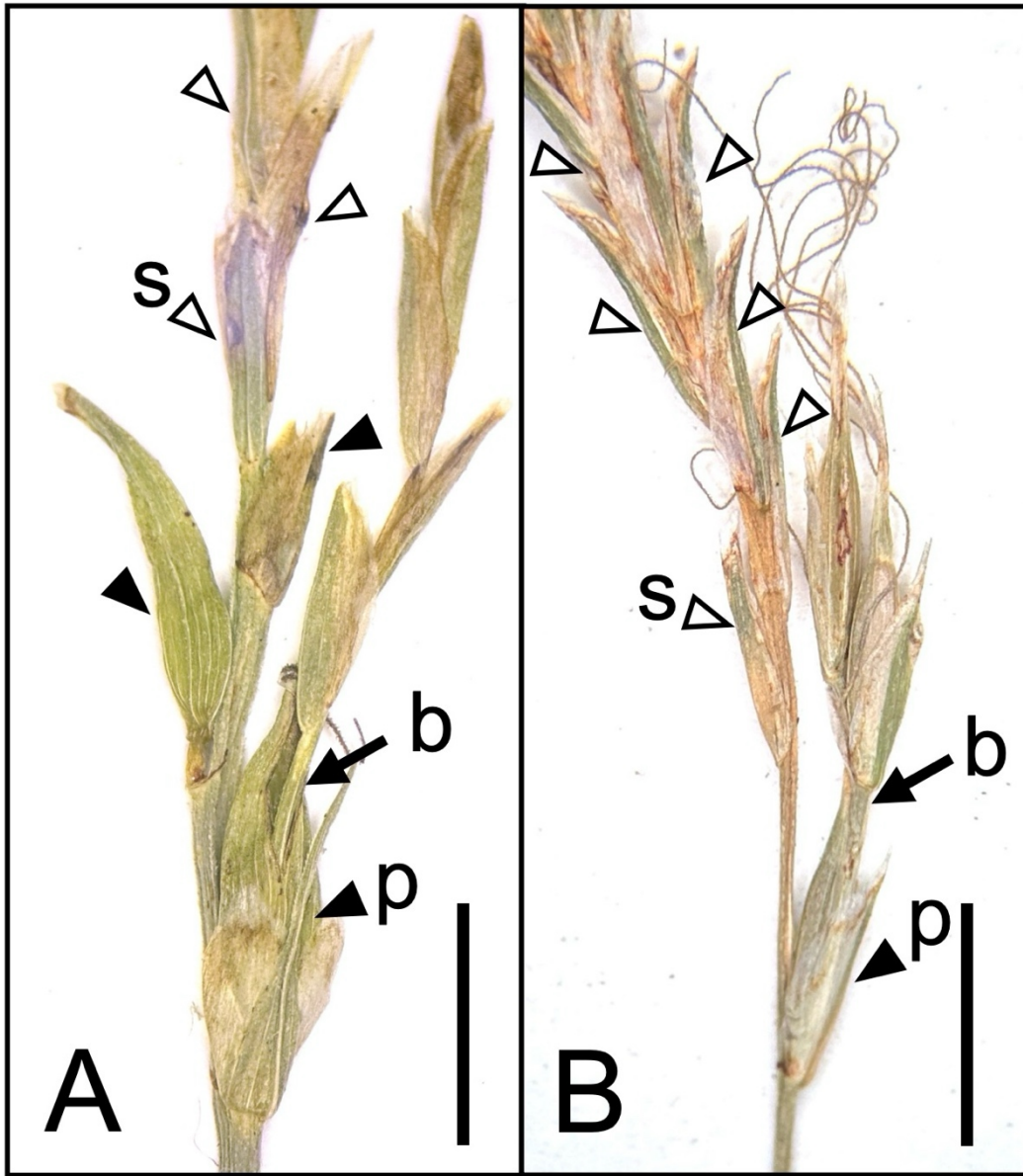


FIG. 6. Terminal spike with a lateral branch. **A**, *Carex dissitiflora* (Takahashi, K.T. & S. Takahashi KT2011, KYO); **B**, *C. bostrychostigma* (Fukuoka, N. & Y. Inamasu, KYO). b (black arrow), lateral branch emerging from a fertile perigynium; p (black arrowhead), pistillate flower; s (white arrowhead), staminate flower. Bars = 4 mm.

Appendix I. Sources of materials of *Carex* subg. *Carex*, used for the present morphological data with the information of classification and phylogenetic position. “-” in “Phylogenetic position”

Species	Classification		Phylogenetic position		Specimens examined morphologically	Literatures used for morphology
	Section of the present study	Section of Dai et al. (2010)	Present study	Roalson et al. (2021)		
<i>C. bostrychostigma</i> Maxim.	<i>Dissitiflorae</i>	<i>Debiles</i>	Dissitiflora Clade	Dissitiflora Clade	<i>Fukuoka, N. & Y. Inamasu</i> (KYO!), <i>Takahashi, K.T. et al.</i> 2012 (KYO!)	Akiyama (1955)
<i>Carex dissitiflora</i> Franch.	<i>Dissitiflorae</i>	<i>Mundae</i>	Dissitiflora Clade	Dissitiflora Clade	<i>Takahashi, K.T. & S. Takahashi</i> 2011 (KYO!)	Hoshino et al. (2021)
<i>C. alba</i> Scop.	<i>Albae</i>	<i>Albae</i>	-	sect. <i>Albae</i>	<i>Townsend, C.C. s.n.</i> (K, digital image!)	Dai et al. (2010)
<i>C. eburnea</i> Boott	<i>Albae</i>	<i>Albae</i>	sect. <i>Albae</i>	sect. <i>Albae</i>	<i>Drummond, T. s.n.</i> (iso- GH, digital image!)	Kükenthal (1909)
<i>C. mckittrickensis</i> P.W. Ball	<i>Albae</i>	<i>Albae</i>	-	-	<i>Correll, D.S. 19187</i> (iso- NY, digital image!)	Ball (1998)
<i>C. ussuriensis</i> Kom.	<i>Albae</i>	<i>Albae</i>	-	-	<i>Komarov, V.L. s.n.</i> (syn- LE, digital image!)	Dai et al. (2010)
<i>C. autumnalis</i> Ohwi	<i>Graciles</i>	<i>Graciles</i>	sect. <i>Graciles</i>	-	<i>Tashiro, Z. s.n.</i> (holo- KYO!), <i>Takahashi, H. & H. Katayama s.n.</i> (OKAY-28218!)	Dai et al. (2010)
<i>C. bilateralis</i> Hayata	<i>Graciles</i>	<i>Graciles</i>	-	-	<i>Nakahara, G. s.n.</i> (holo- TI!)	Dai et al. (2010)
<i>C. bodinieri</i> Franch.	<i>Graciles</i>	<i>Graciles</i>	-	-	<i>Bodinier, E. 1334</i> (holo- P, digital image!)	Dai et al. (2010)
<i>C. brunnea</i> Murray	<i>Graciles</i>	<i>Graciles</i>	sect. <i>Graciles</i>	sect. <i>Graciles</i>	<i>Takahashi, K.T. 2043</i> (KYO!)	Dai et al. (2010)
<i>C. chichijimensis</i> Katsuy.	<i>Graciles</i>	<i>Graciles</i>	sect. <i>Graciles</i>	-	<i>Katsuyama, T. et al. s.n.</i> (iso- TI! & OKAY-19868!)	Hoshino et al. (2021)
<i>C. chuii</i> Nelves	<i>Graciles</i>	<i>Graciles</i>	-	-	<i>Chu, K.L. 3032</i> (holo- K, digital image!)	Dai et al. (2010)
<i>C. ereica</i> Tang & F.T. Wang ex L.K. Dai	<i>Graciles</i>	<i>Graciles</i>	-	-	<i>Chung, K. & R. Li 55-4</i> (MOR, digital image!)	Dai et al. (2010)
<i>C. gentilis</i> Franch.	<i>Graciles</i>	<i>Graciles</i>	-	sect. <i>Graciles</i>	<i>Delavay, J.M. s.n.</i> (-, digital image!)	Dai et al. (2010)
<i>C. gongshanensis</i> Tang & F.T. Wang ex Y.C. Yang	<i>Graciles</i>	<i>Graciles</i>	-	sect. <i>Graciles</i>	<i>Gaoligong Shan Biotic Survey Expedition 34274</i> (CAS, digital image!)	Dai et al. (2010)
<i>C. hattoriana</i> Nakai ex Tuyama	<i>Graciles</i>	<i>Graciles</i>	sect. <i>Graciles</i>	-	<i>Katsuyama, T. s.n.</i> (OKAY-19913!)	Dai et al. (2010)
<i>C. henryi</i> (C.B. Clarke) T. Koyama	<i>Graciles</i>	<i>Graciles</i>	-	sect. <i>Graciles</i>	<i>Ikeda, H. et al. 20814116</i> (TI!)	Dai et al. (2010)
<i>C. lenta</i> D. Don	<i>Graciles</i>	<i>Graciles</i>	sect. <i>Graciles</i>	sect. <i>Graciles</i>	<i>Takahashi, K.T. 2005</i> (KYO!)	Hoshino et al. (2021)
<i>C. lushanensis</i> Kük.	<i>Graciles</i>	<i>Graciles</i>	-	-	<i>Smith, H. 1755</i> (iso- GB, digital image!)	Dai et al. (2010)
<i>C. mosoyensis</i> Franch.	<i>Graciles</i>	<i>Graciles</i>	-	sect. <i>Graciles</i>	<i>Delavay, J.M. s.n.</i> (syn- P, digital image!)	Dai et al. (2010)
<i>C. nachiana</i> Ohwi	<i>Graciles</i>	<i>Graciles</i>	sect. <i>Graciles</i>	-	<i>Ohwi, J. & M. Tagawa 101</i> (holo- KYO!), <i>Yoshino, Y. et al. s.n.</i> (OKAY-23167!)	Dai et al. (2010)
<i>C. omeiensis</i> Tang	<i>Graciles</i>	<i>Graciles</i>	-	-	<i>Wang, F.T. 23391.</i> (GH, digital image!)	Dai et al. (2010)
<i>C. orbicularinucis</i> L.K. Dai	<i>Graciles</i>	<i>Graciles</i>	-	-	<i>Dai, L.K. 50894</i> (holo- PE, digital image!)	Dai et al. (2010)
<i>C. pergracilis</i> Nelves	<i>Graciles</i>	<i>Graciles</i>	-	-	<i>Chu, K.L. 3435</i> (syn- K, digital image!)	Dai et al. (2010)
<i>C. sacrosancta</i> Honda	<i>Graciles</i>	<i>Graciles</i>	sect. <i>Graciles</i>	-	<i>Katsuyama, T. s.n.</i> (OKAY-21660!)	Hoshino et al. (2021)
<i>C. sendaica</i> Franch.	<i>Graciles</i>	<i>Graciles</i>	sect. <i>Graciles</i>	-	<i>Naito, U. s.n.</i> (OKAY-23878!)	Dai et al. (2010)
<i>C. sinodissitiflora</i> Tang & F.T. Wang ex L.K. Dai	<i>Graciles</i>	<i>Graciles</i>	-	-	<i>Chung, K. & R.Li 10-4</i> (MOR, digital image!)	Dai et al. (2010)
<i>C. stipitimus</i> C.B. Clarke ex Franch.	<i>Graciles</i>	<i>Graciles</i>	-	-	<i>Li, Z.Y. et al. 1687</i> (US, digital image!)	Dai et al. (2010)
<i>C. tabatae</i> Katsuy.	<i>Graciles</i>	<i>Graciles</i>	sect. <i>Graciles</i>	-	<i>Katsuyama T. & M. Tabata s.n.</i> (iso- KYO!), <i>Katsuyama, T. s.n.</i> (OKAY-23380!)	Hoshino et al. (2021)
<i>C. tamakii</i> T. Koyama	<i>Graciles</i>	<i>Graciles</i>	sect. <i>Graciles</i>	-	<i>Koyama, T.M. 7437</i> (holo- NY, digital image!), <i>Yano, O. & M. Amano s.n.</i> (OKAY!)	Hoshino et al. (2021)
<i>C. teinogyna</i> Boott	<i>Graciles</i>	<i>Graciles</i>	sect. <i>Graciles</i>	sect. <i>Graciles</i>	<i>Hooker, J.D. & T. Thomson s.n.</i> (iso- P, digital image!), <i>Katayama, H. 50793</i> (OKAY!)	Hoshino et al. (2021)
<i>C. barbayaki</i> Jim. Mejías & Roalson	<i>Mundae</i>	<i>Decorae</i>	-	Indica Clade	<i>Alpine Garden Society China Expedition 554</i> (holo-E, digital image!)	Jiménez-Mejías & Roalson (2017)
<i>C. handelii</i> Kük.	<i>Mundae</i>	<i>Chlorostachyae</i>	Indica Clade I	Indica Clade	<i>Handel-Mazzetti, H.R.E. von 8972</i> (holo- WU, digital image!), <i>Smith, H. 2149</i> (iso- GB, digital image!)	Dai et al. (2010)
<i>C. harrismithii</i> Kük.	<i>Mundae</i>	<i>Molliculae</i>	-	-	<i>Smith, H. 2149</i> (iso- GB, digital image!)	Kükenthal (1929)
<i>C. munda</i> Boott	<i>Mundae</i>	<i>Mundae</i>	Indica Clade I	Indica Clade	<i>Ikeda, H. et al. 1414006</i> (OKAY!)	Dai et al. (2010)
<i>C. atrivaginata</i> Nelves	<i>Indicae</i>	<i>Euprepes</i>	Indica Clade II	Indica Clade	<i>Poilane, E. 27084</i> (holo- MT, digital image!)	Nelmes (1955)
<i>C. balansae</i> Franch.	<i>Indicae</i>	<i>Indicae</i>	Indica Clade II	Indica Clade	<i>Balansa, B. 2828.</i> (holo- P, digital image!)	Kükenthal (1909)
<i>C. baviicola</i> Raymond	<i>Indicae</i>	<i>Mapanifoliae</i>	Indica Clade II	Indica Clade	<i>Petelot, P. A. 8024</i> (holo- MT, digital image!)	Raymond (1959)
<i>C. callista</i> Nelves	<i>Indicae</i>	<i>Indicae</i>	-	-	<i>Maung B.P. 12857</i> (syn- K, digital image!)	Nelmes (1955)
<i>C. cataphyllodes</i> Nelves	<i>Indicae</i>	<i>Indicae</i>	-	-	<i>Kerr, A.F.G. 10371</i> (holo- K, digital image!)	Simpson & Koyama (1998)
<i>C. cirrhulosa</i> Nees	<i>Indicae</i>	<i>Scabrellae</i>	-	-	<i>Cuming, H. 1764</i> (iso- K, digital image!)	Nelmes (1951)
<i>C. cochinchinensis</i> Raymond	<i>Indicae</i>	<i>Mapanifoliae</i>	-	-	<i>Fleury, F. 29884</i> (holo- P, digital image!)	Raymond (1959)
<i>C. commixta</i> Steud.	<i>Indicae</i>	<i>Indicae</i>	-	-	<i>Zollinger, H. 1502</i> (holo- P, digital image!)	Kükenthal (1909)
<i>C. deciduisquama</i> F.T. Wang & Tang ex P.C. Li	<i>Indicae</i>	<i>Indicae</i>	-	-	<i>Zhu, D.-H. et al.</i> (MO, digital image!)	Dai et al. (2010)
<i>C. distracta</i> C.B. Clarke	<i>Indicae</i>	<i>Indicae</i>	-	-	<i>s.c. s.n.</i> (iso- K, digital image!); <i>Masters J.W. s.n.</i> (iso- L, digital image!); <i>Kerr, A.F.G. 20927</i> (holo- K, digital image!)	Kükenthal (1909)
<i>C. euprepes</i> Nelves	<i>Indicae</i>	<i>Euprepes</i>	-	Indica Clade	<i>Kloss, C.B. 7012</i> (holo- K, digital image!), <i>Tamura, M.N. 9230</i> (KYO!)	Nelmes (1955)
<i>C. helferi</i> Boeckeler	<i>Indicae</i>	<i>Mapanifoliae</i>	Indica Clade II	-		Nelmes (1955)

Appendix I. (Continued)

Species	Classification		Phylogenetic position		Specimens examined morphologically	Literatures used for morph
	Section of the present study	Section of Dai et al. (2010)	Present study	Roalson et al. (2021)		
<i>C. horsfieldii</i> Nelm	<i>Indicae</i>	<i>Indicae</i>	-	-	<i>Balansa, B. 2819</i> (holo– P, digital image!)	Kükenthal (1909)
<i>C. indica</i> L.	<i>Indicae</i>	<i>Indicae</i>	Indica Clade II	Indica Clade	<i>Dietrich, A. 653</i> (isolect– MEL, digital image!), <i>Tamura, M.N. et al. 68842</i> (KYO!)	Kükenthal (1909)
<i>C. indiciformis</i> F.T. Wang & Tang ex P.C. Li	<i>Indicae</i>	<i>Indicae</i>	-	-	<i>Lau, S.K. 6219</i> (iso– A, digital image!)	Dai et al. (2010)
<i>C. jeanpertii</i> E.G. Camus	<i>Indicae</i>	<i>Indicae</i>	-	-	<i>Bon, abbé 5361</i> (iso– MT, digital image!)	Nelmes (1955)
<i>C. laosensis</i> Nelm	<i>Indicae</i>	<i>Euprepes</i>	-	-	<i>Kerr, A.F.G. 21202</i> (holo– K, digital image!)	Nelmes (1955)
<i>C. longipes</i> D.Don	<i>Indicae</i>	<i>Graciles</i>	Indica Clade II	-	<i>Clarke, C.B. s.n.</i> (syn– K, digital image!), <i>Ikeda, H. et al. 1215026</i> (OKAY!)	Dai et al. (2010)
<i>C. malaccensis</i> C.B. Clarke	<i>Indicae</i>	<i>Scabrellae</i>	-	sect. <i>Rhomboidales</i>	<i>Curtis, C. 1669</i> (holo– K, digital image!), <i>Burt, B.L. & P.J.B. Woods 1780</i> (E, digital image!)	Nelmes (1951)
<i>C. meeboldiana</i> Kük.	<i>Indicae</i>	<i>Mapanifoliae</i>	-	-	<i>Wichian 591</i> (syn– P, digital image!)	Nelmes (1955)
<i>C. nodiflora</i> Boeckeler	<i>Indicae</i>	<i>Scabrellae</i>	-	Indica Clade	<i>Hugh Cumming 1408</i> (iso– BOT, digital image!)	Nelmes (1951)
<i>C. aff. nodiflora</i>	<i>Indicae</i>	<i>Scabrellae</i>	Indica Clade II	-	<i>Tamura, M.N. et al. 69917</i> (KYO!)	Personal observation
<i>C. pandanophylla</i> C.B. Clarke	<i>Indicae</i>	<i>Mapanifoliae</i>	-	-	<i>Kurz, W.S. 2704</i> (K, digital image!)	Nelmes (1955)
<i>C. perakensis</i> C.B. Clarke	<i>Indicae</i>	<i>Decorae</i>	Indica Clade II	Indica Clade	<i>Tamura, M.N. et al. 68816, 68842</i> (KYO!)	Simpson & Koyama (1998)
<i>C. poilanei</i> Raymond	<i>Indicae</i>	<i>Euprepes</i>	-	-	<i>Poilane, E. 25984</i> (holo– MT, digital image!)	Raymond (1959)
<i>C. repanda</i> C.B. Clarke	<i>Indicae</i>	<i>Scabrellae</i>	-	-	<i>Henry, A. 9971A</i> (K, digital image!)	Nelmes (1951)
<i>C. stramentitia</i> Boott ex Boeckeler	<i>Indicae</i>	<i>Indicae</i>	Indica Clade II	Indica Clade	<i>Hooker, J.D. 6056</i> (iso– P, digital image!), <i>Tamura, M.N. et al. 69935</i> (KYO!)	Simpson & Koyama (1998)
<i>C. taprobanensis</i> T. Koyama	<i>Indicae</i>	<i>Indicae</i>	-	-	<i>Jayasuriya, A.H.M. 2768</i> (para– NY, digital image!)	Koyama (1980)
<i>C. tavoyensis</i> Nelm	<i>Indicae</i>	<i>Euprepes</i>	-	-	<i>Russell, P.T. 1935</i> (holo– K, digital image!)	Nelmes (1955)
<i>C. thailandica</i> T. Koyama	<i>Indicae</i>	<i>Indicae</i>	-	-	<i>Smitinand, T. 7196</i> (iso– BKF!)	Simpson & Koyama (1998)
<i>C. tricephala</i> Boeckeler	<i>Indicae</i>	<i>Scabrellae</i>	Indica Clade II	Indica Clade	<i>Boeckeler, J.O. 1778</i> (syn– L, digital image!), <i>Tamura, M.N. et al. 68843</i> (KYO!)	Kükenthal (1909)
<i>C. trichophylla</i> Nelm	<i>Indicae</i>	<i>Euprepes</i>	-	-	<i>Pételot, P.A. s.n.</i> (holo– P, digital image!)	Nelmes (1955)
<i>C. vietnamica</i> Raymond	<i>Indicae</i>	<i>Mapanifoliae</i>	-	-	-	Raymond (1959)
<i>C. wightiana</i> Nees	<i>Indicae</i>	<i>Scabrellae</i>	-	-	<i>Hooker, J.D. & T. Thomson</i> , (K, digital image!)	Kükenthal (1909)
<i>C. zizanifolia</i> Raymond	<i>Indicae</i>	<i>Euprepes</i>	-	-	<i>Tsai, H.T. 62809</i> (holo– A, digital image!)	Dai et al. (2010)
<i>C. alboboviridis</i> C.B. Clarke	<i>Filicinae</i>	<i>Indicae</i>	-	-	<i>Elliot, G.F. Scott 2534</i> (holo– K, digital image!)	Kükenthal (1909)
<i>C. amicta</i> Boott	<i>Filicinae</i>	<i>Scabrellae</i>	-	-	<i>Purdie s.n.</i> (K, digital image!)	Kükenthal (1909)
<i>C. andringitrensis</i> Cherm.	<i>Filicinae</i>	<i>Indicae</i>	-	Decora Clade	<i>Perrier de la Bâthie, H. 14429</i> (syn– P, digital image!)	Kükenthal (1909)
<i>C. angolensis</i> Nelm	<i>Filicinae</i>	<i>Indicae</i>	-	Decora Clade	<i>Milne-Redhead, E. 4222</i> (syn– K, digital image!)	Nelmes (1940)
<i>C. azteca</i> Mack.	<i>Filicinae</i>	<i>Indicae</i>	-	Decora Clade	<i>Pringle, C.G. 4839</i> (holo– MT, digital image!)	Mackenzie (1931–1935)
<i>C. bathiei</i> H. Lév	<i>Filicinae</i>	<i>Indicae</i>	-	Decora Clade	<i>Perrier de la Bâthie, H. 2615</i> (syn– P, digital image!)	Humbert & Leroy (1936)
<i>C. beckii</i> G.A. Wheeler	<i>Filicinae</i>	<i>Indicae</i>	-	-	<i>Beck, St.G. 17524</i> (holo– MIN, digital image!)	Wheeler (2002)
<i>C. brassii</i> Nelm	<i>Filicinae</i>	<i>Indicae</i>	-	-	<i>Brass, L.J. 16714</i> (iso– BM, digital image!)	Nelmes (1954)
<i>C. burttii</i> Noltie	<i>Filicinae</i>	<i>Indicae</i>	-	-	<i>Lacaita, C.C. 27/30.A</i> (holo– BM, digital image!)	Dai et al. (2010)
<i>C. castanostachya</i> K.Schum. ex Kük.	<i>Filicinae</i>	<i>Indicae</i>	-	-	<i>Richards, H.M. 7834</i> (NY, digital image!)	Kükenthal (1909)
<i>C. ceylanica</i> Boeckeler	<i>Filicinae</i>	<i>Indicae</i>	-	-	<i>Davidse, G. 7633</i> (MO, digital image!)	Kükenthal (1909)
<i>C. chlorosaccus</i> C.B. Clarke	<i>Filicinae</i>	<i>Indicae</i>	-	Decora Clade	<i>Ballings P. & B. Würsten</i> (BR, digital image!)	Kükenthal (1909)
<i>C. condensata</i> Nees	<i>Filicinae</i>	<i>Indicae</i>	Decora Clade I	-	<i>Griffith, W. s.n.</i> (syn– K, digital image!), <i>Tamura, M.N. et al. 68542</i> (KYO!)	Nelmes (1955)
<i>C. continua</i> C.B. Clarke	<i>Filicinae</i>	<i>Indicae</i>	Decora Clade I	Decora Clade	<i>Suzuki M. et al. 9485101</i> (L, digital image!), <i>Tamura, M.N. et al. 68526</i> (KYO!)	Kükenthal (1909)
<i>C. cruciata</i> Wahlenb.	<i>Filicinae</i>	<i>Indicae</i>	Decora Clade I	Decora Clade	<i>Tamura, M.N. et al. 68508</i> (KYO!)	Kükenthal (1909)
<i>C. cubensis</i> Kük.	<i>Filicinae</i>	<i>Indicae</i>	-	-	<i>Ekman, E.L. 11449</i> (iso– NY, digital image!)	Kükenthal (1926)
<i>C. doisutepensis</i> T. Koyama	<i>Filicinae</i>	<i>Indicae</i>	Decora Clade I	Decora Clade	<i>Simpson, D.A. & J.A.N. Parnell 1766</i> (K, digital image!), <i>Tamura, M.N. et al. 68839</i> (KYO!)	Dai et al. (2010)
<i>C. echinoclhoe</i> Kunze	<i>Filicinae</i>	<i>Indicae</i>	-	Decora Clade	<i>Schimper, W. n. 26</i> (iso– M, digital image!)	Kükenthal (1909)
<i>C. ekmanii</i> Kük.	<i>Filicinae</i>	<i>Indicae</i>	-	-	<i>Ekman, E.L. H1453</i> (holo– NY, iso- GH digital image!),	Kükenthal (1926)
<i>C. elatior</i> Boeckeler	<i>Filicinae</i>	<i>Indicae</i>	-	Indica Clade	<i>Perrier de la bâthie, H. 2558</i> (P, digital image!), <i>Razafindraibe, H. & P. Antilahimena 83</i> (P, digital image!)	Kükenthal (1909)
<i>C. euryphylla</i> Cherm.	<i>Filicinae</i>	<i>Indicae</i>	-	-	<i>Perrier de la Bâthie, H. 2528</i> (syn– P, digital image!)	Chermeson et al. (1937)
<i>C. filicina</i> Nees	<i>Filicinae</i>	<i>Indicae</i>	Decora Clade I	Decora Clade	<i>Haviland, G.D. 1402</i> (holo– P, digital image!), <i>Tamura, M.N. et al. 68509</i> (KYO!)	Dai et al. (2010)
<i>C. graminifolia</i> Cherm.	<i>Filicinae</i>	<i>Indicae</i>	-	Decora Clade	<i>Perrier de la Bâthie, H. 2708</i> (holo– P, digital image!)	Chermeson et al. (1937)

Appendix I. (Continued)

Species	Classification		Phylogenetic position		Specimens examined morphologically	Literatures used for morphology
	Section of the present study	Section of Dai <i>et al.</i> (2010)	Present study	Roalson <i>et al.</i> (2021)		
<i>C. guffroyi</i> H. Lév. & H. Perrier	Filicinae	Indicae	-	-	<i>Perrier de la Bâthie, H. 2634</i> (holo– P, digital image!)	Léveillé (1918)
<i>C. haematosaccus</i> C.B. Clarke	Filicinae	Indicae	-	-	<i>Perrier de la Bâthie, H. 6337</i> (isosyn– MO, digital image!)	Kükenthal (1909)
<i>C. heterodoxa</i> Cherm.	Filicinae	Indicae	-	Decora Clade	<i>Perrier de la Bâthie, H. 2634</i> (holo– P, digital image!)	Chermezon <i>et al.</i> (1937)
<i>C. hildebrandtiana</i> Boeckeler	Filicinae	Indicae	-	-	<i>Hildebrandt, J.M. 4014</i> (iso– P, digital image!)	Kükenthal (1909)
<i>C. hirtigluma</i> C.B. Clarke	Filicinae	Indicae	-	Decora Clade	<i>s.c. s.n.</i> (holo– P, digital image!)	Kükenthal (1909)
<i>C. hultenii</i> Aspl.	Filicinae	Indicae	-	Decora Clade	<i>Fröderström, H. & E. Hultén s.n.</i> (iso– MICH, digital image!)	Asplund (1954)
<i>C. humboldtiana</i> Steud.	Filicinae	Indicae	-	Decora Clade	<i>Pringle, C.G. 4840</i> (iso– VT, digital image!)	Mackenzie (1931–1935)
<i>C. humpatensis</i> H.E. Hess	Filicinae	Indicae	-	-	<i>Hess, H.E. 52/1706</i> (holo, iso– ZT, digital image!)	Hess (1953)
<i>C. lamprochlamys</i> S.T.Blake	Filicinae	Indicae	-	-	<i>Brass, L.J. 5323</i> (iso– A, digital image!)	Nelmes (1951)
<i>C. leptocladus</i> C.B. Clarke	Filicinae	Indicae	-	-	<i>Mann, G. s.n.</i> (K, digital image!)	Kükenthal (1909)
<i>C. lindleyana</i> Nees	Filicinae	Indicae	-	-	<i>Wight, R. s.n.</i> (syn– K, digital image!)	Kükenthal (1909)
<i>C. macrophyllidion</i> Nelmes	Filicinae	Indicae	-	Decora Clade	<i>Milne-Redhead, E. 3971</i> (syn– K, digital image!), <i>Biggood, S. et al. 5333</i> (P, digital image!)	Nelmes (1940)
<i>C. madrensis</i> L.H. Bailey	Filicinae	Indicae	-	Decora Clade	<i>Rose, J.N. 2357</i> (iso– NY, digital image!)	Mackenzie (1931–1935)
<i>C. merxmülleri</i> Podlech	Filicinae	Indicae	-	-	<i>Merxmüller, H. 553</i> (holo– M, digital image!)	Podlech (1961)
<i>C. neochevalieri</i> Kük. ex A. Chev.	Filicinae	Indicae	-	Decora Clade	<i>Chevalier, A.J.B. 18776</i> (iso– P, digital image!)	Chevalier (1931)
<i>C. neoguineensis</i> C.B. Clarke	Filicinae	Indicae	-	-	<i>Rainal, J. 17155</i> (P, digital image!)	Kükenthal (1909)
<i>C. neohebridensis</i> Guillaumin & Kük.	Filicinae	Indicae	-	-	<i>Aubert de la Rüe, E. s.n.</i> (holo– P, digital image!)	Guillaumin (1937)
<i>C. novogaliciana</i> Reznicek	Filicinae	Indicae	-	-	<i>González, R.T. 579</i> (holo– MICH, digital image!)	Reznicek (1990)
<i>C. parvigluma</i> C.B. Clarke	Filicinae	Indicae	-	-	<i>Royle, J.F. 37920</i> (K, digital image!)	Kükenthal (1909)
<i>C. polystachya</i> Sw. ex Wahlenb.	Filicinae	Indicae	-	Decora Clade	<i>Hartweg, K.T. 628</i> (iso– BM, digital image!)	Kükenthal (1909)
<i>C. porrecta</i> Reznicek & Camelb.	Filicinae	Indicae	-	-	<i>Lægaard, S. & S. A. Renvoize 70823</i> (iso– US, digital image!)	Camelbeke & Reznicek (1996)
<i>C. proxima</i> Cherm.	Filicinae	Indicae	-	Decora Clade	<i>Perrier de la Bâthie, H. 12600</i> (syn– K, digital image!)	Chermezon <i>et al.</i> (1937)
<i>C. pyramidalis</i> Kük.	Filicinae	Indicae	-	Decora Clade	<i>Hildebrandt, J. M. 3745</i> (iso– P, digital image!)	Kükenthal (1909)
<i>C. rafflesiana</i> Boott	Filicinae	Indicae	-	-	<i>Horsfield, T. s.n.</i> (holo– K, digital image!)	Kükenthal (1909)
<i>C. ramosa</i> Wild.	Filicinae	Indicae	-	Decora Clade	<i>Hoffmann, M.H. 13/327(V, digital image!)</i>	Kükenthal (1909)
<i>C. raphidocarpa</i> Nees	Filicinae	Indicae	-	-	<i>Hohenacker, R.F. 943</i> (syn– K, digital image!)	Kükenthal (1909)
<i>C. resciana</i> Boeckeler	Filicinae	Indicae	-	Decora Clade	<i>Hildebrandt, J.M. 3752</i> (iso– P, digital image!)	Kükenthal (1909)
<i>C. rutenbergiana</i> Cherm.	Filicinae	Indicae	-	Decora Clade	<i>Perrier de la Bâthie, H. 13002</i> (syn– P, digital image!)	Kükenthal (1909)
<i>C. sambiranensis</i> (H. Lév.) Cherm.	Filicinae	Indicae	-	-	<i>Perrier de la Bâthie, H. 2648</i> (holo– P, digital image!)	Chermezon <i>et al.</i> (1937)
<i>C. sanguinea</i> Boott	Filicinae	Indicae	-	-	<i>Griffith, W. 6094</i> (syn– P, digital image!)	Kukkonen (2001)
<i>C. sarawaketensis</i> Kük.	Filicinae	Indicae	-	-	<i>Brass, L. J. & E. Meijer-Drees 9828</i> (type– NL, digital image!)	Blake (1947)
<i>C. scabrella</i> Wahlenb.	Filicinae	Scabrellae	-	-	<i>Eggers, H.F.A. 2650</i> (iso– CORD, digital image!), <i>Thomas, H. et al. 993</i> (FLAS, digital image!)	Kükenthal (1909)
<i>C. scabripes</i> Cherm.	Filicinae	Indicae	-	-	<i>Perrier de la Bâthie, H. 13390</i> (holo– P, digital image!)	Chermezon <i>et al.</i> (1937)
<i>C. schliebenii</i> Podlech	Filicinae	Indicae	-	-	<i>Schlieben, H. J. 598</i> (iso– Z, digital image!)	Podlech (1961)
<i>C. sodiroi</i> Kük.	Filicinae	Indicae	-	-	<i>Sodiro s.n.</i> (iso– P, digital image!)	Kükenthal (1909)
<i>C. spicatopaniculata</i> Boeckeler ex C.B. Clarke	Filicinae	Indicae	-	-	<i>Schlieben, H. J. 598</i> (iso– M, digital image!)	Kükenthal (1909)
<i>C. spicigera</i> Nees	Filicinae	Scabrellae	-	-	<i>Wight, R. 1299</i> (K, digital image!), <i>Thwaites, G.H.K. 822</i> (P, digital image!)	Kükenthal (1909)
<i>C. standleyana</i> Steyer.	Filicinae	Indicae	-	Decora Clade	<i>Williams, L.O. 13178</i> (holo– F, digital image!)	Steyermark (1952)
<i>C. steudneri</i> Boeckeler	Filicinae	Indicae	-	-	<i>Schimper, W. 545</i> (iso– K, digital image!)	Kükenthal (1909)
<i>C. subfilicinoides</i> Kük.	Filicinae	Indicae	Decora Clade I	Decora Clade	<i>Smith, H. 2401</i> (iso– GB, digital image!), <i>Ikedo, H. et al. 1414095</i> (OKAY!)	Dai <i>et al.</i> (2010)
<i>C. subscabrella</i> Kük.	Filicinae	Scabrellae	-	-	-	Urban (1929)
<i>C. tenuipaniculata</i> P.C. Li	Filicinae	Indicae	-	-	<i>Liou, T.N. 14237</i> (holo– PE, digital image!)	Dai <i>et al.</i> (2010)
<i>C. tricholepis</i> Nelmes	Filicinae	Indicae	-	-	<i>Hess, H.E. 52/1120</i> (syn– ZT, digital image!)	Nelmes (1940)
<i>C. tsatananensis</i> Cherm.	Filicinae	Indicae	-	Decora Clade	<i>Perrier de la Bâthie, H. 16397</i> (holo– P, digital image!)	Chermezon <i>et al.</i> (1937)
<i>C. valbrayi</i> H. Lév.	Filicinae	Indicae	-	Decora Clade	<i>Perrier de la Bâthie, H. 2578</i> (syn– P, digital image!)	Chermezon <i>et al.</i> (1937)

Appendix I. (Continued)

Species	Classification		Phylogenetic position		Specimens examined morphologically	Literatures used for morphology
	Section of the present study	Section of Dai <i>et al.</i> (2010)	Present study	Roalson <i>et al.</i> (2021)		
<i>C. vesiculosa</i> Boott	Filicinae	Indicae	-	Decora Clade	Hooker, J.D. & T. Thomson s.n. (P, digital image!), Ikeda, H. <i>et al.</i> 20814118 (TI!)	Kükenthal (1909)
<i>C. wahlenbergiana</i> Boott	Filicinae	Indicae	-	Decora Clade	Boivin, L.H. 994 (syn- P, digital image!)	Kükenthal (1909)
<i>C. zuluensis</i> C.B. Clarke	Filicinae	Indicae	-	Decora Clade	Baur, R. 1156 (lecto- K, digital image!)	Clarke (1908)
<i>C. anomoea</i> Hand.-Mazz.	Decorae	Decorae	-	-	Hooker, J.D. s.n. (holo- P, digital image!)	Dai <i>et al.</i> (2010)
<i>C. baiposhanensis</i> P.C. Li	Decorae	Decorae	-	-	Qinghai-Tibet Expedition 11795 (holo- PE, digital image!)	Dai <i>et al.</i> (2010)
<i>C. celebica</i> Kük.	Decorae	Decorae	-	-	Kjellberg, G. 3730 (holo- BO, digital image!)	Nelmes (1951)
<i>C. daltoni</i> Boott	Decorae	Decorae	-	Decora Clade	Hooker, J.D. s.n. (holo- P, digital image!)	Kükenthal (1909)
<i>C. decora</i> Boott	Decorae	Decorae	Decora Clade II	Decora Clade	Hooker, J.D. s.n. (isosyn- BOT, digital image!), Ikeda, H. 1225016 (OKAY!)	Kükenthal (1909)
<i>C. dielsiana</i> Kük.	Decorae	Decorae	-	Decora Clade	Forrest, G. 2147 (holo- P, digital image!)	Dai <i>et al.</i> (2010)
<i>C. gandakiensis</i> Katsuy.	Decorae	Decorae	-	-	Stainton, S. <i>et al.</i> 921 (holo- TI!, iso- BOT, digital image!)	Katsuyama (2010)
<i>C. inclinis</i> Boott ex C.B. Clarke	Decorae	Decorae	Decora Clade II	Decora Clade	Hooker, J.D. s.n. (holo- K, digital image!), Ikeda H. 1225066 (OKAY!)	Kükenthal (1909)
<i>C. insignis</i> Boott	Decorae	Decorae	-	Decora Clade	Hooker, J.D. & T. Thomson (syn- P, digital image!)	Dai <i>et al.</i> (2010)
<i>C. jizhuangensis</i> S.Yun Liang	Decorae	Decorae	-	-	-	Dai <i>et al.</i> (2010)
<i>C. kumaonensis</i> Kük.	Decorae	Decorae	-	Decora Clade	Ikeda, H. <i>et al.</i> 1215018 (OKAY!)	Kükenthal (1909)
<i>C. morii</i> Hayata	Decorae	Decorae	-	-	Ohwi, J. 1349 (K, digital image!)	Dai <i>et al.</i> (2010)
<i>C. munipoorensis</i> C.B. Clarke	Decorae	Hymenochlaenae	-	-	Watt, G. 7462 (K, digital image!), Watt, G. 6894 (E, digital image!)	Kükenthal (1909)
<i>C. neopolycephala</i> Tang & F.T. Wang ex L.K. Dai	Decorae	Hymenochlaenae	-	Decora Clade	Wang, C. W. 63329 (iso- A, digital image!)	Kükenthal (1909)
<i>C. phyllocaula</i> Nelmes	Decorae	Decorae	-	-	Kerr, A.F.G. 6615 (holo- K, digital image!)	Nelmes (1955)
<i>C. polycephala</i> Boott	Decorae	Hymenochlaenae	-	Decora Clade	Hooker, J.D. s.n. (iso- P, digital image!)	Dai <i>et al.</i> (2010)
<i>C. pomiensis</i> Y.C. Yang	Decorae	Decorae	-	-	PE-Xizang Expedition PE6048 (PE, digital image!)	Dai <i>et al.</i> (2010)
<i>C. pontica</i> Albov	Decorae	Decorae	-	-	Alboff, N. 353 (P, digital image!), Olgun, A.H. 71 (H, digital image!)	Kükenthal (1909)
<i>C. pulchra</i> Boott	Decorae	Decorae	-	Decora Clade	Hooker, J.D. s.n. (syn- P, digital image!)	Kükenthal (1909)
<i>C. scitula</i> Boott	Decorae	Decorae	-	-	Griffith, W. 6097 (iso- P, digital image!)	Kükenthal (1909)
<i>C. shuangbaiensis</i> L.K. Dai	Decorae	Hymenochlaenae	-	-	H. T. Tsai 58894 (iso- A, digital image!)	Dai <i>et al.</i> (2010)
<i>C. stracheyi</i> Boott ex C.B. Clarke	Decorae	Decorae	Decora Clade II	-	Strachey, R. & J. E. Winterbottom 18 (isosyn- GH, BR, digital image!)	Kükenthal (1909)
<i>C. subperakensis</i> L.K. Ling & Y.Z. Huang	Decorae	Decorae	-	-	-	Dai <i>et al.</i> (2010)
<i>C. turrita</i> Kük.	Decorae	Decorae	-	-	Merrill, E.D. 6623 (iso- P, digital image!)	Kükenthal (1909)
<i>C. urelytra</i> Ohwi	Decorae	Decorae	-	-	Ohwi J. 3351 (holo- KYO!)	Dai <i>et al.</i> (2010)
<i>C. verticillata</i> Zoll. & Moritzi	Decorae	Decorae	-	-	Jeswiet, J 1929/ 40 (holo- NL, digital image!)	Kükenthal (1909)
<i>C. walkeri</i> Am. ex Boott	Decorae	Decorae	-	-	Thwaites, G.H.K. 2751 (holo- K, digital image!)	Kükenthal (1909)
<i>C. winterbottomii</i> C.B. Clarke	Decorae	Decorae	-	-	Strachey, R. & J. E. Winterbottom 16 (iso- GH, digital image!)	Kükenthal (1909)
<i>C. zhenkangensis</i> Tang & F.T. Wang ex S.Yun Liang	Decorae	Decorae	-	-	Li, X.W. 2422 (KUN, digital image!)	Dai <i>et al.</i> (2010)

Chapter II

Phylogenetic Analyses of the *Carex filipes* complex (sect. *Paniceae*) in East Asia, with Reference to Morphology, Karyology and Taxonomy

Abstract

Carex filipes (Cyperaceae) and its closely related species, which we tentatively refer to as the *C. filipes* complex, are greatly diverse morphologically. The closely allied species have often been included within *C. filipes* where they have been treated by as many as seven infraspecific taxa at the rank of variety. To better determine the taxonomic distinctness of the individual species and infraspecific taxa, we undertook molecular phylogenetic analyses of individuals of the *C. filipes* complex using the plastid DNA regions *atpB-rbcL*, *rpl16-rpl16-rps3*, *rps16*, *matK*, and *trnL-trnL-trnF* and nuclear DNA regions ITS and ETS. We compared the morphology of as many individuals as possible and determined the somatic chromosome number of some individuals to supplement missing information. It was concluded that the *C. filipes* complex comprises ten species, two varieties and one hybrid, *i.e.* *C. arisanensis*, *C. curtispica*, *C. egena*, *C. filipes* var. *filipes*, *C. filipes* var. *tremula*, *C. kujuzana*, *C. ×kurogii*, *C. kuzakaiensis*, *C. rouyana* var. *arakiana*, *C. rouyana* var. *rouyana*, *C. sohayakiensis*, *C. sparsinix* and *C. vaniotii*. A diagnostic key to separate them, based on morphology, and chromosome numbers are presented. Two new species, *C. curtispica* and *C. sohayakiensis* and one new hybrid, *C. ×kurogii*, are described. The origin of the hybrid is also discussed.

Introduction

Carex L. (Cyperaceae), the fifth largest genus of angiosperms (Frodin 2004), comprises about 2,000 species (Roalson *et al.* 2021). *Carex* is distinguished from other genera of Cyperaceae Juss. by unisexual flowers and a perigynium, the latter of which corresponds to an achene enclosed by a

prophyll (Goetghebeur 1998, Starr *et al.* 2004). Six subgenera, *Siderosticta* M. J. Waterway, *Carex*, *Euthyceras* Peterm., *Psyllophorae* (Degland) Peterm., *Uncinia* (Pers.) Peterm. and *Vigneae* (P. Beauv. ex T. Lestib.) Heer., were recognized in the genus based on a recent molecular phylogenetic analysis (Villaverde *et al.* 2020). Martín-Bravo *et al.* (2019) showed that the subgeneric lineages differentiated in East Asia. Therefore, *Carex* in eastern Asia is important for understanding the history its diversification, which is still unclear since previous classifications based on morphology differ from one another (Kükenthal 1909, Ohwi 1936, Koyama 1962, Liang *et al.* 2000, Dai *et al.* 2010, Hoshino *et al.* 2020). The classifications have seldom been reexamined in the light of molecular phylogenetic analyses.

Carex filipes Franch. & Sav., which is included in *C. sect. Paniceae* G. Don, occurs in Japan, Korea and China. The morphology of *C. filipes* is diverse, resulting in several varieties being recognized within the species (Table 1). Koyama (1962) circumscribed *Carex filipes* the most widely, recognizing seven varieties. Kükenthal (1909) included *C. sparsinix* C. B. Clarke ex Franch. and *C. vaniotii* H. Lév. in *C. filipes* and recognized them as *C. filipes* var. *sparsinix* (C. B. Clarke ex Franch.) Kük. and *C. filipes* var. *vaniotii* (H. Lév.) Kük. Most of the infraspecific taxa, treated as varieties, have been recognized as distinct species (Table 1). We provisionally treated these nine taxa together as the *C. filipes* complex (Fig. 1), with the first objective being to determine the delimitation of *C. filipes* itself.

Our second objective was to solve several taxonomic problems concerning the varieties of *C. filipes*. Liang *et al.* (2000) considered *C. filipes* var. *sparsinix* to be endemic to China and distinguished it from *C. filipes* var. *filipes*, which they considered to be solely in Japan and Korea. Dai *et al.* (2010) reduced *C. filipes* var. *sparsinix* to *C. filipes* var. *filipes*, which they considered to be widely distributed in eastern Asia. Most Japanese taxonomists (Ohwi 1936, Akiyama 1955, Hoshino *et al.* 2020) considered *C. filipes* var. *sparsinix* to be restricted to China. Koyama (1962) reduced *C. filipes* var. *sparsinix* to *C. filipes* var. *rouyana* (Franch.) Kük., and considered it to be in both Japan and China.

The status of *Carex filipes* var. *tremula* Ohwi is also unclear. It has been reported from Kyushu and Shikoku, western Japan, and morphologically differs from *C. filipes* var. *filipes* in having short

staminate spike (Ohwi 1936). Although var. *tremula* was recognized by Japanese researchers until the mid-1900s (Ohwi 1936, Akiyama 1955, Koyama 1962), Katsuyama (2015) and Kurogi (2019) reported that the differences to separate it were ambiguous and Hoshino *et al.* (2020) did not recognize it.

Morphological distinctions between *C. filipes* var. *kuzakaiensis* M. Kikuchi and *C. arakiana* Ohwi are also uncertain. *Carex filipes* var. *kuzakaiensis*, which resembles *C. arakiana* morphologically, was reported to be endemic to northeastern Honshu, Japan (Katsuyama 2015). Based on Hoshino *et al.* (2020), *Carex arakiana* is distinguishable from *C. filipes* var. *kuzakaiensis* in having a longer, pedunculate staminate spike, and reddish staminate scales. Numakunai (2017), however, reported that several individuals of *C. filipes* var. *kuzakaiensis* had reddish staminate scales, as in *C. arakiana*, and Ohmori (2019) pointed out that the peduncle of the terminal spike of *C. filipes* var. *kuzakaiensis* was sometimes as long as that of *C. arakiana*.

Although interspecific hybrids within *C. filipes* complex have never been reported, the recent papers listed below reported that hybridization was one of the important factors behind the high specific diversity of *Carex* and thus made classification controversial. In North America, Cayouette & Catling (1992) described about 300 intraspecific hybrids, and as many hybrids were described in Europe by Koopman (2011), although Řepka & Mendel (2022) indicated that the number of hybrids was overestimated since Koopman (2011) relied only on morphological differences. In contrast, in Japan, Hoshino *et al.* (2020) described few hybrids because intraspecific hybrids had been based on the absence of fertile achenes or pollen (Fujiwara 1999, Abe 2003, Nagamasu & Kinoshita 2010, Oda *et al.* 2018). Molecular analyses, however, revealed that several intraspecific hybrids were fertile (Więclaw & Wilhelm 2014, Nagasawa *et al.* 2021, Řepka & Mendel 2022), implying that intraspecific hybridization in *Carex* in Japan is underestimated. Therefore, our third subject was to determine whether diversification in the *C. filipes* complex was due to hybridization.

To answer these systematic questions underlying the *Carex filipes* complex, we first undertook a phylogenetic analysis of the *C. filipes* complex and related species of *C.* sect. *Paniceae* from East Asia.

We constructed two separate phylogenetic trees based on the plastid DNA (cpDNA) regions and nuclear DNA (nrDNA) regions, respectively, to detect evidence of interspecific hybridization. We then compared the morphology and chromosomes with results from the phylogenetic analysis. *Carex* displays remarkable aneuploid series among and within species due to holocentric chromosomes (Hoshino 1981, Roalson 2008, Hipp *et al.* 2009), so that differences in chromosome number could potentially provide important evidence for reproductive isolation in *Carex* (Takahashi *et al.* 2021). Finally, we revised the taxonomy of the *C. filipes* complex based on evidence from these analyses.

Materials and Methods

Phylogenetic analysis

For the phylogenetic analysis, 106 individuals of 15 species and two varieties of *Carex* sect. *Paniceae* from East Asia and 1 individual of *C. curvicolis* Franch. & Sav. (sect. *Racemosae* G. Don) were examined. The individuals from Japan were identified following Hoshino *et al.* (2020) and those from Korea and China followed Liang *et al.* (2000). Exceptionally, *C. nasuensis* K. T. Takah., T. Nog. & M. N. Tamura and *C. tsukudensis* (T. Koyama) K. T. Takah. & M. N. Tamura were identified based on our previous studies (Takahashi *et al.* 2021, 2022). All of them are listed in Table 2. The sample code in Table 2 consists of two parts: the part before the hyphen indicates the name of the haplotype; the part after the hyphen indicates the number of the individual identification; the uppercase letter within the haplotype name indicates the cpDNA clade in which the haplotype is included (for the haplotype, see under Results: *Phylogenetic analysis for cpDNA regions*). Sixty seven individuals of six species and two varieties from the *C. filipes* complex were included. Their collection sites are shown in Fig. 2. Among the species of sect. *Paniceae*, *C.*

vaginata Tausch and *C. pilosa* Scop. were used as outgroups along with *C. curvicolis* Franch. & Sav. of sect. *Racemosae*; they were proven to be excluded from the clade comprising other species of sect. *Paniceae* from eastern Asia (Jiménez Mejías *et al.* 2016, Dorey 2019).

Total DNA was extracted from silica gel dried leaves or from leaves removed from herbarium

specimens by the CTAB method used in our previous studies (Takahashi *et al.* 2021, 2022). The polymerase chain reaction (PCR) amplification of five plastid regions (*atpB-rbcL*, *rpl16-rpl16-rps3*, *rps16*, *trnK-matK-trnK* and *trnL-trnL-trnF*) and two nuclear regions (ITS and ETS) using the primers listed in Table 3 and DNA sequencing as described by Takahashi *et al.* (2021, 2022).

Phylogenetic trees were constructed for both the plastid and nuclear regions. Maximum parsimony (MP) and maximum likelihood (ML) analyses were used to reconstruct the phylogeny in PAUP* v.4.0 beta 10 (Swofford 2002).

In the MP analysis, both the base substitutions and insertions/deletions (indels), both equally weighted, were treated as characters for phylogenetic reconstruction. Repeats of adenine (poly-A), cytosine (poly-C), and thymine (poly-T) and simple sequence repeats (SSRs) were excluded from the phylogenetic analyses because of the difficulty in identifying homologous bases and indels. In the ML analysis, only base substitutions were treated as character states for phylogenetic reconstruction. The DNA substitution model was selected by jModelTest (Darriba *et al.* 2012). TIMI+G was selected as the best model by Akaike's Information Criterion (AIC) and Akaike's Information Criterion Corrected (AICC). ML analysis was performed using a heuristic search option of as-is addition sequence. The MP analysis was performed that of simple addition sequence. Bootstrap analysis was based on 1000 replications. Because the phylogenetically informative sites of the nuclear DNA region of some individuals were heterozygous, those individuals were considered putative hybrids and were not included in the phylogenetic analyses. Using the sequence data along with morphological and chromosomal data, the putative parental species were determined for the suspected hybrids.

We further performed relaxed molecular clock analyses for the trees in BEAST v.1.10.4 software (Suchard *et al.* 2018) under the uncorrelated log-normal clock settings, birth-death model and 40 million generations. The tree topology was fixed to the strict consensus tree resulting from the MP analysis. We adopted secondary calibration points for the root of sect. *Paniceae* (mean 7.19 Mya) and the divergence node between sect. *Paniceae* and *Carex curvicolis* of sect. *Racemosae* (mean 20.44 Mya) based on the study of Martín-Bravo *et al.* (2019). We confirmed the run convergence in Tracer

v.1.7.1 (Rambaut *et al.* 2018) and determined trees after burn-in (10%) in TreeAnnotator v.1.10.4 (Suchard *et al.* 2018).

Observations of morphological characters

To compare the morphology with the results from the phylogenetic analyses, we examined 16 morphological characters for all 104 specimens used in the molecular analyses, except for the outgroup, to determine the range of variation (Table 2). The morphology of the perigynia and pistillate scales was observed in pistillate spikes preserved in 70% ethanol, while other morphological characters were observed on herbarium specimens.

Twelve of 16 characters (length of staminate spikes, number of staminate scales, color of staminate scales, length of peduncles of staminate spike, maximum number of peryginia per spike, length of peryginia, length of beak of peryginia, length of pistillate scales, shape of apex of pistillate scales, shape of apex of perigynia, color of pistillate scales, ratio of length of bract to length of paracladia) were measured on each culm; 4 additional characters (maximum width of leaves, maximum height of culms, maximum length of rhizome on culms, and color of basal part of sheaths) were measured on each individual. Because the length of the staminate scales and pistillate scales changes according to the location on the spike, each scale in the center of a spike was measured (*e.g.*, if a spike had 9 scales, we measured the 5th one). The length of the bracts and length of the paracladia (branching units including the pistillate spike and peduncle, shown in Fig. 1A) were measured for the uppermost pistillate spikes.

Observation of somatic chromosomes and estimation of genome size

Thirty-two individuals of six species and one variety cultivated in the Botanical Gardens, Graduate School of Science, Kyoto University, were examined to determine the somatic chromosome number and to estimate genome size (Table 2). To observe the somatic chromosomes, the root tips were prepared by the squash method used in our previous study (Takahashi *et al.* 2021). To estimate genome

size, we conducted flow cytometry (FCM) (Doležel *et al.* 2007), *i.e.* 1 cm² of a leaf from each individual was mixed with nuclei extraction buffer of CyStain UV Precise P (Sysmex Partec, Gorlitz, Germany). The nuclei were separated from the leaves by chopping with a razor blade and stained with the buffer CyStain UV Precise P (Sysmex Partec, Gorlitz, Germany). The stained nuclei were counted using Cyflow® PA (Sysmex Partec, Gorlitz, Germany). We estimate the 1C DNA value (pg/1C) by using *Raphanus sativus* (1C=0.555 pg DNA; Doležel *et al.* 1992) as a reference and coefficient of variation (CV). To test for significant differences among the species, we performed a Mann-Whitney *U* test, which is a non-parametric test between two samples. The level of significance was set at $P < 0.01$. The analyses were run in R (R Core Team 2019).

Results

Phylogenetic analysis for cpDNA regions

The aligned DNA sequence of the cpDNA regions (*atpB-rbcL*, *rpl16-rpl16-rps3*, *rps16*, *trnK-matK-trnK* and *trnL-trnL-trnF*) was 6,160 bp long and distinguished 47 haplotypes among 106 individuals. Of the 6,160 bp, 362 bp were variable and 82 bp were phylogenetically informative. In addition, 10 indels were used as phylogenetically informative characters for the MP analysis. The topology of the strict consensus of the 423 equally MP trees (Fig. 3) was identical with that of ML tree except for three branches (represented by arrowheads in Fig. 3), which were not present in the ML tree.

Thirteen clades (A–M) were formed in the cpDNA tree (Fig. 3). Individuals with the same DNA sequence were put together in a single OTU as the same haplotype. The sample code name consists of two parts: the part before the hyphen indicates the name of the haplotype; the part after the hyphen indicates number of the individual identification; the uppercase letter within the name of the haplotype indicates the cpDNA clade in which the haplotype is included. *Carex filipes* var. *filipes* was divided into three clades: Clade A (72% MPBS / <50% MLBS), Clade B (57% / <50%) and Clade D (100% / 97%). *Carex filipes* var. *kuzakaiensis* was in Clade B and four individuals of *C. arakiana* (B1-1–B1-

4) had the same haplotype (B1) as two individuals of *C. filipes* var. *kuzakaiensis* (B1-5 & B1-6). *Carex rouyana* was in both Clade C (100% / 95%) and Clade E (100% / 93%); each of these clades contained individual(s) of *C. arakiana*. *Carex filipes* var. *sparsinux* was in Clade G (100% / 99%); the individual from Hangzhou, China (G2-1), was sister to *C. kujuzana* from Japan (G1-1–G1-4). *Carex arisanensis* Hayata was monophyletic and formed Clade H (100% / 99%). *Carex vaniotii* H. Lév. was in Clade K (99% / 94%) along with *C. parciflora* Boott. *Carex egena* H. Lév. & Vaniot was sister to all of the other ingroup (*C. filipes* complex) species.

Phylogenetic analysis for nrDNA regions

The aligned nrDNA sequences (ITS and ETS) was 1,201 bp and distinguished 53 different haplotypes among 106 individuals. Of the 1,201 bp, 213 bp were variable, and 121 bp were phylogenetically informative. No informative indels were found in the dataset. The topology of strict consensus of the 359 equally MP trees (Fig. 4) was identical with that of ML tree. Seven individuals had heterozygous sites (Table 4) and thus were considered putative hybrids; they were excluded from the analyses.

The topology of the nrDNA tree (Fig. 4) differed from that of the cpDNA tree (Fig. 3) in several respects. Although *Carex filipes* var. *filipes* was divided into three clades in the cpDNA tree (Fig. 3), it was monophyletic (97% / 92%) in the nrDNA tree (Fig. 4). The individuals of Clade B in the cpDNA tree (Fig. 3) were polyphyletic in the nrDNA tree (Fig. 4). *Carex rouyana* (C1-1 & C1-2) and *C. rouyana* (E4-1 & E4-2) had the same DNA sequence of ITS and ETS (Fig. 4), although they were included in different clades in the cpDNA tree, *i.e.* Clade C and E (Fig. 3). Six individuals of *C. rouyana* and one individual of *C. arakiana* had five heterozygous sites (Table 4). The phylogenetic positions of *C. filipes* var. *kuzakaiensis* were significantly different between cpDNA and nrDNA trees. It was included in Clade B with *C. filipes* var. *filipes* in the cpDNA tree (Fig. 3), while it was related to *C. rouyana* (E3-1, E4-3, E4-4 & E4-6), and remote from *C. filipes* var. *filipes* in the nrDNA tree (Fig. 4).

Observations of morphological characters

Results of the morphological comparisons are presented in Table 5. They were mainly summarized for each taxon, but occasionally divided into different summaries according to the different clades of the cpDNA tree. Exceptionally, individuals of *C. arakiana* (C5-1 & E3-1) and *C. rouyana* (C4-1, C4-2, E2-1, E2-2 & E4-5), which have heterozygosity sites in their DNA sequences of ITS and ETS (Table 4), and individuals of *C. rouyana* (E4-1 & E4-2, included in Clade E of cp tree), which have the same ITS and ETS nrDNA sequences with individuals of *C. rouyana* (C1-1 & C1-2, included in Clade C of cp tree), were separated from the core members *C. arakiana* and *C. rouyana* as putative hybrids and positioned at the bottom of each species (Table 5).

Individuals of *C. filipes* var. *filipes* of Clade A had a longer staminate spike (0.8–1.4 cm) than did those in Clades B and D (0.4–0.7 cm). Individuals of *C. filipes* var. *filipes* of Clade D had a shorter beak of the perigynia (1.3–1.7 mm) (Fig. 5C) than those in Clades A and B (2.0–4.0 mm) (Fig. 5A, B), and wider leaves (5.0–7.2 mm) than those in Clades A and B (2.3–4.2 mm). *Carex arakiana* of Clade B also had a shorter beak of the perigynia (1.2–1.8 mm) (Fig. 5F) than taxa in Clade E (2.5–3.6 mm) (Fig. 5E); this trait was consistent with *C. filipes* var. *kuzakaiensis* (1.1–1.6 mm).

Carex rouyana of Clade C had longer bracts than paracladia (1.5–2.7 times), while *C. rouyana* of Clade E had almost equal or shorter bracts than paracladia (0.5–1.0 times); putative hybrids between Clades C and E had paracladia of intermediate length (Fig. 6A). *Carex rouyana* of Clade C (12–25) had fewer staminate scales than *C. rouyana* of Clade E (28–43); the number of staminate scales in the putative hybrids was intermediate in number (12–36) (Fig. 6B). The number of perigynia per spike in putative hybrids was also intermediate between the putative parents (Fig. 6C). The rhizome of putative hybrids (Fig. 7E) was mostly longer than in both putative parents (Figs. 6D & 7B, C). A similar tendency was observed in the relationship of *C. filipes* var. *kuzakaiensis* to *C. filipes* var. *filipes* of Clade B and *C. rouyana* of Clade E (Figs. 7A, B, D & 8A–D). *Carex filipes* var. *kuzakaiensis* had intermediate traits between the latter two in the length of the bracts (Fig. 8A), number of staminate

scales (Fig. 8B) and number of perigynia (Fig. 8C), but had elongate rhizomes (Figs. 7D & 8D).

The individuals of *C. filipes* var. *sparsinux* and *C. kujuzana* had longer pistillate scales (4.0–4.6 mm) (Fig. 9E–G) than those of *C. filipes* var. *filipes* and *C. rouyana* (2.8–3.4 mm) (Fig. 9A–D), and the apex of the pistillate scales of the former two was aristate (Fig. 9E–G). Furthermore, one individual of *C. filipes* var. *sparsinux* (G2-1) (Fig. 7G), which was sister to *C. kujuzana*, differed from the other individuals of *C. filipes* var. *sparsinux* (G3-1, G3-2) (Fig. 7F) in having a stolon for each culm: this trait was consistent with that of *C. kujuzana* (Fig. 7H).

Observation of somatic chromosomes and estimation of genome size

Chromosome numbers for taxa in the *Carex filipes* complex were previously reported as follows: $2n = 48$ and 54 for *C. filipes* (Tanaka 1948), $2n = 38$ for *Carex rouyana* (Tanaka 1948), $2n = 30$ for *C. arakiana* (Hoshino 1981), and $2n = 44$ for *C. arisanensis* (Yano *et al.* 2008). We did not use, however, the previous counts in the present discussion because we could not locate voucher specimens for them and were unable to confirm the identification of the taxa. Accordingly, we tried to determine the chromosome numbers for as many taxa as possible through our own efforts in the present study.

Results of observation of somatic chromosomes and FCM are summarized in Table 6. The chromosome numbers of *Carex filipes* var. *filipes* differed greatly among the clades in the cpDNA tree. In *C. filipes* var. *filipes* of Clade A, we found three different numbers: $2n = 52$, 54 and 58 (Fig. 10A–C). Among them, $2n = 52$ and 58 are new counts for this variety. We found $2n = 40$, 42 , 44 , 46 and 50 (Fig. 10D–H) in *C. filipes* var. *filipes* of Clade B and $2n = 60$ (Fig. 10I) in *C. filipes* var. *filipes* of Clade D. All of them ($2n = 40$, 42 , 44 , 46 , 50 and 60) are new counts for this variety. The longest chromosome of *C. filipes* var. *filipes* of Clade B (1.6 – 2.2 μm) was longer than the longest ones in Clade A (1.3 – 1.6 μm) and Clade D (1.3 – 1.4 μm) (Table 6). The chromosome numbers of *C. rouyana* were not so different between Clades C and E. We found $2n = 52$ in *C. rouyana* of Clade C (Fig. 10N), and $2n = 50$ and 52 in *C. rouyana* of Clade E (Fig. 10L, M). The putative hybrid (C4-1, E2-1 and E3-1) between Clades C and E showed the number of $2n = 52$ (Fig. 10O) (Table 6). The chromosome number

of *C. filipes* var. *kuzakaiensis* ($2n = 52$, Fig. 10K), *C. nasuensis* ($2n = 50$, Fig. 10P), *C. kujuzana* ($2n = 58$, Fig. 10Q) and *C. vaniotii* ($2n = 44$, Fig. 10R) were observed for the first time in this study. *Carex arakiana* of Clade B (B1-1, Fig. 10J) showed the same chromosome number ($2n = 52$) as *C. filipes* var. *kuzakaiensis* (B2-1, Fig. 10K), which was also included in the same clade as *C. arakiana* of Clade B in the cp tree (Fig. 3).

The mean DNA contents were variable, 0.350–0.387 pg/1C, among these species. No significant difference in mean DNA content existed among the species or clades (Mann-Whitney *U* test, $P < 0.01$).

Discussion

Revisiting the taxonomy of Carex filipes and related species based on phylogeny and morphology

Based on Ohwi (1936) and Akiyama (1955), *Carex filipes* var. *tremula* had been distinguished from *C. filipes* var. *filipes* in having short staminate spikes. In this study, the individuals of *Carex filipes* var. *filipes* of Clade B (B3-1, B3-2, B4-1–B4-6 & B5-1) were morphologically similar to *C. filipes* var. *tremula* in having short staminate spikes (Table 5) and the individual from the type locality of *C. filipes* var. *tremula* (Tsushima Isls., Pref. Nagasaki, Japan, *Tashiro s.n.*, 1909, holo–KYO!), was also included in Clade B (B4-6). Therefore, the individuals of Clade B correspond to *C. filipes* var. *tremula*. The distribution ranges of Clades A and B primarily differ from each other, although they overlap to some extent (West Shikoku, Fig. 2A). The individuals of Clade A are distributed on the Pacific side of Honshu and Shikoku, Japan, while those of Clade B are on the Kii Peninsula, in western Shikoku and in Kyushu, Japan. Many endemic species of land plants are known from the distribution range, called the Sohayaki region (Koidzumi 1931, Murata & Koyama 1976), of Clade B. *Carex filipes* var. *filipes* of Clade A is weakly supported both in the cpDNA (Fig. 3) and nrDNA (Fig. 4) trees. In comparison, *C. filipes* var. *filipes* of Clade B is paraphyletic both in the cpDNA (Fig. 3) and nrDNA (Fig. 4) trees. The differentiation between Clade A-*filipes* and Clade B-*filipes*, which can be considered to have occurred in the Quaternary (Figs. 3 & 4), might be incomplete. The chromosome number of Clade B-*filipes* ($2n = 42, 44, 46$ and 50) differs from that of Clade A-*filipes* ($2n = 52, 54$ and 58), but there is

no clear gap between the two ranges in variation of the chromosome number ($2n = 42-50$ and $52-58$), and reproductive isolation cannot be verified based on chromosome number. Therefore, we conclude that differences between Clade A-*filipes* and Clade B-*filipes* remain within the range of variation of the species. We therefore recognize Clade A-*filipes* and Clade B-*filipes* to be *C. filipes* var. *filipes* and *C. filipes* var. *tremula*, respectively.

In contrast, *Carex filipes* var. *filipes* of Clade D was remotely positioned from *C. filipes* var. *filipes* of Clade A and B in the cpDNA tree (Fig. 3). Although several individuals of Clade B (B4-1–B4-5) were closely related to Clade D in the nrDNA tree (Fig. 4), the chromosome numbers of B4-1–B4-5 ($2n = 40, 42$ and 44) differ from those of Clade D ($2n = 60$) to a high degree. Therefore, it is difficult to attribute this paraphyly to introgression, but rather to incomplete lineage sorting. Further, Clade D was also distinguished morphologically from Clade A and B in having a shorter beak of the perigynia (Fig. 5A–C) and wider leaves (see above under Results: *Observations of morphological characters*). Those traits also differ from those of the type specimen of *C. filipes* (Faurie 3484, holo–P, digital image!). Therefore, we conclude it to be a new species, *C. curtispica*.

DNA sequences of four individuals of *Carex arakiana* (B1-1–B1-4) were completely identical to those of two individuals of *C. filipes* var. *kuzakaiensis* (B1-5 and B1-6) in both regions of the cpDNA and nrDNA. Thus, the peduncle length of the staminate spikes and color of the staminate scales, which were used by Hoshino *et al.* (2020) as diagnostic characteristics between *Carex arakiana* and *C. filipes* var. *kuzakaiensis*, proved to be of no diagnostic value. The former four individuals (B1-1–B1-4) differ from the other individuals of *C. arakiana* in having a shorter beak of the perigynia (1.2–1.8 mm vs. 2.5–3.6 mm), and were similar to *C. filipes* var. *kuzakaiensis* in the length of the perigynium beak (1.2–1.8 mm vs. 1.1–1.6 mm). The type specimen of *C. arakiana* (Mt. Oe, Kyoto Pref., Japan, *Araki s.n.*, 22 May 1932, holo–KYO!) has a long beak of the perigynia (2.6–3.1 mm); the beak of *C. filipes* var. *kuzakaiensis* (Kikuchi 28504, holo–IW T, digital image!) has a short beak (1.3–1.5 mm). Therefore, *C. arakiana* of Clade B (B1-1–B1-4) should be identified as *C. filipes* var. *kuzakaiensis*. The diagnostic characteristics between *C. arakiana* and *C. filipes* var. *kuzakaiensis* cannot be based on the length of

the peduncle of the staminate spikes and color of the staminate scales (Hoshino *et al.* 2020), but length of the beak of the perigynia. This view generally agrees with that of Ohmori (2019).

Carex filipes var. *kuzakaiensis*, including individuals of B1-1–B1-4, was monophyletic in both the cp and nr trees (Figs. 3 & 4). Although monophyly was robust in nr tree (Fig. 4), in the cp tree it was weak and closely similar to *C. filipes* var. *tremula* (Fig. 3). However, *C. filipes* var. *kuzakaiensis* occurs in environments with low temperatures, such as at high elevations and high latitudes (Fig. 2C). Its habitats significantly differ from those of other varieties of *C. filipes* (Fig. 2A). It also differs from the other varieties of *C. filipes* in the number of staminate scales and length of the perigynium beak to a high degree. Therefore, we conclude that *C. filipes* var. *kuzakaiensis* can be recognized as a species distinct from *C. filipes*, i.e. *C. kuzakaiensis*, comb. & stat. nov.

Carex rouyana was divided into Clade C and E in the cp tree (Fig. 3). Those two clades were also remotely positioned in the nr tree (Fig. 4), although five individuals (C4-1, C4-2, E2-1, E2-2 & E4-5) of *Carex rouyana* had heterozygous sites in the nrDNA regions. One base of the base pair was from Clade C, and the other from Clade E (Table 4). They were morphologically intermediate between the two clades and thus can be considered to be putative hybrids between them. Except for the putative hybrids, there are clear differences between the two clades. Individuals of Clade C have bracts that are longer than the pistillate spikes (1.5–2.7 times) and fewer staminate scales (12–25), while those of Clade E have bracts that are shorter than or as long as the pistillate spikes (0.5–1.0 times) and a greater number of staminate scales (28–43). Thus, we considered *C. rouyana* of Clades C and E to differ at the species level. As in the type specimen of *C. rouyana* (Prov. Musashi, Japan, *sine coll.*, May 1887, holo-P, digital image!) the bracts do not exceed the pistillate spikes and the number of staminate scales is more than 30 as far as we observed. We consider the type specimen of *C. rouyana* to be included in Clade E. Thus, *C. rouyana* of Clade C should be treated as a different species, which we name *C. sohayakiensis*, sp. nov. Putative hybrids between these two species were unusual in having much longer rhizomes than both parental species, but elongation of the rhizomes due to hybridization has been reported also in the Poaceae (Matsumura *et al.* 1987, Kopecký *et al.* 2018). We recognize these

putative hybrids as *C. ×kurogii*, hybr. nov. (*C. rouyana* × *C. sohayakiensis*).

Individuals of *C. arakiana* that were excluded from *C. kuzakaiensis* (C5-1, E3-1 & E5-1) were phylogenetically sister to *C. rouyana* or its hybrid, *C. ×kurogii* (Figs. 3 & 4). Those individuals of *C. arakiana* can be morphologically distinguished from *C. rouyana* in having the basal portion of the sheath reddish-purple. They are restricted to the Kinki district, western Japan. Therefore, we consider *Carex arakiana* to be a variety of *C. rouyana*, *C. rouyana* var. *arakiana*. This view supports Ohwi (1936), Akiyama (1955), and Koyama (1962) (Table 1).

Both phylogenetic trees (Figs. 3 & 4) indicated that *Carex filipes* var. *sparsinux* was related to *C. kujuzana* rather than to *C. filipes* or *C. rouyana*. The morphology of the pistillate scales also supported the phylogeny, *i.e.* the pistillate scales of *C. filipes* var. *sparsinux* and *C. kujuzana* were longer and apically aristate (Fig. 9E–G), while those of *C. filipes* and *C. rouyana* were shorter and apically acute (Fig. 9A–D). Therefore, *C. filipes* var. *sparsinux* should be recognized as a separate species from *C. filipes*. Furthermore, some individuals identified as *C. filipes* var. *sparsinux* (G2-1) were morphologically similar to *C. kujuzana* (G1-3) in having a stolon arising from each culm (Fig. 7G, H). They were phylogenetically sister to each other in both the cp and nr trees (Figs. 3 & 4). The type specimen of *C. sparsinux* (China, Hupeh, *Henry 3745*; holo-K, digital image!) has no stolon. Therefore, we concluded that individuals of *C. filipes* var. *sparsinux* lacking a stolon (G3-1 & G3-2) should be recognized as *C. sparsinux* while those with a stolon on each culm (G2-1) are conspecific with *C. kujuzana*.

Carex arisanensis, *C. vaniotii*, *C. egena* and *C. filipes* were phylogenetically distant from one another in both the cp and nr trees (Figs. 3 & 4).

Therefore, the former three should be retained as separate species from *C. filipes*. Accordingly, we recognized ten species, two varieties and one hybrid in the *C. filipes* complex, *i.e.* *C. arisanensis*, *C. curtispica*, *C. egena*, *C. filipes* var. *filipes*, *C. filipes* var. *tremula*, *C. kujuzana*, *C. ×kurogii*, *C. kuzakaiensis*, *C. rouyana* var. *arakiana*, *C. rouyana* var. *rouyana*, *C. sohayakiensis*, *C. sparsinux*, and *C. vaniotii* (Table 1).

Hybrid origin of Carex kuzakaiensis

Carex kuzakaiensis (B1-1–B1-6, B2-1 & B2-2) was closely related to *C. filipes* var. *tremula* (B3-1, B3-2, B4-1–B4-6 & B5-1) in the cpDNA tree (Fig. 3), while it was close to *C. rouyana* (E43) in the nrDNA tree (Fig. 4). Disagreement in topology between the cp and nr trees might be attributed to incomplete lineage sorting (ILS) or introgressive hybridization (Kobayashi *et al.* 2022), and ILS might not be expected if insufficient time has passed since the prior speciation event (Twyford & Ennos 2012, Feng *et al.* 2022). In the cpDNA tree (Fig. 3), *C. kuzakaiensis* and *C. rouyana* differentiated long ago (at 2.84 Ma), although *C. kuzakaiensis* and *C. filipes* var. *tremula* diverged recently (at 0.375 Ma). Additionally, in the nrDNA tree (Fig. 4), *C. kuzakaiensis* and *C. filipes* var. *tremula* deviated not long ago (1.05 Ma), although *C. kuzakaiensis* and *C. filipes* var. *tremula* diverged long ago (3.45 Ma). Therefore, it is difficult to consider that the difference in phylogenetic position of *Carex kuzakaiensis* was caused by ILS. In addition, *C. kuzakaiensis* is morphologically intermediate between *C. filipes* and *C. rouyana* and shows elongation of the rhizome in the same manner as another hybrid, *C. rouyana* × *C. sohayakiensis*. This evidence suggests that *C. kuzakaiensis* originated as a hybrid of *C. filipes* (maternal) and *C. rouyana* (paternal).

Usually, speciation due to intraspecific hybridization is accompanied by polyploidization (Rieseberg 1997). However, the genome size of *C. kuzakaiensis* was not a multiple of the parental species and polyploidization does not appear to have occurred (Table 6). Hybrid speciation without polyploidization is called homoploid hybrid speciation, but it generally appears to be more difficult than hybrid speciation with polyploidization because homoploid hybrids may easily backcross with their parental species and cannot escape the dissolving effects of introgression (Buerkle *et al.* 2000). Homoploid hybrid speciation has been reported among several species of *Carex* (Pedersen *et al.* 2016, Nowak *et al.* 2020). Those sedges have sterile pollen or ovules and survive by vegetative propagation via stolons, and are reproductively isolated from their parents. However, the achenes of *C. kuzakaiensis* usually mature, and appear to be fertile. This can be explained by the fact that when F1

hybrids repeatedly self-fertilize, alleles from both parental species are stabilized throughout the genome and fertility of the hybrid lineage might be acquired (Buerkle & Rieseberg 2008). Therefore, it might be possible that *Carex kuzakaiensis* maintained its hybrid lineage through vegetative propagation by stolon, and acquired fertility through repeated self-fertilization.

Taxonomic treatment

Key to the species of the *Carex filipes* complex

- 1a. Perigynia tapered to apex 2
- 1b. Perigynia abruptly narrowed to apex 4
- 2a. Peduncle of staminate spike 21–40 mm long; staminate scales reddish brown **11. *C. egena***
- 2b. Peduncle of staminate spike 5–10 mm long; staminate scales pale green 3
- 3a. Staminate spike 0.4–0.7 cm long; perigynia 2–4 per spike..... **9. *C. arisanensis***
- 3b. Staminate spike 0.8–1.3 cm long; perigynia 5–10 per spike **10. *C. vaniotii***
- 4a. Scales at central part of pistillate spike 4.0–4.6 mm long, apex aristate..... 5
- 4b. Scales at central part of pistillate spike 2.8–3.4 mm long, apex acute 6
- 5a. Rhizome 0.2–0.5 cm long **7. *C. sparsinux***
- 5b. Rhizome 1.5–4.0 cm long **8. *C. kujuzana***
- 6a. Staminate scales 4–9 per staminate spike..... 7
- 6b. Staminate scales 12–50 per staminate spike 9
- 7a. Beak of perigynia 1.3–1.7 mm long; maximum width of leaves 5.0–7.2 mm..... **5. *C. curtispica***
- 7b. Beak of perigynia 2.0–3.0 mm long; maximum width of leaves 2.3–4.2 mm 8
- 8a. Staminate spikes 0.8–1.4 cm long **1a. *C. filipes* var. *filipes***
- 8b. Staminate spikes 0.4–0.6 cm long **1b. *C. filipes* var. *tremula***
- 9a. Beak of perigynia 1.1–1.8 mm long, less than half as long as perigynium **2. *C. kuzakaiensis***
- 9b. Beak of perigynia 2.5–4.2 mm long, more than half as long as perigynium 10

- 10a. Bract of uppermost pistillate spike 1.5–2.7 times as long as spike **3.** *C. sohayakiensis*
- 10b. Bract of uppermost pistillate spike 0.5–1.2 times as long as spike 11
- 11a. Plants loosely caespitose, often stoloniferous; rhizome 0.5–4.2 cm long **4.** *C. ×kurogii*
- 11b. Plants tightly caespitose, not stoloniferous; rhizome 0.2–0.5 cm long 12
- 12a. Basal part of sheath pale brown **6a.** *C. rouyana* var. *rouyana*
- 12b. Basal part of sheath reddish purple **6b.** *C. rouyana* var. *arakiana*

1. *Carex filipes* Franch. & Sav., Enum. Pl. Jap. 2: 148 (1877), cum diagn. Franc., 576 (1878)—*Typus*: Japan, Mt. Fuji, *U. Faurie* 3484 (holo—P, digital image!).

1a. var. *filipes*

Distribution. Japan: Honshu (westward from Kanto Dist.), Shikoku, and Korea

Japanese name. Tamatsuri-suge.

Habitat. In open places at forest margin, 100–1700 m alt.

Chromosome number. $2n = 52, 54, 58$.

Additional specimens examined. Japan: Tochigi Pref., Nikko-shi, *K. T. Takahashi* 1026 (KYO). Ibaraki Pref., Hitachiota-shi, *K. T. Takahashi* 1025 (KYO), Hitachi-shi, *K. T. Takahashi* 1027 (KYO). Gunma Pref., Kawaba-mura, *T. Ohmori* 9515 (GMNHJ). Tokyo, Hachioji-shi, *K. T. Takahashi* 1186 (KYO). Nagano Pref., Karuizawa-cho, *K. T. Takahashi* 1184 (KYO). Gifu Pref., Seki-shi, *K. T. Takahashi* 1013 (KYO). Aichi Pref., Tahara-shi, *K. T. Takahashi* 1183 (KYO). Shiga Pref., Maibara-shi, *K. T. Takahashi* 1201 (KYO). Osaka Pref., Chihayaakasaka-mura, *K. T. Takahashi* 1200 (KYO). Okayama Pref., Mimasaka-shi, *K. T. Takahashi et al.* 1180 (KYO), Okayama-shi, *K. T. Takahashi & O. Yano* 1125 (KYO). Hiroshima Pref., Miyoshi-shi, *K. T. Takahashi* 1017 (KYO). Tokushima Pref., Miyoshi-shi, *K. T. Takahashi et al.* 1114 (KYO). Ehime Pref., Seiyo-shi, *K. T. Takahashi* 1159 (KYO). Kumakogen-cho, *K. T. Takahashi* 1034 (KYO).

1b. var. *tremula* (Ohwi) Ohwi in Mem. Coll. Sci. Kyoto Imp. Univ., Ser. B, Biol. 11: 423 (1936).

— *Carex arisanensis* Hayata var. *tremula* Ohwi in Mem. Coll. Sci. Kyoto Imp. Univ., Ser. B, Biol. 5: 255 (1930). — *Carex tremula* (Ohwi) Ohwi in Acta Phytotax. Geobot. 2: 28 (1933). — *Typus*: Japan, Pref. Nagasaki, Tsushima Isl., Mt. Yatate, Z. Tashiro s.n., 1909 (holo– KYO !).

Carex filipes (non Franch. & Sav.) H. Lév. & Vaniot in Bull. Acad. Intern. Géogr. Bot. 11: 112 (1902).

Distribution. Endemic to Japan: Honshu (Kii Peninsula), Shikoku (Pref. Ehime), Kyushu (Prefs. Oita, Kumamoto, Miyazaki and Kagoshima).

Japanese name. Hime-juzu-suge.

Habitat. In open places at forest margin, 500–1000 m alt.

Chromosome number. $2n = 40, 42, 44, 46, 50$.

Additional specimens examined. Japan: Nara Pref., Totsukawa-mura, K. T. Takahashi & J. Oda 1166 (KYO). Wakayama Pref., Tanabe-shi, K. T. Takahashi 1002 (KYO). Ehime Pref., Kumakogen-cho, K. T. Takahashi 1163 (KYO). Oita Pref., Beppu-cho, K. T. Takahashi 1169 (KYO). Kumamoto Pref., Aso-shi, K. T. Takahashi 1170 (KYO). Miyazaki Pref., Shiiba-son, K. T. Takahashi 1216 (KYO), Miyakonojo-shi, K. T. Takahashi 1217 (KYO). Nagasaki Pref., Tsushima-shi, K. T. Takahashi & O. Yano 1209 (KYO). Kagoshima Pref., Satsuma-cho, J. Oda 4109 (KYO).

2. *Carex kuzakaiensis* (M. Kikuchi) K. T. Takahashi & M. N. Tamura, **comb. & stat. nov.**

Basionym: *Carex filipes* Franch. & Sav. subsp. *kuzakaiensis* M. Kikuchi in Ann. Rep. Gakugei Fac. Iwate Univ. 13: 47, pl. 2 (1958). — *Carex filipes* Franch. & Sav. var. *kuzakaiensis* (M. Kikuchi) T. Koyama in J. Fac. Sci. Univ. Tokyo, Sect. 3, Bot. 8: 229 (1962). — *Typus*: Japan, Pref. Iwate, Mt. Kabuto-myojin, M. Kikuchi 28504 (holo–IWT, digital image!).

Distribution. Endemic to Japan: Honshu (northward from Japan Sea side of Chugoku Dist.).

Japanese name. Oku-tamatsuri-suge.

Habitat. In open places at forest margin, 200–1500 m alt.

Chromosome number. $2n = 52$.

Additional specimens examined. Japan: Iwate Pref., Takizawa-shi, *K. T. Takahashi 1024* (KYO), Miyako-shi, *K. T. Takahashi 1212* (KYO). Tochigi Pref., Nikko-shi, *K. T. Takahashi 1058* (KYO). Gunma Pref., Katashina-mura, *K. T. Takahashi 1060* (KYO). Niigata Pref., Myoko-shi, *K. T. Takahashi 1061* (KYO). Gifu Pref., Takayama-shi, *K. T. Takahashi 1035* (KYO). Hiroshima Pref., Shobara-shi, *K. T. Takahashi et al. 1181* (KYO). Tottori Pref., Kofu-cho, *K. T. Takahashi et al. 1182* (KYO).

3. *Carex sohayakiensis* K. T. Takahashi & M. N. Tamura, *sp. nov.* (Figs. 5D & 11)

Carex sohayakiensis is similar to *C. filipes* and *C. rouyana*, but differs from *C. filipes* in having long staminate spike (1.5–2.0 cm long in *C. sohayakiensis*; 0.8–1.4 cm long in *C. filipes*), and many staminate scales (15–25 in *C. sohayakiensis*; 7–12 in *C. filipes*), and from *C. rouyana* in having longer pistillate spikes than bracts, and few staminate scales (15–25 in *C. sohayakiensis*; 28–43 in *C. rouyana*).

Typus. Japan. Pref. Nara, Totsukawa, 15 May 2021, *K. T. Takahashi & J. Oda 1168* (holo-KYO!).

Herbs, perennial, loosely caespitose. Rhizome 0.5–2 cm long. Culm 23–30 cm long, erect, trigonous, smooth. Leaves basal, 10–35 cm long, 2–4 mm broad, light green, basal part of sheath pale brown or partly reddish purple. Bracts leaf-like, ca. twice as long as spikes, long sheathed. Spikes 3 or 4; terminal spike staminate, clavate, 1.5–2 cm long, 12–25-flowered, long-pedunculate, erect; staminate scales obovate, 4–5 mm long, acute, pale brown or partly reddish purple, midrib green; lateral spikes pistillate, linear, 1.5–3 cm long, loosely 3–6-flowered, long-pedunculate, drooping; pistillate scales ovate, 3–4 mm long, acute at apex, reddish purple, midrib green. Perigynia obtusely trigonous, 5.6–7.1 mm long, 1.5–2 mm broad, membranaceous, smooth, several-veined, long-beaked, abruptly narrowed to apex; beak 2.8–4 mm long. Achene tightly enveloped by perigynium, ellipsoid, trigonous, 2–2.5 mm long. Stigma 3. Anthesis April to May.

Distribution. Endemic to Japan: Honshu (westward from Kanto Dist.), Shikoku, Kyushu.

Japanese name. Usuiro-tamatsuri-suge (nov.).

Habitat. In open places at forest margin, 800–1200 m alt.

Chromosome number. $2n = 52$.

Additional specimens examined. Japan: Nara Pref., Totsukawa-mura, *K. T. Takahashi & J. Oda* 1167 (KYO). Kochi Pref., Niyodogawa-cho, *K. T. Takahashi* 1165 (KYO). Miyazaki Pref., Hinokage-cho, *K. T. Takahashi & S. Kurogi* 1178 (KYO).

4. *Carex* ×*kurogii* *K. T. Takahashi & M. N. Tamura*, **hybr. nov.** (*C. rouyana* × *C. sohayakiensis*) (Fig. 12)

Carex ×*kurogii* is similar to *C. rouyana* and *C. sohayakiensis*, but differs from *C. rouyana* in having long rhizome, longer than 5 mm, and from *C. sohayakiensis* in having bracts shorter than or as long as pistillate spikes.

Typus. Japan. Pref. Miyazaki, Takachiho-cho, 10 May 2021, *K. T. Takahashi & S. Kurogi* 1174 (holo-KYO!).

Herbs, perennial, loosely caespitose, sometimes stoloniferous. Rhizome 0.5–4.2 cm long. Culm 21–40 cm long, erect, trigonous, smooth. Leaves basal, 15–30 cm long, 1.5–6 mm broad, shorter than culm, light green, basal part of sheath pale brown or partly reddish purple. Bracts leaf-like, shorter than or as long as spikes, long sheathed. Spikes 3 or 4; terminal spike staminate, clavate, 1.5–2.7 cm long, 16–30-flowered, long-pedunculate, erect; staminate scales obovate, 4–5 mm long, acute, pale brown or partly reddish purple, midrib green; lateral spikes pistillate, linear, 1.5–3 cm long, loosely 2–6-flowered, long-pedunculate, drooping; pistillate scales ovate, 3–4 mm long, acute at apex, reddish purple, midrib green. Perigynia obtusely trigonous, 5.5–8.0 mm long, 1.5–2 mm broad, membranaceous, smooth, several-veined, long-beaked, abruptly narrowed to apex; beak 2.3–4.5 mm long. Achene tightly enveloped by perigynium, ellipsoid, trigonous, 2–2.5 mm long. Stigma 3. Anthesis April to May.

Distribution. Endemic to Japan: Honshu (westward from Kanto Dist.), Shikoku, Kyushu (Pref. Miyazaki).

Japanese name. Takachiho-tamatsuri-suge (nov.).

Habitat. In open places at forest margin, 300–900 m alt.

Chromosome number. $2n = 52$.

Additional specimens examined. Japan: Tochigi Pref., Utsunomiya-shi, K. T. Takahashi 1203 (KYO). Kyoto Pref., Oe-cho, K. T. Takahashi et al. 1015 (KYO). Hyogo Pref., Kobe-shi, K. T. Takahashi 1197 (KYO). Hiroshima Pref., Miyoshi-shi, Sera 19846 (OKAY). Kagawa Pref., Takamatsu-shi, K. T. Takahashi et al. 1113 (KYO). Tokushima Pref., Mima-shi, K. T. Takahashi 1009 (KYO). Miyazaki Pref., Shiiba-son, K. T. Takahashi & S. Kurogi 1176 (KYO), Takachiho-cho, K. T. Takahashi & S. Kurogi 1044 (KYO).

5. *Carex curtispica* K. T. Takahashi & M. N. Tamura, *sp. nov.* (Figs. 5C & 13)

Carex curtispica is similar to *C. filipes* var. *tremula*, but differs from it in having short, curved beak of perigynia, shorter than half of perigynia, and wide leaves, wider than 5 mm.

Typus. Japan. Pref. Ehime, Kumakogen, 9 June 2019, K. T. Takahashi et al. 1032 (holo-KYO!).

Herbs, perennial, caespitose. Rhizome 2–5 mm long. Culm 10–23 cm long, erect, trigonous, smooth. Leaves basal, 10–20 cm long, 2.6–5 mm broad, shorter than or as long as culm, light green, basal part of sheath pale brown or partly reddish purple. Bracts leaf-like, ca. twice as long as spikes, long sheathed. Spikes 2 or 3; terminal spike staminate, linear, 3–5 mm long, 4–7-flowered, shortly pedunculate, erect; staminate scales obovate, 3.5–4.5 mm long, acute, pale brown or partly reddish purple, midrib green; lateral spikes pistillate, ellipsoid to shortly cylindrical, 5–10 mm long, 2–4-flowered, long-pedunculate, drooping; pistillate scales ovate, 2.5–4 mm long, acute at apex, reddish purple, midrib green. Perigynia obtusely trigonous, 3.2–4.2 mm long, 1.2–1.5 mm broad, membranaceous, smooth, several-veined; beak of perigynia short, curved, abruptly narrowed to apex; beak 1.3–1.7 mm long. Achene tightly enveloped by perigynium, ellipsoid, trigonous, 2–2.5 mm long. Stigma 3. Anthesis April to May.

Distribution. Endemic to Japan: Shikoku (Pref. Ehime), Kyushu (Prefs. Saga and Miyazaki).

Japanese name. Mame-tamatsuri-suge (nov.).

Habitat. In open places at forest margin, 500–1000 m alt.

Chromosome number. $2n = 60$.

Additional specimens examined. Japan: Ehime Pref., Kumakogen-cho, K. T. Takahashi 1033 (KYO), Uchiko-cho, J. Oda 2233 (KYO). Saga Pref., Kanzaki-shi, K. T. Takahashi et al. 1158 (KYO). Miyazaki Pref.: Hinokage-cho, K. T. Takahashi & S. Kurogi 1177 (KYO).

6. *Carex rouyana* Franch. in Bull. Soc. Philom. Paris, Ser. 8, 7 : 51 (1895). — *Carex filipes* Franch. & Sav. var. *rouyana* (Franch.) Kük. in Pflanzenr. (Engler) IV, 20 (Heft 38) [Cyper. Caric.]: 640 (1909). — *Carex filipes* Franch. & Sav. subsp. *rouyana* (Franch.) T. Koyama in J. Jap. Bot. 29: 42 (1954). — *Typus*: Japan, Prov. Musashi, Chichibu, *sine coll.* (holo-P, digital image!).

6a. var. *rouyana*

Distribution. Endemic to Japan: Honshu (Tohoku to Chugoku Dists.), Shikoku, Kyushu.

Japanese name. O-tamatsuri-suge.

Habitat. In open places at forest margin, 200–1000 m alt.

Chromosome number. $2n = 38, 50, 52$.

Additional specimens examined. Japan: Tochigi Pref., Nikko-shi, K. T. Takahashi 1005 (KYO), Utsunomiya-shi, K. T. Takahashi 1201 (KYO). Saitama Pref., Iino-shi, K. T. Takahashi 1211 (KYO). Mie Pref., Toba-shi, K. T. Takahashi 1126 (KYO).

6b. var. *arakiana* Ohwi in Acta. Phytotax. Geobot. 1: 299 (1932). — *Carex arakiana* (Ohwi) Ohwi in Mem. Coll. Sci. Kyoto Imp. Univ., Ser. B, Biol. 11: 424 (1936). — *Typus*: Japan, Pref. Kyoto, Mt. Oe, Y. Araki s.n., 22 May 1932 (holo– KYO!).

Distribution. Endemic to Japan: Honshu (Kinki Dist.), Shikoku.

Japanese name. Hirohano-o-tamatsuri-suge.

Habitat. In open places at forest margin, 500–800 m alt.

Chromosome number. $2n = 30$.

Additional specimens examined. Japan: Shiga Pref., Nagahama-shi, T. Noguchi 00424 (KYO). Mie

Pref., Inabe-shi, *J. Oda* 9801 (KYO).

7. *Carex sparsinix* C. B. Clarke ex Franch. in Nouv. Arch. Mus. Paris III, 10 : 66 (1898). — *Carex filipes* Franch. & Sav. var. *sparsinix* (C. B. Clarke ex Franch.) Kük. in Pflanzenr. (Engler) IV, 20 (Heft 38) [Cyper. Caric.]: 639 (1909). — *Typus*: China, Hupeh, Patung, *A. Henry* 3745 (holo-K, digital image!).

Distribution. Endemic to China: Jiangsu, Anhui, Zhejiang, Fujian, Hubei, Guizhou.

Japanese name. Shina-tsurisuge.

Habitat. In open places at forest margin, 300–900 m alt.

Chromosome number. Unknown.

Additional specimens examined. China: Jiangsu, Jurong, *X.-F. Jin et al.* 160513240 (OKAY).
Zhejiang, Jinhua, *Chen et al.* 15050205 (OKAY).

8. *Carex kujuzana* Ohwi in Acta Phytotax. Geobot. 1: 298 (1932). — *Typus*: Japan, Pref. Oita, Mt. Kuju, *J. Ohwi s.n.*, 1932 (holo– KYO!).

Carex dissitispicula Ohwi in Acta Phytotax. Geobot. 7: 35 (1938). — *Carex kujuzana* Ohwi var. *dissitispicula* (Ohwi) T. Koyama in J. Fac. Sci. Univ. Tokyo, Sect. 3, Bot. 8: 227 (1962). — *Typus*: Korea, Mt. Mutozan, *K. T. Cho s. n.*, 6 May 1937 (holo– KYO!)

Carex kunioi T. Koyama in J. Jap. Bot. 30: 131 (1955) — *Typus*: Japan, Pref. Nagano, Karuizawa, Mt. Kabutoyama, *K. Sato* 11061 (holo– TI!)

Carex fujimakii M. Kikuchi in Ann. Rep. Gakugei Fac. Iwate Univ. 13: 47, pl. 1 (1958) — *Typus*: Japan, Pref. Iwate, Kuroishino, *M. Kikuchi s.n.*, 6 June 1951 (holo– IUM, digital image!).

Distribution. Japan: Honshu (northward from Kanto Dist.), Kyushu, Korea, and China: Hangzhou.

Japanese name. Kuju-tsurisuge.

Habitat. In open places at forest margin, 400–900 m alt.

Chromosome number. $2n = 58$.

Additional specimens examined. Japan: Gunma Pref., Tsumagoi-mura, *K. T. Takahashi et al.* 1201 (KYO). Tokyo, Fuchu-shi, *K. T. Takahashi* 1187 (KYO). Oita Pref., Taketa-shi, *K. T. Takahashi* 1021 (KYO). Kumamoto Pref., Takamori-cho, *O. Yano et al.* (OKAY). China: Zhejiang, Hangzhou, *H. Ikeda et al.* 11050354 (OKAY).

9. *Carex arisanensis* Hayata in J. Coll. Sci. Imp. Univ. Tokyo 30: 378 (1911). — *Carex filipes* Franch. & Sav. subsp. *arisanensis* (Hayata) T. Koyama in J. Jap. Bot. Bot. 29: 41 (1954). — *Carex filipes* Franch. & Sav. Var. *arisanensis* (Hayata) T. Koyama in J. Fac. Sci. Univ. Tokyo, Sect. 3, Bot. 8: 228 (1962). — *Typus*: Taiwan, Mt. Arisan, *T. Kawakami & U. Mori* 3697 (holo– TI!).

Distribution. Japan: Ryukyu (Pref. Okinawa, Ishigaki Isl.), China: Fujian, Guangxi, Hunan, Zhejiang, and Taiwan.

Japanese name. Arisan-tamatsuri-suge.

Habitat. In open places at forest margin, 400–500 m alt.

Chromosome number. $2n = 44$.

Additional specimens examined. Japan: Okinawa Pref., Ishigaki-shi, *J. Oda* 1750 (KYO). China: Guangdong, Shaoguan, *Chen et al.* 14042257 (OKAY). Taiwan: Taipei, *J. Oda* 1680 (KYO).

10. *Carex vaniotii* H. Lév. in Bull. Acad. Int. Géogr. Bot. 11: 108 (1902). — *Carex filipes* Franch. & Sav. var. *vaniotii* (H. Lév.) Kük. in Pflanzenr. (Engler) IV, 20 (Heft 38) [Cyper. Caric.]: 641 (1909). — *Carex parciflora* Boott var. *vaniotii* (H. Lév.) Ohwi in Mem. Coll. Sci. Kyoto Imp. Univ., Ser. B, Biol. 5: 290 (1930), 'vanioti'. — *Carex jakiana* Boott subsp. *parciflora* (Boott) Kük. var. *vaniotii* (H. Lév.) T. Koyama in Bot. Mag. (Tokyo) 70: 356 (1957). — *Typus* : Japan, Mt. Iwate (Ganju), *U. Faurie* 1701 (holo–P!; iso– KYO !).

Distribution. Endemic to Japan: Honshu (northward from Chubu Dist.).

Japanese name. Nagabono-kojuzu-suge.

Habitat. Wet forests in subalpine zone, 1200–2000 m alt.

Chromosome number. $2n = 44$.

Additional specimens examined. JAPAN: Aomori Pref., Aomori-shi, *J. Oda & K. Yonekura* 8001 (KYO). Gifu Pref., Takayama-shi, *K. T. Takahashi et al.* 1205 (KYO). Fukui Pref., Katsuyama-shi, *J. Oda* 9783 (KYO).

11. *Carex egena* H. Lév. & Vaniot in Repert. Spec. Nov. Regni Veg. 4: 227 (1907). — *Typus*: Korea, Mont des diamants, *U. Faurie* 1366 (holo-P, digital image!).

Carex oligostachys Meinsh. ex Maxim. in Bull. Acad. Imp. Sci. Saint-Pétersbourg 31 : 117 (1887). — *Carex filipes* Franch. & Sav. var. *oligostachys* (Meinsh. ex Maxim.) Kük. in Pflanzenr. (Engler) IV, 20 (Heft 38) [Cyper. Caric.] : 641 (1909). — *Typus*: China, Mandshuria austroorientalis, *C. J. Maximowicz s.n.* 1860 (holo-LE, digital image !).

Distribution. Korea and China.

Japanese name. Hane-suge.

Habitat. In open places at forest margin, 400–1500 m alt.

Chromosome number. Unknown.

Additional specimens examined. Korea: Gyeonggi, Namyangju, *J. Oda* 9103 (KYO).

Acknowledgement

We express our sincere gratitude to Mr. Tatsunari Noguchi, Mr. Takashi Ajima, Dr. Hiroshi Ikeda, Dr. Akihiro Kakezawa, Mr. Satoru Kinoshita, Mr. Shuichi Kurogi, Ms. Nobuko Kuroiwa, Mr. Narihisa Matsuoka, Mr. Nobuyuki Numakunai, Mr. Takehiro Ohmori, Ms. Tsurae Ojio, Mr. Masayuki Ozawa, Mr. Takayuki Shibata, Mr. Hidehiko Shimizu, Mr. Shigenari Takahashi, Dr. Seita T. Watanabe and Mr. Kazuya Yamawaki for collecting samples. We are grateful to Dr. Chung Kyong-Sook for allowing us to use a specimen collected from Korea. Thanks are also due to the directors and staff members of GMNHJ, HYO, IUM, KPM, KYO, OKAY, OSA, P, SAPS, TI and TNS for allowing us to use their facilities and collections. We are grateful to Dr. Hiroshi Tobe and Dr. Hiroshi Ikeda for critically

reading the manuscript. This study was supported in part by JSPS KAKENHI (Grants-in-Aid for Scientific Research), Grant Numbers JP16H05763 and 22K05697, and JSPS-NRCT Joint Research Program, Grant number JPJSBP120229202.

References

- Abe, Y. 2003. Taxinomic notes of subsect. *Leucochlorae* (gen. *Carex*) in Akita Prefecture. Ann. Rep. Akita Pref. Mus. 28: 15–22 (in Japanese).
- Akiyama, S. 1955. Carices of the Far Eastern Region of Asia, Hokkaido Univ. Press, Sapporo (in Japanese).
- Buerkle, C. A. & L. H. Rieseberg. 2008. The rate of genome stabilization in homoploid hybrid species. Evolution 62: 266–275.
- Buerkle, C. A., R. J. Morris, M. A. Asmussen & L. H. Rieseberg. 2000. The likelihood of homoploid hybrid speciation. Heredity 84: 441–451.
- Cayouette J. & P. M. Catling. 1992. Hybridization in the genus *Carex* with special reference to North America. Bot. Rev. 58: 351–438.
- Dai, L. K., S. Y. Liang, S. R. Zhang, Y. C. Tang, T. Koyama & G. C. Tucker. 2010. *Carex* L. In: Wu, Z. Y., P. H. Raven & D. Y. Hong (eds.), Flora of China, vol. 23, pp. 285–461. Science Press, Beijing, and Missouri Botanical Garden Press, St. Louis.
- Darriba, D., G. L. Taboada, R. Doallo & D. Posada. 2012. jModelTest 2: more models, new heuristics and parallel computing. Nature, Meth. 9: 772.
- Doležel, J., S. Sgorbati & S. Lucretti. 1992. Comparison of three DNA fluorochromes for flow cytometric estimation of nuclear DNA content in plants. Physiol. Pl. 85: 625–631.
- Doležel, J., J. Greilhuber & J. Suda. 2007. Estimation of nuclear DNA content in plants using flow cytometry. Nature Protocols 2: 2233–2244.
- Dorey, J. E. 2019. Systematics of *Carex* Section *Laxflorae* (Cyperaceae). The City University of New York.

ProQuest Number: 27539862.

- Feng, S., M. Bai, I. Rivas-González, C. Li, S. Liu, Y. Tong, H. Yang, G. Chen, D. Xie, K. E. Sears, L. M. Franco, J. D. Gaitan-Espitia, R. F. Nespolo, W. E. Johnson, H. Yang & P. A. Brandies. 2022. Incomplete lineage sorting and phenotypic evolution in marsupials. *Cell* 185: 1–15.
- Franchet, A. 1898. Les *Carex* de l'Asie orientale III. *Nouv. Arch. Mus. Hist. Nat., Sér. 3*, 10: 39–120.
- Frodin, D. G. 2004. History and concepts of big plant genera. *Taxon* 53: 753–776.
- Fujiwara, R. 1999. A new hybrid of *Carex* from the Azumino plain, Nagano prefecture [Japan]. *Bull. Nagano Nature Conserv. Res. Inst.*: 23–26 (in Japanese).
- Gebauer, S., J. R. Starr & M. H. Hoffmann. 2015. Molecular phylogeny of the species-rich *Carex* sect. *Racemosae* (Cyperaceae) based on four nuclear and chloroplast markers. *Syst. Bot.* 40: 433–447.
- Goetghebeur, P. 1998. Cyperaceae. *In*: Kubitzki, K. (ed.), *The Families and Genera of Vascular Plants*, vol. IV. Flowering Plants: Monocotyledons, pp. 141–190. Springer, Berlin.
- Hipp, A. L., P. E. Rothrock & E. H. Roalson. 2009. The evolution of chromosome arrangements in *Carex* (Cyperaceae). *Bot. Rev.* 75: 96–109.
- Hoshino, T. 1981. Karyomorphological and cytogenetical studies on aneuploidy in *Carex*. *J. Sci. Hiroshima Univ., Ser. B, Div. 2, Bot.* 17: 155–238.
- Hoshino, T., T. Katsuyama, T. Masaki & M. Michikawa. 2020. *Carex* L. *In*: Iwatsuki, K., D. E. Boufford & H. Ohba (eds.), *Flora of Japan*, vol. IVa. Angiospermae: Monocotyledoneae (a), pp. 226–344. Kodansha, Tokyo.
- Jiménez Mejías, P., M. Hahn, K. Lueders, J. R. Starr, B. H. Brown, B. N. Chouinard, K. S. Chung, M. Escudero, B. A. Ford, K. A. Ford, S. Gebauer, B. Gehrke, M. H. Hoffmann, X. F. Jin, J. Jung, S. Kim, M. Luceño, E. Maguilla, S. Martín-Bravo, M. Míguez, A. Molina, R. F. C. Naczi, J. E. Pender, A. A. Reznicek, T. Villaverde, M. J. Waterway, K. L. Wilson, J. C. Yang, S. R. Zhang, A. L. Hipp & E. H. Roalson. 2016. Megaphylogenetic specimen-level approaches to the *Carex* (Cyperaceae) phylogeny

- using ITS, ETS, and *matK* sequences: Implications for classification. *Syst. Bot.* 41: 500–518.
- Katsuyama, T. 2015. *Carex* of Japan (enlarged and revised ed.). Bunichi Sogo Shuppan, Tokyo (in Japanese).
- Kobayashi, Y. H., S. Fuse & M. N. Tamura. 2022. Biosystematic studies on the family Piperaceae (Piperales) II. Incongruence between plastid and nuclear ITS phylogenetic trees of *Peperomia* subgenus *Micropiper* and revision of the section-equivalent groups based on ITS data. *Acta Phytotax. Geobot.* 73: 183–193.
- Koidzumi, G. 1931. Zengen (Preface). *In*: Mayebar, K., *Florula Austro-Higoensis*. Private publication (in Japanese).
- Koopman J. 2011. *Carex* Europaea. The genus *Carex* L. (*Cyperaceae*) in Europe, 1. – Margraf Publishers, Weikersheim.
- Kopecký, D., T. Felder, F. X. Schubiger, V. Mahelka, J. Bartos, J. Dolezel & B. Boller. 2018. Frequent occurrence of triploid hybrids *Festuca pratensis* × *F. apennina* in the Swiss Alps. *Alpine Bot.* 128: 121–132.
- Koyama, T. 1962. Classification of the family Cyperaceae (2). *J. Fac. Sci. Univ. Tokyo, Sect. 3, Bot.* 8: 149–278.
- Kükenthal, G. 1909. Cyperaceae-Caricoideae. *In*: Engler, H. G. A. (ed.), *Das Pflanzenreich* IV, 20 (Heft 38). W. Engelmann, Leipzig.
- Kurogi, S. 2019. A checklist of *Carex* of Miyazaki Prefecture. *Bull. Miyazaki Pref. Mus. Nat. Hist.* 39: 21–34 (in Japanese).
- Liang S. Y., L. K. Dai & Y. C. Tang. 2000. Caricoideae. *In*: Dai, L. K. & S. Y. Liang (eds.), *Flora Reipublicae Popularis Sinicae*, vol. 12, pp. 338–350. Science Press, Beijing (in Chinese).
- Martín Bravo, S., P. Jiménez Mejías, T. Villaverde, M. Escudero, M. Hahn, D. Spalink, E. H. Roalson, A. L. Hipp & the Global *Carex* Group (C. Benítez Benítez, L. P. Bruederle, E. Fitzek, B. A. Ford, K. A. Ford, M. Garner, S. Gebauer, M. H. Hoffmann, X. F. Jin, I. Larridon, É. Lévillé Bourret, Y. F. Lu, M.

- Luceño, E. Maguilla, J. I. Márquez Corro, M. Míguez, R. Naczi, A. A. Reznicek & J. R. Starr). 2019. A tale of worldwide success: Behind the scenes of *Carex* (Cyperaceae) biogeography and diversification. *J. Syst. Evol.* 57: 695–718.
- Murata, G. & H. Koyama. 1976. Sohayaki Element, defined by Dr. G. Koidzumi. *Mem. Natl. Sci. Mus.* (Tokyo) 9: 111–121 (in Japanese).
- Nagamasu, H. & S. Kinoshita. 2010. A natural hybrid between *Carex curvicollis* and *C. forficula* (Cyperaceae), and the lectotypification of *C. ×musashiensis*. *J. Jap. Bot.* 85: 297–302.
- Nagasawa, K., K. Sawa, H. Setoguchi, M. Maki & S. Sakaguchi. 2021. Two new natural hybrids between *Carex angustisquama* (Cyperaceae) and related species from solfatara fields in the Tohoku region, Japan. *Acta Phytotax. Geobot.* 72: 241–251.
- Nowak, M. D., A. T. M. Pedersen, A. K. Brysting, A. Schröder-Nielsen, E. Eleven & C. S. Bjorå. 2020. Testing hypotheses of hybrid origins for two seashore species of *Carex* section *Phacocystis* (Cyperaceae). *Bot. J. Linn. Soc.* 194: 100–117.
- Numakunai, N. 2017. Reddish staminate scales of *Carex filipes* var. *kuzakaiensis* (Cyperaceae). *J. Jap. Bot.* 92: 50–52 (in Japanese with English summary).
- Oda, J. & H. Nagamasu. 2008. Two new species of sect. *Capitellatae* (Cyperaceae) from Japan. *Acta Phytotax. Geobot.* 59: 55–66.
- Oda, J., S. Kinoshita & H. Nagamasu. 2018. *Carex ×ishimaensis*, a hybrid between *Carex boottiana* and *C. subdita* (Cyperaceae, sect. *Rhomboidales*) from Ishima Island, Tokushima Prefecture, Japan. *J. Jap. Bot.* 93: 269–277.
- Oda, J., S. Fuse, J. Yamashita & M. N. Tamura. 2019. Phylogeny and taxonomy of *Carex* (Cyperaceae) in Japan I. *C. sect. Rarae*. *Acta Phytotax. Geobot.* 70: 69–85.
- Ohmori, T. 2019. Revisional notes on the morphology and distribution of *Carex filipes* var. *kuzakaiensis* (Cyperaceae). *J. Jap. Bot.* 94: 165–172 (in Japanese with English summary).

- Ohwi, J. 1936. Cyperaceae Japonicae I. Mem. Coll. Sci. Kyoto Imp. Univ., Ser. B, Biol. 11: 417–428.
- Pedersen, A. T. M., M. D. Nowak, A. K. Brysting, R. Elven & C. S. Bjorå. 2016. Hybrid origins of *Carex rostrata* var. *borealis* and *C. stenolepis*, two problematic taxa in *Carex* section *Vesicariae* (Cyperaceae). PLoS One 12: e0171398.
- R Core Team. 2019. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.
- Rambaut, A., A. J. Drummond, D. Xie, G. Baele & M. A. Suchard. 2018. Posterior summarisation in Bayesian phylogenetics using Tracer 1.7. Syst. Biol. 67: 901–904.
- Rieseberg, L. H. 1997. Hybrid origins of plant species. Ann. Rev. Ecol. Syst. 28: 359–389.
- Řepka, R. & P. Mendel. 2022. *Carex ×favratii* (Cyperaceae), new record for Romania and evidence of its hybrid origin. Phytotaxa 550: 159–170.
- Roalson, E. H. 2008. A synopsis of chromosome number variation in the Cyperaceae. Bot. Rev. 74: 209–393.
- Roalson, E. H., J. T. Columbus & E. A. Friar. 2001. Phylogenetic relationships in Cariceae (Cyperaceae) based on ITS (nrDNA) and *trnT-L-F* (cpDNA) region sequences: Assessment of subgeneric and sectional relationships in *Carex* with emphasis on section *Acrocystis*. Syst. Bot. 26: 318–341.
- Roalson E. H., P. Jiménez-Mejías, A. L. Hipp, C. Benítez-Benítez, L. P. Bruederle, K.S. Chung, M. Escudero, B. A. Ford, K. Ford, S. Gebauer, B. Gehrke, M. Hahn, M. Q. Hayat, M. H. Hoffmann, X.-F. Jin, S. Kim, I. Larridon, É. Lévillé-Bourret, Y.-F. Lu, M. Luceño, E. Maguilla, J. I. Márquez-Corro, S. Martín-Bravo, T. Masaki, M. Míguez, R. F. C. Naczi, A. A. Reznicek, D. Spalink, J. R. Starr, Uzma, T. Villaverde, M. J. Waterway, K. L. Wilson & S.-R. Zhang. 2021. A framework infrageneric classification of *Carex* (Cyperaceae) and its organizing principles. J. Syst. Evol. 59: 726–762.
- Shaw, J., E. B. Lickey, J. T. Beck, S. B. Farmer, W. Liu, J. Miller, K. C. Siripun, C. T. Winder, E. E. Schilling & R. L. Small. 2005. The tortoise and the hare II: relative utility of 21 noncoding chloroplast DNA

- sequences for phylogenetic analysis. *Amer. J. Bot.* 92: 142–166.
- Starr, J. R., S. A. Harris & D. A. Simpson. 2003. Potential of the 5' and 3' ends of the intergenic spacer (IGS) of rDNA in the Cyperaceae: New sequences for lower level phylogenies in sedges with an example from *Uncinia* Pers. *Int. J. Pl. Sci.* 164: 213–227.
- Starr, J. R., S. A. Harris & D. A. Simpson. 2004. Phylogeny of the unispicate taxa in Cyperaceae tribe Cariceae I: genetic relationship and evolutionary Scenarios. *Syst. Bot.* 29: 528–544.
- Starr, J. R., R. F. C. Naczi, & B. N. Chouinard. 2009. Plant DNA barcodes and species resolution in sedges (*Carex*, Cyperaceae). *Molec. Ecol. Resources* 9 (Suppl. 1): 151–163.
- Stewart, C. N. Jr. & L. E. Via. 1993. A rapid CTAB DNA isolation technique useful for RAPD fingerprinting and other PCR applications. *BioTechniques* 14: 748–750.
- Suchard M. A., P. Lemey, G. Baele, D. L. Ayres, A. J. Drummond & A. Rambaut. 2018. Bayesian phylogenetic and phylodynamic data integration using BEAST 1.10. *Virus Evolution* 4: vey016.
- Swofford, D. L. 2002. PAUP*: Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4 beta 10. Sinauer, Sunderland.
- Taberlet, P., L. Gielly, G. Pautou & J. Bouvet. 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molec. Biol.* 17: 1105–1109.
- Takahashi, K. T., T. Noguchi, J. Oda, S. Fuse & M. N. Tamura. 2022. *Carex nasuensis*, a new species from Japan. *Acta Phytotax. Geobot.* 73: 107–118.
- Takahashi, K. T., J. Oda, S. Fuse & M. N. Tamura. 2021. Biosystematic studies of *Carex* (Cyperaceae) I. Molecular phylogenetic analysis of the *C. macroglossa* complex with reference to variation in morphology, chromosomal features and species delimitation. *Acta Phytotax. Geobot.* 72: 81–91.
- Tanaka, N. 1948. The Problem of Aneuploidy. *Biological Contributions in Japan*, vol. 4, pp. 1–327. Hokuryukan, Tokyo (in Japanese).
- Twyford, A. D. & R.A. Ennos. 2012. Next-generation hybridization and introgression. *Heredity*. 108: 179–

- Villaverde T., P. Jiménez-Mejías, M. Luceño, M. J. Waterway, S. Kim, B. Lee, M. Rincón-Barrado, M. Hahn, E. Maguilla, E. H. Roalson, A. L. Hipp & the Global Carex Group. 2020. A new classification of *Carex* (Cyperaceae) subgenera supported by a HybSeq backbone phylogenetic tree. Bot. J. Linn. Soc. 194: 141–163.
- Więclaw, H. & M. Wilhelm. 2014. Natural hybridization within the *Carex flava* complex (Cyperaceae) in Poland: Morphometric studies. Ann. Bot. Fennici 51: 129–147.
- Xu, D. H., J. Abe, M. Sakai, A. Kanazawa & Y. Shimamoto. 2000. Sequence variation of non-coding regions of chloroplast DNA of soybean and related wild species and its implications for the evolution of different chloroplast haplotypes. Theor. Appl. Genet. 101: 724–732.
- Yano, O., T. Katsuyama & T. Hoshino. 2008. Cytological studies of Japanese *Carex* (Cyperaceae) I. J. Jpn. Cyperol. (13): 9–21 (in Japanese).
- Yano, O., H. Ikeda & T. Hoshino. 2010. Phylogeography of the Japanese common sedge, *Carex conica* complex (Cyperaceae), based on chloroplast DNA sequence data and chromosomal variation. Amer. J. Bot. 97: 1365–1376.

Tables

Table 1. Comparison of classifications of *Carex filipes* complex among the previous and present studies. Shaded cells indicate the taxa that were/are assigned to the species of *C. filipes*.

Original description of taxa	Franchet (1898)	Kükenthal (1909)	Ohwi (1936)	Akiyama (1955)	Koyama (1962)	Liang <i>et al.</i> (2000)	Dai <i>et al.</i> (2010)	Hoshino <i>et al.</i> (2020)	The present study
<i>Carex filipes</i> Franch. & Sav.	<i>C. filipes</i>	<i>C. filipes</i> var. <i>filipes</i>	<i>C. filipes</i> var. <i>filipes</i>	<i>C. filipes</i> var. <i>filipes</i>	<i>C. filipes</i> var. <i>filipes</i>	<i>C. filipes</i> var. <i>filipes</i>	<i>C. filipes</i> var. <i>filipes</i>	<i>C. filipes</i> var. <i>filipes</i>	<i>C. filipes</i> var. <i>filipes</i>
<i>C. sparsinux</i> C. B. Clarke ex Franch.	<i>C. sparsinux</i>	<i>C. filipes</i> var. <i>sparsinux</i>	-	-	(= <i>C. filipes</i> var. <i>rouyana</i>)	<i>C. filipes</i> var. <i>sparsinux</i>	(= <i>C. filipes</i> var. <i>filipes</i>)	-	<i>C. sparsinux</i> <i>C. kujuzana</i>
<i>C. rouyana</i> Franch.	<i>C. rouyana</i>	<i>C. filipes</i> var. <i>rouyana</i>	<i>C. filipes</i> var. <i>rouyana</i>	<i>C. filipes</i> var. <i>rouyana</i>	<i>C. filipes</i> var. <i>rouyana</i>	(= <i>C. filipes</i> var. <i>sparsinux</i>)	(= <i>C. filipes</i> var. <i>oligostachys</i>)	<i>C. rouyana</i>	<i>C. rouyana</i> var. <i>rouyana</i> <i>C. sohayakiensis</i>
<i>C. oligostachys</i> Meinsh. ex Maxim.	<i>C. oligostachys</i>	<i>C. filipes</i> var. <i>oligostachys</i>	<i>C. filipes</i> var. <i>oligostachys</i>	<i>C. filipes</i> var. <i>oligostachys</i>	<i>C. filipes</i> var. <i>oligostachys</i>	<i>C. egena</i>	<i>C. filipes</i> var. <i>oligostachys</i>	-	<i>C. egena</i>
<i>C. vaniotii</i> H. Lév.	-	<i>C. filipes</i> var. <i>vaniotii</i>	<i>C. vaniotii</i>	<i>C. vaniotii</i>	<i>C. jakiana</i> subsp. <i>parciflora</i> var. <i>vaniotii</i>	-	-	<i>C. vaniotii</i>	<i>C. vaniotii</i>
<i>C. arisanensis</i> Hayata var. <i>tremula</i> Ohwi	-	-	<i>C. filipes</i> var. <i>tremula</i>	<i>C. filipes</i> var. <i>tremula</i>	<i>C. filipes</i> var. <i>tremula</i>	-	-	(= <i>C. filipes</i> var. <i>filipes</i>)	<i>C. filipes</i> var. <i>tremula</i> <i>C. curtispica</i>
<i>C. rouyana</i> Franch. var. <i>arakiana</i> Ohwi	-	-	<i>C. filipes</i> var. <i>arakiana</i>	<i>C. filipes</i> var. <i>arakiana</i>	<i>C. filipes</i> var. <i>arakiana</i>	-	-	<i>C. arakiana</i>	<i>C. rouyana</i> var. <i>arakiana</i>
<i>C. filipes</i> Franch. & Sav. subsp. <i>kuzakaiensis</i> M. Kikuchi	-	-	-	-	<i>C. filipes</i> var. <i>kuzakaiensis</i>	-	-	<i>C. filipes</i> var. <i>kuzakaiensis</i>	<i>C. kuzakaiensis</i>
<i>C. arisanensis</i> Hayata	-	<i>C. arisanensis</i>	<i>C. arisanensis</i>	<i>C. arisanensis</i>	<i>C. filipes</i> var. <i>arisanensis</i>	<i>C. arisanensis</i>	<i>C. arisanensis</i>	<i>C. arisanensis</i>	<i>C. arisanensis</i>

Table 2. Sources of materials used in the present morphological and molecular analyses with accession numbers

deposited in DDBJ (DNA Data Bank of Japan). Sample codes correspond to those of Figs. 3 and 4.

Sample	Taxon before the Code present study	Taxon after the Code present study	Locality	Voucher	Accession Number									
					atpB-rbcL	psbA-trnK	rpl16-rps3	rpl6	trnK-trnK	trnL-trnF	ITS	ETS		
A1-1	<i>C. filipes</i> var. <i>filipes</i>	<i>C. filipes</i> var. <i>filipes</i>	Japan: Pref. Tochigi: Nikko-shi	<i>Takahashi 1026</i> (KYO)	LC715304	LC715323	LC715337	LC715351	LC715374	LC715388	LC715402			
A1-2	<i>C. filipes</i> var. <i>filipes</i>	<i>C. filipes</i> var. <i>filipes</i>	Japan: Pref. Ibaraki: Hitachiotashi	<i>Takahashi 1025</i> (KYO)	LC630617	LC630663	LC630755	LC630709	LC630999	LC631045	LC631091			
A1-3	<i>C. filipes</i> var. <i>filipes</i>	<i>C. filipes</i> var. <i>filipes</i>	Japan: Pref. Ibaraki: Hitachi-shi	<i>Takahashi 1027</i> (KYO)	LC71609	LC717153	LC717161	LC717197	LC722051	LC721825	LC721897			
A1-4	<i>C. filipes</i> var. <i>filipes</i>	<i>C. filipes</i> var. <i>filipes</i>	Japan: Pref. Gunma: Kawaba-mura	<i>Ohmori 9515</i> (GMNHJ)	LC71620	LC717164	LC717162	LC717190	LC722062	LC721836	LC721908			
A1-5	<i>C. filipes</i> var. <i>filipes</i>	<i>C. filipes</i> var. <i>filipes</i>	Japan: Pref. Tokyo: Hachioji-shi	<i>Takahashi 1186</i> (KYO)	LC715309	LC715329	LC715343	LC715357	LC715380	LC715394	LC715408			
A1-6	<i>C. filipes</i> var. <i>filipes</i>	<i>C. filipes</i> var. <i>filipes</i>	<i>Takahashi 1183</i> (KYO)		LC715314	LC715327	LC715342	LC715355	LC715378	LC715392	LC715406			
A1-7	<i>C. filipes</i> var. <i>filipes</i>	<i>C. filipes</i> var. <i>filipes</i>	Japan: Pref. Hiroshima: Miyoshi-shi	<i>Takahashi 1017</i> (KYO)	LC71606	LC717150	LC717167	LC717196	LC722048	LC721822	LC721894			
A1-8	<i>C. filipes</i> var. <i>filipes</i>	<i>C. filipes</i> var. <i>filipes</i>	Japan: Pref. Osaka: Chihayaakasaki-mura	<i>Takahashi 1200</i> (KYO)	LC71667	LC717181	LC717179	LC722037	LC722109	LC721883	LC721955			
A1-9	<i>C. filipes</i> var. <i>filipes</i>	<i>C. filipes</i> var. <i>filipes</i>	Japan: Pref. Shiga: Maibara-shi	<i>Takahashi 1201</i> (KYO)	LC71659	LC7171803	LC717171	LC722029	LC722101	LC721875	LC721947			
A2-1	<i>C. filipes</i> var. <i>filipes</i>	<i>C. filipes</i> var. <i>filipes</i>	Japan: Pref. Nagano: Karuizawa-cho	<i>Takahashi 1184</i> (KYO)	LC715315	LC715328	LC715339	LC715356	LC715379	LC715393	LC715407			
A3-1	<i>C. filipes</i> var. <i>filipes</i>	<i>C. filipes</i> var. <i>filipes</i>	Japan: Pref. Gifu: Seki-shi	<i>Takahashi 1013</i> (KYO)	LC715313	LC715322	LC715336	LC715350	LC715373	LC715387	LC715401			
A4-1	<i>C. filipes</i> var. <i>filipes</i>	<i>C. filipes</i> var. <i>filipes</i>	Japan: Pref. Okayama: Mimasaka-shi	<i>Takahashi et al. 1180</i> (KYO)	LC71650	LC717194	LC717122	LC722020	LC722092	LC721866	LC721938			
A4-2	<i>C. filipes</i> var. <i>filipes</i>	<i>C. filipes</i> var. <i>filipes</i>	Japan: Pref. Okayama: Okayama-shi	<i>Takahashi & Yano 1125</i> (KYO)	LC715308	LC715326	LC715341	LC715354	LC715377	LC715391	LC715405			
A5-1	<i>C. filipes</i> var. <i>filipes</i>	<i>C. filipes</i> var. <i>filipes</i>	Japan: Pref. Tokushima: Miyoshi-shi	<i>Takahashi et al. 1114</i> (KYO)	LC715312	LC715325	LC715340	LC715353	LC715376	LC715390	LC715404			
A5-2	<i>C. filipes</i> var. <i>filipes</i>	<i>C. filipes</i> var. <i>filipes</i>	Japan: Pref. Ehime: Seiyu-shi	<i>Takahashi 1159</i> (KYO)	LC71638	LC717182	LC717170	LC722008	LC722108	LC721850	LC721926			
A5-3	<i>C. filipes</i> var. <i>filipes</i>	<i>C. filipes</i> var. <i>filipes</i>	Japan: Pref. Ehime: Kumakogen-cho	<i>Takahashi 1031</i> (KYO)	LC71610	LC717154	LC717162	LC717180	LC722052	LC721826	LC721898			
A5-4	<i>C. filipes</i> var. <i>filipes</i>	<i>C. filipes</i> var. <i>filipes</i>	Japan: Pref. Ehime: Kumakogen-cho	<i>Takahashi 1034</i> (KYO)	LC715311	LC715324	LC715338	LC715352	LC715375	LC715389	LC715403			
A5-5	<i>C. filipes</i> var. <i>filipes</i>	<i>C. filipes</i> var. <i>filipes</i>	Japan: Pref. Tokushima: Miyoshi-shi	<i>Takahashi 1114</i> (KYO)	LC71628	LC717172	LC717170	LC722007	LC722070	LC721844	LC721916			
B1-1	<i>C. arakiana</i>	<i>C. kuzakaisensis</i>	Japan: Pref. Hiroshima: Shobara-shi	<i>Takahashi et al. 1181</i> (KYO)	LC71651	LC717195	LC717123	LC722021	LC722093	LC721867	LC721939			
B1-2	<i>C. arakiana</i>	<i>C. kuzakaisensis</i>	Japan: Pref. Tottori: Kofu-cho	<i>Takahashi et al. 1182</i> (KYO)	LC71652	LC717196	LC717124	LC722022	LC722094	LC721868	LC721940			
B1-3	<i>C. arakiana</i>	<i>C. kuzakaisensis</i>	Japan: Pref. Gifu: Takayama-shi	<i>Takahashi 1035</i> (KYO)	LC71613	LC717157	LC717165	LC717183	LC722055	LC721829	LC721901			
B1-4	<i>C. arakiana</i>	<i>C. kuzakaisensis</i>	Japan: Pref. Niigata: Myoko-shi	<i>Takahashi 1061</i> (KYO)	LC71619	LC717163	LC717161	LC717189	LC722061	LC721831	LC721907			
B1-5	<i>C. filipes</i> var. <i>kuzakaisensis</i>	<i>C. kuzakaisensis</i>	Japan: Pref. Gunma: Katashina-mura	<i>Takahashi 1060</i> (KYO)	LC71618	LC717162	LC717160	LC717188	LC722060	LC721834	LC721906			
B1-6	<i>C. filipes</i> var. <i>kuzakaisensis</i>	<i>C. kuzakaisensis</i>	Japan: Pref. Tochigi: Nikko-shi	<i>Takahashi 1058</i> (KYO)	LC71617	LC717161	LC717159	LC717187	LC722059	LC721833	LC721905			
B2-1	<i>C. filipes</i> var. <i>kuzakaisensis</i>	<i>C. kuzakaisensis</i>	Japan: Pref. Iwate: Takizawa-shi	<i>Takahashi 1024</i> (KYO)	LC71608	LC717152	LC717160	LC717178	LC722050	LC721824	LC721896			
B2-2	<i>C. filipes</i> var. <i>kuzakaisensis</i>	<i>C. kuzakaisensis</i>	Japan: Pref. Iwate: Miyako-shi	<i>Takahashi 1212</i> (KYO)	LC71666	LC717180	LC717178	LC722036	LC722108	LC721882	LC721954			
B3-1	<i>C. filipes</i> var. <i>filipes</i>	<i>C. filipes</i> var. <i>tremula</i>	Japan: Pref. Nara: Totsukawa-mura	<i>Takahashi & Oda 1166</i> (KYO)	LC71641	LC717185	LC717173	LC722011	LC722083	LC721857	LC721929			
B3-2	<i>C. filipes</i> var. <i>filipes</i>	<i>C. filipes</i> var. <i>tremula</i>	Japan: Pref. Wakayama: Tanabe-shi	<i>Takahashi 1002</i> (KYO)	LC71600	LC717144	LC717162	LC717190	LC722042	LC721816	LC721888			
B4-1	<i>C. filipes</i> var. <i>filipes</i>	<i>C. filipes</i> var. <i>tremula</i>	Japan: Pref. Ehime: Kumakogen-cho	<i>Takahashi 1163</i> (KYO)	LC71639	LC717183	LC717171	LC722009	LC722081	LC721855	LC721927			
B4-2	<i>C. filipes</i> var. <i>filipes</i>	<i>C. filipes</i> var. <i>tremula</i>	Japan: Pref. Ehime: Beppu-cho	<i>Takahashi 1169</i> (KYO)	LC71644	LC717188	LC717176	LC722014	LC722086	LC721869	LC721942			
B4-3	<i>C. filipes</i> var. <i>filipes</i>	<i>C. filipes</i> var. <i>tremula</i>	Japan: Pref. Kumamoto: Aso-shi	<i>Takahashi 1170</i> (KYO)	LC71645	LC717189	LC717177	LC722015	LC722087	LC721861	LC721933			
B4-4	<i>C. filipes</i> var. <i>filipes</i>	<i>C. filipes</i> var. <i>tremula</i>	Japan: Pref. Miyazaki: Shiiba-son	<i>Takahashi 1216</i>	LC71668	LC717182	LC717170	LC722038	LC722110	LC721884	LC721956			
B4-5	<i>C. filipes</i> var. <i>filipes</i>	<i>C. filipes</i> var. <i>tremula</i>	Japan: Pref. Miyazaki: Miyakonojo-shi	<i>Takahashi 1217</i>	LC71663	LC717180	LC717178	LC722033	LC722105	LC721879	LC721951			
B4-6	<i>C. filipes</i> var. <i>filipes</i>	<i>C. filipes</i> var. <i>tremula</i>	Japan: Pref. Nagasaki: Tsushima-shi	<i>Takahashi & Yano 1209</i>	LC71664	LC717180	LC717176	LC722034	LC722106	LC721880	LC721952			
B5-1	<i>C. filipes</i> var. <i>filipes</i>	<i>C. filipes</i> var. <i>tremula</i>	Japan: Pref. Kagoshima: Satsuma-cho	<i>Oda 4109</i> (KYO)	LC71621	LC717165	LC717163	LC717191	LC722063	LC721837	LC721909			
C1-1	<i>C. roaryana</i>	<i>C. sohayakiensis</i>	Japan: Pref. Nara: Totsukawa-mura	<i>Takahashi & Oda 1167</i> (KYO)	LC71642	LC717186	LC717174	LC722012	LC722084	LC721858	LC721930			
C1-2	<i>C. roaryana</i>	<i>C. sohayakiensis</i>	Japan: Pref. Nara: Totsukawa-mura	<i>Takahashi & Oda 1168</i> (KYO)	LC71643	LC717187	LC717175	LC722013	LC722085	LC721859	LC721931			
C2-1	<i>C. roaryana</i>	<i>C. sohayakiensis</i>	Japan: Pref. Miyazaki: Hinokage-cho	<i>Takahashi & Kurogi 1178</i> (KYO)	LC71649	LC717193	LC717121	LC722019	LC722091	LC721866	LC721937			
C3-1	<i>C. roaryana</i>	<i>C. sohayakiensis</i>	Japan: Pref. Kochi: Niyodogawa-cho	<i>Takahashi 1165</i> (KYO)	LC71640	LC717184	LC717172	LC722010	LC722082	LC721856	LC721928			
C4-1	<i>C. roaryana</i>	<i>C. kurogii</i>	Japan: Pref. Kagawa: Takamatsu-shi	<i>Takahashi et al. 1113</i> (KYO)	LC71627	LC717171	LC717169	LC722007	LC722069	LC721843	LC721915			
C4-2	<i>C. roaryana</i>	<i>C. kurogii</i>	Japan: Pref. Tokushima: Mima-shi	<i>Takahashi 1009</i> (KYO)	LC71602	LC717146	LC717164	LC722012	LC722084	LC721818	LC721890			
C5-1	<i>C. roaryana</i>	<i>C. kurogii</i>	Japan: Pref. Kyoto: Oe-cho	<i>Takahashi et al. 1015</i> (KYO)	LC71604	LC717148	LC717176	LC722016	LC722088	LC721862	LC721934			
D1-1	<i>C. filipes</i> var. <i>filipes</i>	<i>C. curtispica</i>	Japan: Pref. Ehime: Kumakogen-cho	<i>Takahashi 1033</i> (KYO)	LC71611	LC717155	LC717163	LC717181	LC722053	LC721827	LC721899			
D1-2	<i>C. filipes</i> var. <i>filipes</i>	<i>C. curtispica</i>	Japan: Pref. Ehime: Kumakogen-cho	<i>Takahashi 1033</i> (KYO)	LC71612	LC717156	LC717164	LC722054	LC722086	LC721828	LC721900			
D2-1	<i>C. filipes</i> var. <i>filipes</i>	<i>C. curtispica</i>	Japan: Pref. Ehime: Uchiko-cho	<i>Oda 2233</i> (KYO)	LC71625	LC717169	LC717167	LC722027	LC722067	LC721841	LC721913			
D3-1	<i>C. filipes</i> var. <i>filipes</i>	<i>C. curtispica</i>	Japan: Pref. Saga: Kanzaki-shi	<i>Takahashi et al. 1158</i> (KYO)	LC71637	LC717181	LC717179	LC722007	LC722079	LC721853	LC721925			
D4-1	<i>C. filipes</i> var. <i>filipes</i>	<i>C. curtispica</i>	Japan: Pref. Miyazaki: Hinokage-cho	<i>Takahashi & Kurogi 1177</i> (KYO)	LC71648	LC717192	LC717120	LC722018	LC722090	LC721864	LC721936			
E1-1	<i>C. nasuensis</i>	<i>C. nasuensis</i>	Japan: Pref. Ibaraki: Shirosato-cho	<i>Takahashi 1007</i> (KYO)	LC715306	LC715320	LC715334	LC715348	LC715371	LC715385	LC715399			
E1-2	<i>C. nasuensis</i>	<i>C. nasuensis</i>	Japan: Pref. Tochigi: Nasushiobara-shi	<i>Takahashi 1012</i> (KYO)	LC715304	LC715318	LC715332	LC715346	LC715369	LC715383	LC715397			
E1-3	<i>C. nasuensis</i>	<i>C. nasuensis</i>	Japan: Pref. Tochigi: Tochigi-shi	<i>Takahashi 1003</i> (KYO)	LC715305	LC715319	LC715333	LC715347	LC715370	LC715384	LC715398			
E1-4	<i>C. nasuensis</i>	<i>C. nasuensis</i>	Japan: Pref. Kyoto: Oyamazaki-cho	<i>kurosaki 23052</i> (KYO)	LC715307	LC715321	LC715335	LC715349	LC715372	LC715386	LC715400			
E2-1	<i>C. roaryana</i>	<i>C. kurogii</i>	Japan: Pref. Miyazaki: Takachiho-cho	<i>Takahashi & Kurogi 1174</i> (KYO)	LC71646	LC717190	LC717178	LC722016	LC722088	LC721862	LC721934			
E2-2	<i>C. roaryana</i>	<i>C. kurogii</i>	Japan: Pref. Miyazaki: Shiiba-son	<i>Takahashi & Kurogi 1176</i> (KYO)	LC71647	LC717191	LC717179	LC722017	LC722089	LC721863	LC721935			
E3-1	<i>C. roaryana</i>	<i>C. roaryana</i> var. <i>roaryana</i>	Japan: Pref. Mie: Toba-shi	<i>Takahashi 1126</i> (KYO)	LC71629	LC717173	LC717171	LC722019	LC722071	LC721845	LC721917			
E3-2	<i>C. arakiana</i>	<i>C. roaryana</i> var. <i>arakiana</i>	Japan: Pref. Mie: Inabe-shi	<i>Oda 9801</i> (KYO)	LC71599	LC717143	LC717161	LC722016	LC722041	LC721815	LC721887			
E4-1	<i>C. roaryana</i>	<i>C. kurogii</i>	Japan: Pref. Hyogo: Kobe-shi	<i>Takahashi 1197</i> (KYO)	LC71657	LC717180	LC717179	LC722027	LC722099	LC721873	LC721945			
E4-2	<i>C. roaryana</i>	<i>C. kurogii</i>	Japan: Pref. Hiroshima: Miyoshi-shi	<i>Sera 19846</i> (OKAY)	LC71655	LC717199	LC717127	LC722025	LC722097	LC721871	LC721943			
E4-3	<i>C. roaryana</i>	<i>C. roaryana</i> var. <i>roaryana</i>	Japan: Pref. Tochigi: Nikko-shi	<i>Takahashi 1005</i> (KYO)	LC71601	LC717145	LC717163	LC717191	LC722043	LC721817	LC721889			
E4-4	<i>C. roaryana</i>	<i>C. roaryana</i> var. <i>roaryana</i>	Japan: Pref. Tochigi: Utsunomiya-shi	<i>Takahashi 1201</i> (KYO)	LC71669	LC717183	LC717141	LC722039	LC722071	LC721885	LC721957			
E4-5	<i>C. roaryana</i>	<i>C. kurogii</i>	Japan: Pref. Tochigi: Utsunomiya-shi	<i>Takahashi 1203</i> (KYO)	LC71661	LC717189	LC717143	LC722031	LC722050	LC721878	LC721949			
E4-6	<i>C. roaryana</i>	<i>C. roaryana</i> var. <i>roaryana</i>	Japan: Pref. Saitama: Iino-shi	<i>Takahashi 1211</i>	LC71665	LC717180	LC717137	LC722035	LC722107	LC721871	LC721953			
E5-1	<i>C. arakiana</i>	<i>C. roaryana</i> var. <i>arakiana</i>	Japan: Pref. Shiga: Nagahama-shi	<i>Noguchi 00424</i> (KYO)	LC71656	LC717180	LC717128	LC722026	LC722098	LC721842	LC721944			
F1-1	<i>C. laxa</i>	<i>C. laxa</i>	Japan: Hokkaido: Tomakomai-shi	<i>Takahashi 1137</i> (KYO)	LC71630	LC717174	LC717102	LC722000	LC722072	LC721876	LC721918			
F2-1	<i>C. laxa</i>	<i>C. laxa</i>	Japan: Pref. Tochigi: Nikko-shi	<i>Hasegawa</i> (OKAY)	LC71636	LC717180	LC717108	LC722006	LC722078	LC721852	LC721924			
G1-1	<i>C. kujuzana</i>	<i>C. kujuzana</i>	Japan: Pref. Gunma: Tsumagoi-mura	<i>Takahashi et al. 1201</i> (KYO)	LC71660	LC717184	LC717132	LC722030	LC722102	LC721876	LC721942			
G1-2	<i>C. kujuzana</i>	<i>C. kujuzana</i>	Japan: Pref. Tokyo: Fuchu-shi	<i>Takahashi 1187</i> (KYO)	LC71653	LC717197	LC717125	LC722023	LC722095	LC721869	LC721941			
G1-3	<i>C. kujuzana</i>	<i>C. kujuzana</i>	Japan: Pref. Kumamoto: Takamori-cho	<i>Yano et al.</i> (OKAY)	LC71654	LC717198	LC717126	LC722024	LC722096	LC721870	LC721942			
G1-4	<i>C. kujuzana</i>	<i>C. kujuzana</i>	Japan: Pref. Ehime: Taketa-shi	<i>Takahashi 1021</i> (KYO)	LC71607	LC717151	LC717169	LC722017	LC722049	LC721823	LC721895			
G2-1	<i>C. filipes</i> var. <i>sparsinax</i>	<i>C. kujuzana</i>	China: Prov. Zhejiang: Hangzhou	<i>Iheda et al. 110503</i>										

Table 3.. Name, direction, nucleotide sequence and source of primers used in this study. Primers with an asterisk were used only for DNA sequencing; those without an asterisk were used for both PCR amplification and DNA sequencing.

Primer name	Direction	Sequence (5'–3')	Source
[<i>atpB–rbcL</i>]			
f1	Forward	GTACTGGGCCAATAATTGA	Yano <i>et al.</i> (2010)
r	Reverse	CAACACTTGCTTAGTCTCTG	Xu <i>et al.</i> (2000)
[<i>rpl16–rpl16–rps3</i>]			
rpL16F71	Forward	GCTATGCTTAGTGTGTGACTCGTTG	Shaw <i>et al.</i> (2005)
rpL16R1516	Reverse	CCCTTCATTCTTCCTCTATGTTG	Shaw <i>et al.</i> (2005)
rpL–03F*	Forward	CCAACCTATTGCTTCGTATTATC	Oda <i>et al.</i> (2019)
rpL–04R*	Reverse	CATAGCTTCTCTATTTCATGATTAGG	Oda <i>et al.</i> (2019)
[<i>rps16</i>]			
rpS16F	Forward	AAACGATGTGGTARAAAGCAAC	Shaw <i>et al.</i> (2005)
rpS16R	Reverse	AACATCWATTGCAASGATTGAT	Shaw <i>et al.</i> (2005)
[<i>trnL–trnL–trnF</i>]			
trnL–c*	Forward	CGAAATCGGTAGACGCTACG	Taberlet <i>et al.</i> (1991)
trnF–f*	Reverse	ATTTGAAGTGGTGACACGAG	Taberlet <i>et al.</i> (1991)
trnL–e	Forward	GGTTCAAGTCCCTCTATCCC	Taberlet <i>et al.</i> (1991)
trnL–d	Reverse	GGGGATAGAGGGACTTGAAC	Taberlet <i>et al.</i> (1991)
[<i>trnK–matK–trnK</i>]			
trnK–66f	Forward	AATTCGTCCAGACTTTTGG	newly designed
trnK–940R	Reverse	ATATAAGCATTGTTGTTGACG	Gebauer <i>et al.</i> (2015)
matK–2.1f	Forward	CCTATCCATCTGGAAATCTTAG	Starr <i>et al.</i> (2009)
matK–5f	Reverse	GTTCTAGCACAAGAAAGTCG	Starr <i>et al.</i> (2009)
matK–2180F	Forward	GATATTATTAGTCGATTGGTCG	newly designed
trnK–2690R	Reverse	GAGCTAATCGGATGGAGTAG	newly designed
[ITS]			
ITS5i	Forward	AGGTGACCTGCGGAAGGATCATT	Roalson <i>et al.</i> (2001)
ITS4i	Reverse	GGTAGTCCCCTGACCTGG	Roalson <i>et al.</i> (2001)
[ETS]			
ETS–1F	Forward	CTGTGGCGTCGCATGAGTTG	Starr <i>et al.</i> (2003)
18S–R	Reverse	AGACAAGCATATGACTACTGGCAGG	Starr <i>et al.</i> (2003)

Table 4.. Putative hybrids between taxa positioned in the different clades, and the heterozygous sites along nr (ITS and ETS) DNA sequences. For the voucher specimen of each sample, see information of the same sample code in Table 2.

Taxon before the present study	Taxon after the present study	Sample code	Locality	Heterozygous sites and their positions (bp)*				
				ITS		ETS		
				65	416	81	84	288
Putative parents								
<i>Carex rouyana</i>	<i>C. sohayakiensis</i>	C2-1	Pref. Miyazaki: Hinokage-cho	T	C	G	T	T
<i>C. rouyana</i>	<i>C. rouyana</i>	E4-3	Pref. Tochigi: Nikko-shi	C	G	T	C	C
Putative hybrids								
<i>C. rouyana</i>	<i>C. ×kurogii</i>	C4-1	Pref. Kagawa: Takamatsu-shi	T/C	C/G	G/T	T/C	T/C
<i>C. rouyana</i>	<i>C. ×kurogii</i>	C4-2	Pref. Tokushima: Mima-shi	T/C	C/G	G/T	T/C	T/C
<i>C. arakiana</i>	<i>C. ×kurogii</i>	C5-1	Pref. Kyoto: Oe-cho	T/C	C/G	G/T	T/C	T/C
<i>C. rouyana</i>	<i>C. ×kurogii</i>	E2-1	Pref. Miyazaki: Takachiho-cho	T/C	C/G	G/T	T/C	T/C
<i>C. rouyana</i>	<i>C. ×kurogii</i>	E2-2	Pref. Miyazaki: Shiba-son	T/C	C/G	G/T	T/C	T/C
<i>C. arakiana</i>	<i>C. ×kurogii</i>	E3-2	Pref. Mie: Inabe-shi	T/C	C/G	G/T	T/C	T/C
<i>C. rouyana</i>	<i>C. ×kurogii</i>	E4-5	Pref. Tochigi: Utsunomiya-shi	T/C	C/G	G/T	T/C	T/C

*counted from the first bp of ITS and ETS, respectively

Table 5. Morphological characters and character states of taxa before/after the present study and haplotypes. *Pistillate scales attached at the central part of spike were measured. **Bracts and paracladia for the uppermost pistillate spike were measured.

Taxon before the present study	Taxon after the present study	Haplotype in cpDNA tree	Length of staminate spike (cm)	Number of staminate scales	Color of staminate scales	Length of peduncles of staminate spike (mm)	Maximum Number of peryginia per spike	Length of beak of peryginia (mm)	Length of pistillate scales* (mm)	Shape of pistillate scales*	Shape of apex of pistillate peryginia	Color of pistillate scales	Length of bracts / length of paracladia**	Maximum width of leaves (mm)	Maximum height of culms (cm)	Maximum length of rhizome on culms (cm)	Color of basal part of sheaths
<i>Carex filipes</i> var. <i>filipes</i>	<i>C. filipes</i> var. <i>tremula</i>	A1-A5	0.8-1.4	6-9	reddish purple	2-30	3-6	2.5-4.0	3.0-3.4	acute	abruptly narrowed	reddish brown	1.4-5.2	2.3-4.2	17-44	0.2-1.0	reddish purple
		B3-B5	0.4-0.7	4-7	partly reddish purple	4-12	2-5	2.0-3.0	2.9-3.3	acute	abruptly narrowed	reddish brown	1.6-5.0	2.5-4.0	16-25	0.3-1.5	reddish purple
		D1-D4	0.4-0.6	4-9	pale or partly reddish purple	5-10	2-4	1.3-1.7	2.8-3.2	acute	abruptly narrowed	reddish brown	1.7-5.6	5.0-7.2	10-23	0.2-0.5	pale brown or reddish purple
<i>C. filipes</i> var. <i>kuzakatsensis</i>	<i>C. kuzakatsensis</i>	B2-B3	1.1-1.7	13-24	partly reddish purple	9-25	4-8	3.8-4.8	2.9-3.2	acute	abruptly narrowed	reddish brown	0.82-2.0	4.8-7.2	43-64	0.2-4.6	pale brown
		B1	1.2-1.8	20-30	pale or partly reddish purple	17-94	4-7	3.5-4.8	3.0-3.3	acute	abruptly narrowed	reddish brown	0.86-1.8	4.5-10.3	30-55	0.3-3.5	reddish purple
		E5	2.0-2.0	30-36	reddish purple	32-66	4-9	5.2-6.2	3.1-3.4	acute	abruptly narrowed	reddish brown	0.63-0.92	5.0-9.2	40-54	0.3-0.4	reddish purple
<i>C. arakiana</i>	<i>C. roiyana</i> var. <i>arakiana</i>	C5, E3 (hybrids)	1.5-2.0	25-34	partly reddish purple	28-67	4-7	5.5-6.1	2.3-3.3	acute	abruptly narrowed	reddish purple	0.88-1.2	4.0-7.8	35-44	0.5-1.0	pale brown or reddish purple
		C4, E2, E4 (hybrids)	1.5-2.3	12-36	partly reddish purple	28-59	3-7	5.5-8.0	3.0-3.5	acute	abruptly narrowed	reddish purple	0.78-1.1	1.5-4.0	21-54	0.6-4.2	pale brown
		C1-C4	1.2-2.0	12-25	pale or partly reddish purple	18-33	3-6	5.6-7.1	3.0-3.4	acute	abruptly narrowed	reddish brown	1.5-2.7	2.3-3.0	23-36	0.5-2.0	pale brown or partly reddish
<i>C. roiyana</i>	<i>C. roiyana</i> var. <i>roiyana</i>	E2, E4	1.8-2.5	28-43	reddish purple	20-79	4-9	5.6-6.5	3.0-3.3	acute	abruptly narrowed	reddish brown	0.53-1.0	3.4-4.5	35-56	0.2-0.5	pale brown
		E1	0.5-0.8	4-8	pale	5-14	2-4	6.7-8.0	2.9-3.4	acute	tapered	pale	1.2-5.9	3.0-9.8	24-35	0.2-0.4	pale brown
		F1-F2	1.2-1.8	12-20	reddish brown	35-76	9-13	3.1-4.0	2.9-3.4	acute	abruptly narrowed	reddish brown	0.67-0.93	1.6-2.0	20-38	0.2-0.4	pale brown
<i>C. kujuzana</i>	<i>C. kujuzana</i>	G1	2.0-2.5	22-34	reddish purple	43-52	4-8	5.1-5.4	4.0-4.5	aristate	abruptly narrowed	reddish purple	0.93-2.1	3.0-5.0	32-44	1.5-4.0	reddish purple
		G2	2.0-2.5	25-25	reddish purple	50-50	4-5	5.0-5.5	4.3-4.5	aristate	abruptly narrowed	reddish brown	1.2-1.5	2.5-3.8	41-41	1.6-4.6	reddish purple
		G3	2.5-3.5	38-58	reddish purple	50-68	5-10	5.2-6.3	4.2-4.6	aristate	abruptly narrowed	reddish brown	1.1-1.8	5.1-8.3	61-86	0.2-0.5	reddish purple
<i>C. filipes</i> var. <i>sparsimax</i>	<i>C. arisanensis</i>	H1-H3	0.4-0.7	4-9	pale	6-10	2-4	5.2-6.4	3.3-3.8	acute	tapered	pale	0.72-1.6	8.0-12.5	38-70	0.2-0.4	pale brown
		I1-I4	0.8-1.2	10-19	pale	8-11	4-10	5.0-7.2	2.8-3.4	acute	tapered	pale	1.7-7.9	1.2-5.8	12-44	0.2-0.5	pale brown
		J1-J2	0.5-1.2	6-18	pale	8-16	5-10	5.2-6.5	3.0-3.5	acute	tapered	pale	1.8-5.9	1.9-6.8	23-71	0.3-0.5	pale brown
<i>C. papulosa</i>	<i>C. papulosa</i>	K3	0.8-1.3	8-19	pale	5-9	5-10	4.0-5.0	3.5-3.9	aristate	tapered	pale	2.2-4.5	3.0-5.1	20-40	0.2-0.4	pale brown
		K1-K2	0.9-1.5	10-25	pale	1-8	5-18	3.7-4.5	3.7-4.2	aristate	tapered	pale	2.0-6.9	2.8-6.5	22-65	0.2-0.5	pale brown
		L1-L2	1.5-3.0	28-39	yellowish brown	24-90	4-10	5.0-5.8	4.1-4.7	aristate	tapered	yellowish brown	0.94-1.7	3.2-6.0	35-66	0.3-0.5	pale brown
<i>C. egena</i>	<i>C. egena</i>	M1	1.5-2.0	21-40	reddish purple	21-40	3-5	5.7-6.5	3.9-4.4	aristate	tapered	pale	0.88-1.6	9.0-12.7	31-45	0.2-0.4	pale brown

Table 6. Chromosome information of *Carex filipes* complex from Japan: number and length of somatic chromosomes and DNA contents measured with flow cytometry. The chromosome numbers with an asterisk were first counted in this study. Mean DNA contents (pg/1C) were calculated using *Raphanus sativus* (1C= 0.555 pg DNA) as the reference. CV indicates coefficient of variation. For the voucher specimen of each sample, see information of the same sample code in Table 2.

Taxon before the present study (Taxon after the present study) & locality	Sample code	2n	Length of chromo- some (μm)	Mean DNA content (pg/1C)	Mean CV (%)
<i>Carex filipes</i> var. <i>filipes</i> (<i>C. filipes</i> var. <i>filipes</i>)					
Pref. Ibaraki: Hitachiota-shi	A1-2	52*	0.9–1.5	0.381	4.0
Pref. Tokyo: Hachioji-shi	A1-5	52*	0.8–1.6	0.366	4.0
Pref. Shiga: Maibara-shi	A1-9	52*	0.9–1.6	0.381	6.0
Pref. Okayama: Mimasaka-shi	A4-1	52*	0.9–1.4	0.374	6.3
Pref. Hiroshima: Miyoshi-shi	A1-7	54	0.9–1.5	0.370	3.9
Pref. Ehime: Seijo-shi	A5-2	54	0.8–1.3	0.373	6.4
Pref. Aichi: Tahara-shi	A1-6	58*	0.8–1.3	0.383	4.9
Pref. Gifu: Seki-shi	A3-1	58*	0.8–1.4	0.375	4.8
<i>C. filipes</i> var. <i>filipes</i> (<i>C. filipes</i> var. <i>tremula</i>)					
Pref. Kumamoto: Aso-shi	B4-3	40*	0.8–2.2	0.380	4.5
Pref. Ohita: Beppu-cho	B4-2	42*	0.9–2.0	0.387	5.0
Pref. Miyazaki: Miyakonojo-shi	B4-5	42*	0.7–1.9	0.378	4.5
Pref. Miyazaki: Shiiba-son	B4-4	44*	0.9–2.0	0.382	6.3
Pref. Kagoshima: Satsuma-cho	B5-1	46*	0.8–1.6	0.365	5.9
Pref. Wakayama: Tanabe-shi	B3-2	46*	0.9–1.7	0.374	5.8
Pref. Nara: Totsukawa-mura	B3-1	50*	0.9–1.7	0.378	6.6
<i>C. filipes</i> var. <i>filipes</i> (<i>C. curtispica</i>)					
Pref. Ehime: Kumakogen-cho	D1-1	60*	0.7–1.4	0.365	5.5
Pref. Saga: Kanzaki-shi	D3-1	60*	0.8–1.3	0.357	4.7
<i>C. filipes</i> var. <i>kuzakaiensis</i> (<i>C. kuzakaiensis</i>)					
Pref. Tochigi: Nikko-shi	B1-6	52*	0.8–1.4	0.344	6.1
Pref. Iwate: Takizawa-shi	B2-1	52*	0.9–1.5	0.362	5.2
<i>C. arakiana</i> (<i>C. kuzakaiensis</i>)					
Pref. Hiroshima: Shobara-shi	B1-1	52*	0.8–1.3	0.364	4.4
Pref. Gifu: Takayama-shi	B1-3	52*	0.9–1.4	0.356	5.9
<i>C. rouyana</i> (<i>C. rouyana</i> var. <i>rouyana</i>)					
Pref. Tochigi: Nikko-shi	E4-3	50*	0.8–1.5	0.351	5.5
Pref. Mie: Toba-shi	E3-1	52*	0.9–1.6	0.356	5.4
<i>C. rouyana</i> (<i>C. ×kurogii</i>)					
Pref. Miyazaki: Takachiho-cho	E2-1	52*	0.8–1.6	0.359	4.7
Pref. Hyogo: Kobe-shi	E4-1	52*	0.8–1.6	0.370	4.4
<i>C. rouyana</i> (<i>C. sohayakiensis</i>)					
Pref. Nara: Totsukawa-mura	C1-2	52*	0.9–1.4	0.363	4.9
Pref. Kagawa: Takamatsu-shi	C4-1	52*	1.0–1.5	0.366	6.0
<i>C. nasuensis</i> (<i>C. nasuensis</i>)					
Pref. Ibaraki: Shirosato-cho	E1-1	50*	0.8–1.6	0.351	5.0
Pref. Tochigi: Tochigi-shi	E1-3	50*	0.9–1.4	0.364	5.4
<i>C. kujuzana</i> (<i>C. kujuzana</i>)					
Pref. Tokyo: Fuchu-shi	G1-2	58*	0.8–1.3	0.355	6.1
Pref. Ohita: Taketa-shi	G1-4	58*	0.8–1.4	0.366	6.0
<i>C. vaniotii</i> (<i>C. vaniotii</i>)					
Pref. Gifu: Takayama-shi	K3-3	44*	1.0–2.1	0.370	4.9

Figures



Fig. 1. Comparison of typical staminate spikes and the uppermost pistillate spikes among taxa of *Carex filipes* complex. **A**, *C. filipes* var. *filipes* (sample code: A1-2) (*C. filipes* var. *filipes*); **B**, *C. filipes* var. *filipes* (B4-3) (*C. filipes* var. *tremula*); **C**, *C. filipes* var. *filipes* (D1-1) (*C. curtispica*); **D**, *C. filipes* var. *kuzakaiensis* (B1-6) (*C. kuzakaiensis*); **E**, *C. arakiana* (B1-1) (*C. kuzakaiensis*); **F**, *C. arakiana* (E5-1) (*C. rouyana* var. *arakiana*); **G**, *C. rouyana* (E4-3) (*C. rouyana* var. *rouyana*); **H**, *C. rouyana* (C1-2) (*C. sohayakiensis*); **I**, *C. filipes* var. *sparsinux* (G3-1) (*C. sparsinux*); **J**, *C. arisanensis* (H1-1) (*C. arisanensis*); **K**, *C. vaniotii* (K3-1) (*C. vaniotii*); **L**, *C. egena* (M1-1) (*C. egena*). Bars = 5 mm. Taxon names without parentheses indicate the names initially used in floras. Taxon names in parentheses indicate names proposed here. See information for the same sample code in Table 2 for voucher specimens for each taxon.

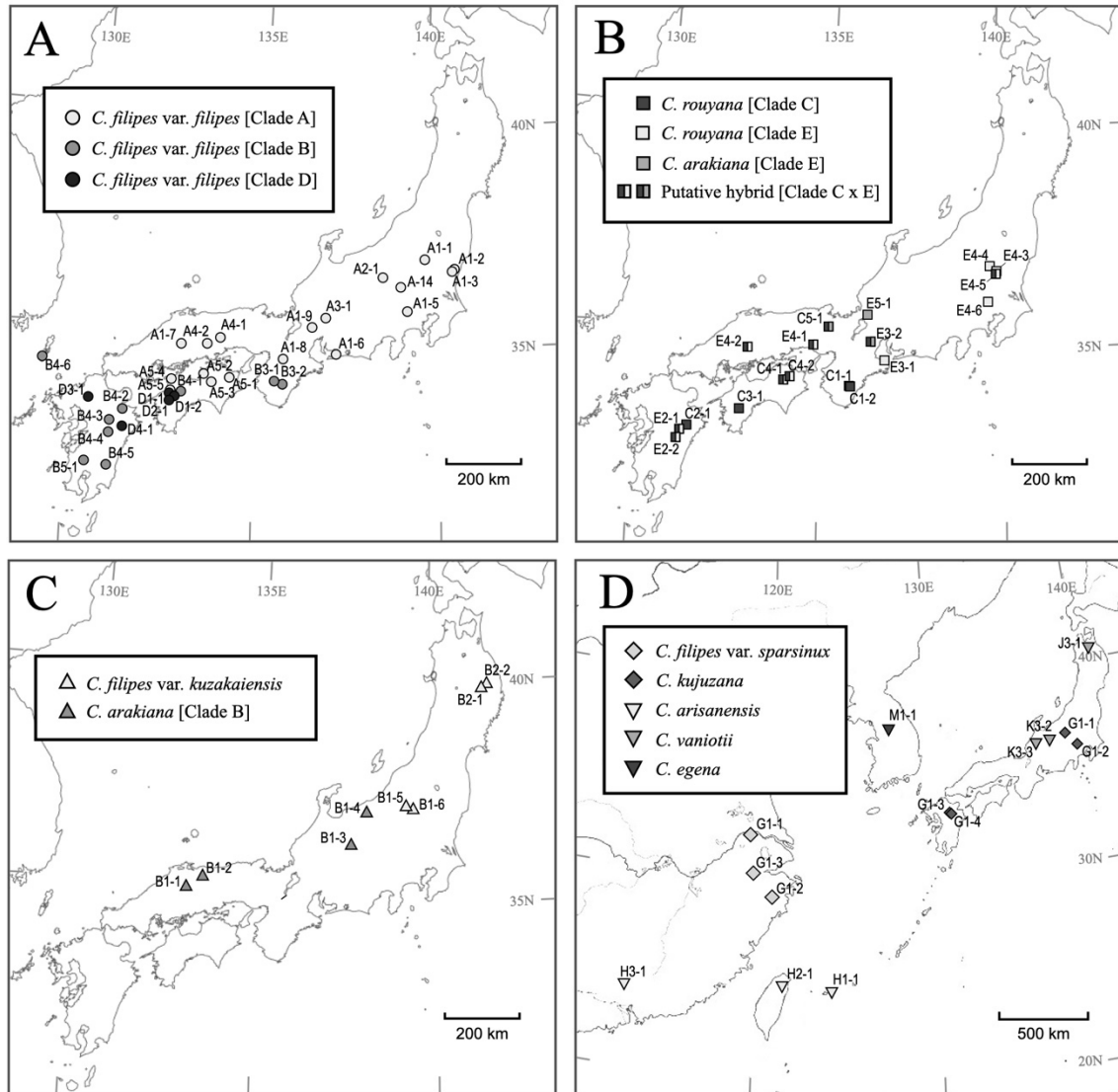


Fig. 2. Collection sites of specimens of the *Carex filipes* complex and putative hybrids. Clade names in square brackets represent the cpDNA clade (Fig. 3) where part of the taxon is included. The code for each symbol corresponds to the sample code listed in Table 2. **A**, *C. filipes* var. *filipes* included in Clade A (*C. filipes* var. *filipes*), Clade B (*C. filipes* var. *tremula*) and Clade D (*C. curtispica*), respectively; **B**, *C. rouyana* in Clade C (*C. sohayakiensis*) and Clade E (*C. rouyana* var. *rouyana*), respectively, *C. arakiana* in Clade E (*C. rouyana* var. *arakiana*) and putative hybrids between Clades C and E (*C. ×kurogii*); **C**, *C. filipes* var. *kuzakaiensis* and *C. arakiana* in Clade B (*C. kuzakaiensis*); **D**, *C. filipes* var. *sparsinix* (*C. sparsinix*), *C. kujuzana* (*C. kujuzana*), *C. arisanensis* (*C. arisanensis*), *C. vaniotii* (*C. vaniotii*) and *C. egena* (*C. egena*). Taxon names without parentheses indicate names initially used in floras. Taxon names in parentheses indicate names proposed here.

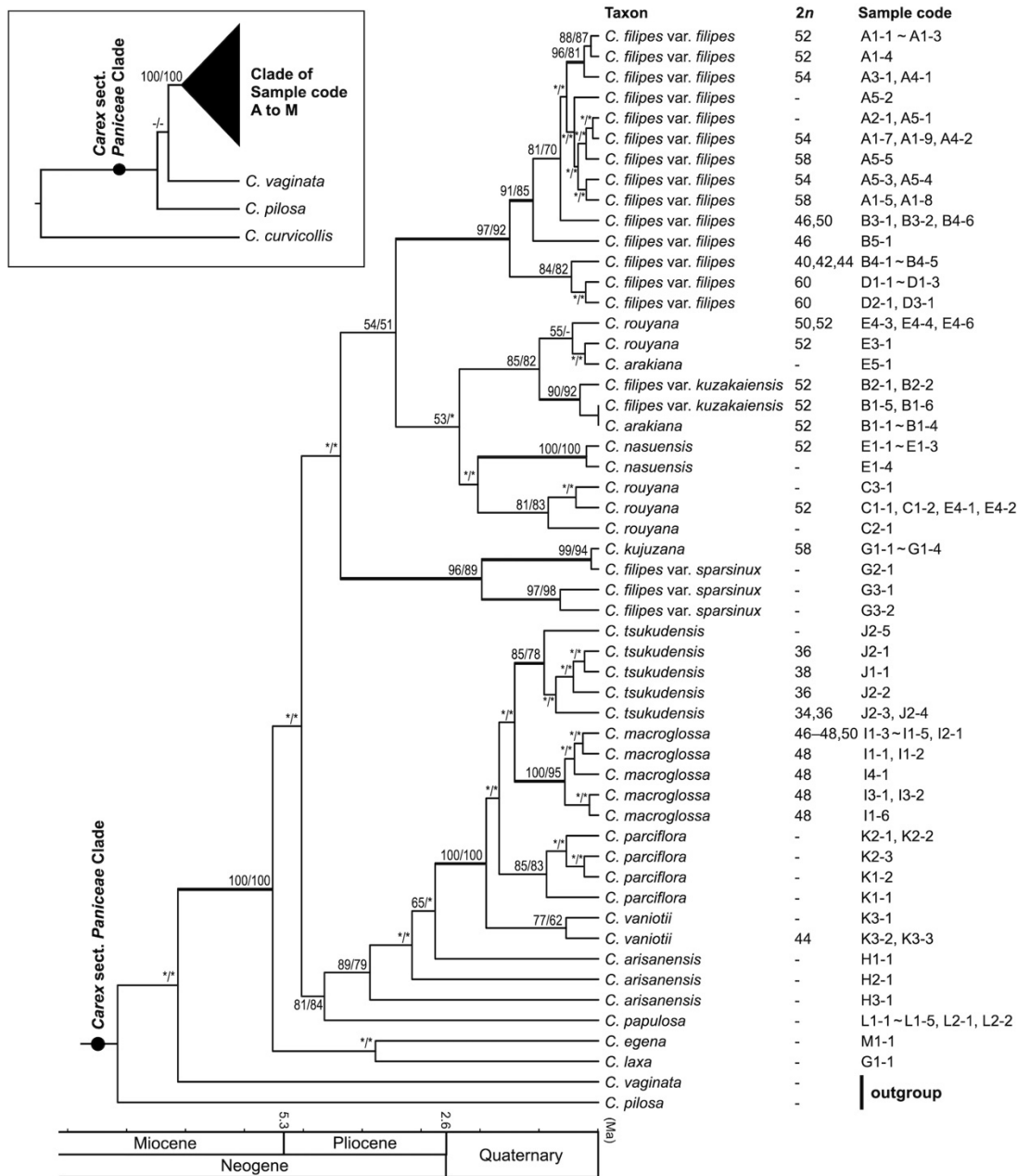


Fig. 4. Phylogenetic tree from BEAST analysis of 106 individuals of the *Carex filipes* complex and outgroups in eastern Asia based on nuclear ITS and ETS regions. The tree topology was fixed to that of the MP strict consensus tree and is identical with that of the ML tree. Individuals with the same DNA sequence are combined in a single OTU. Sample codes are the same as those in Table 2 and Fig. 3. Numbers above and below branches indicate MP / ML (hLRT) bootstrap values, respectively; an asterisk (*) represents bootstrap values of less than 50%. Branches of bold lines are supported by more than 90% bootstrap values. A geological timescale is provided at the bottom. See information for the same sample codes in Table 2 for voucher specimens of each OTU.

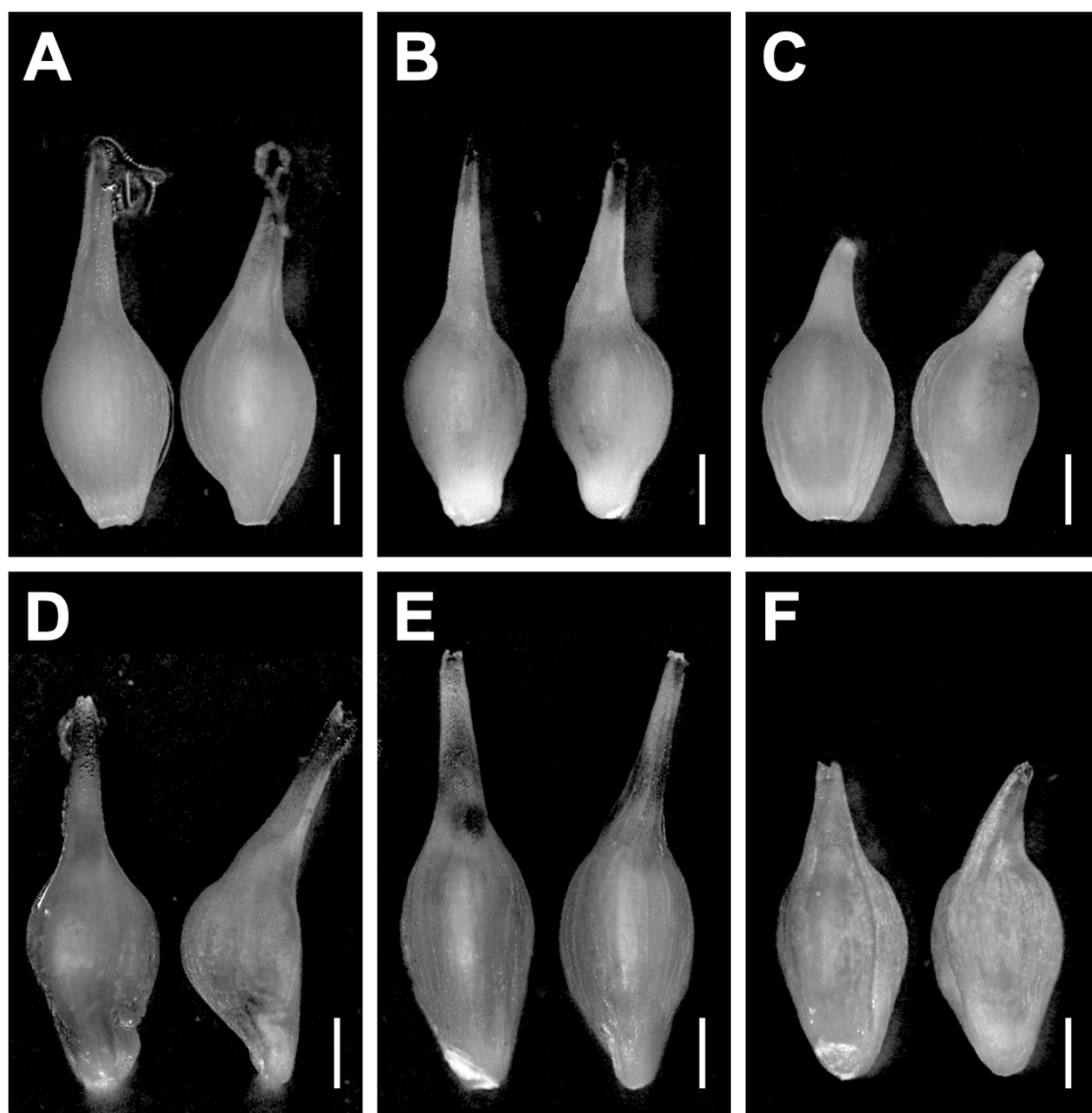


Fig. 5. Morphology of abaxial surface (left) and right side of perigynia in *Carex filipes* complex. **A**, *C. filipes* var. *filipes* (sample code: A1-2) (*C. filipes* var. *filipes*); **B**, *C. filipes* var. *filipes* (B4-3) (*C. filipes* var. *tremula*); **C**, *C. filipes* var. *filipes* (D1-1) (*C. curtispica*); **D**, *C. rouyana* (C1-2) (*C. sohayakiensis*); **E**, *C. rouyana* (E4-3) (*C. rouyana* var. *rouyana*); **F**, *C. arakiana* (B1-1) (*C. kuzakaiensis*). Bars = 1 mm. Taxon names without parentheses indicate names initially used in floras. Taxon names in parentheses indicate names proposed here. see information for the same sample code in Table 2 for voucher specimens for each taxon.

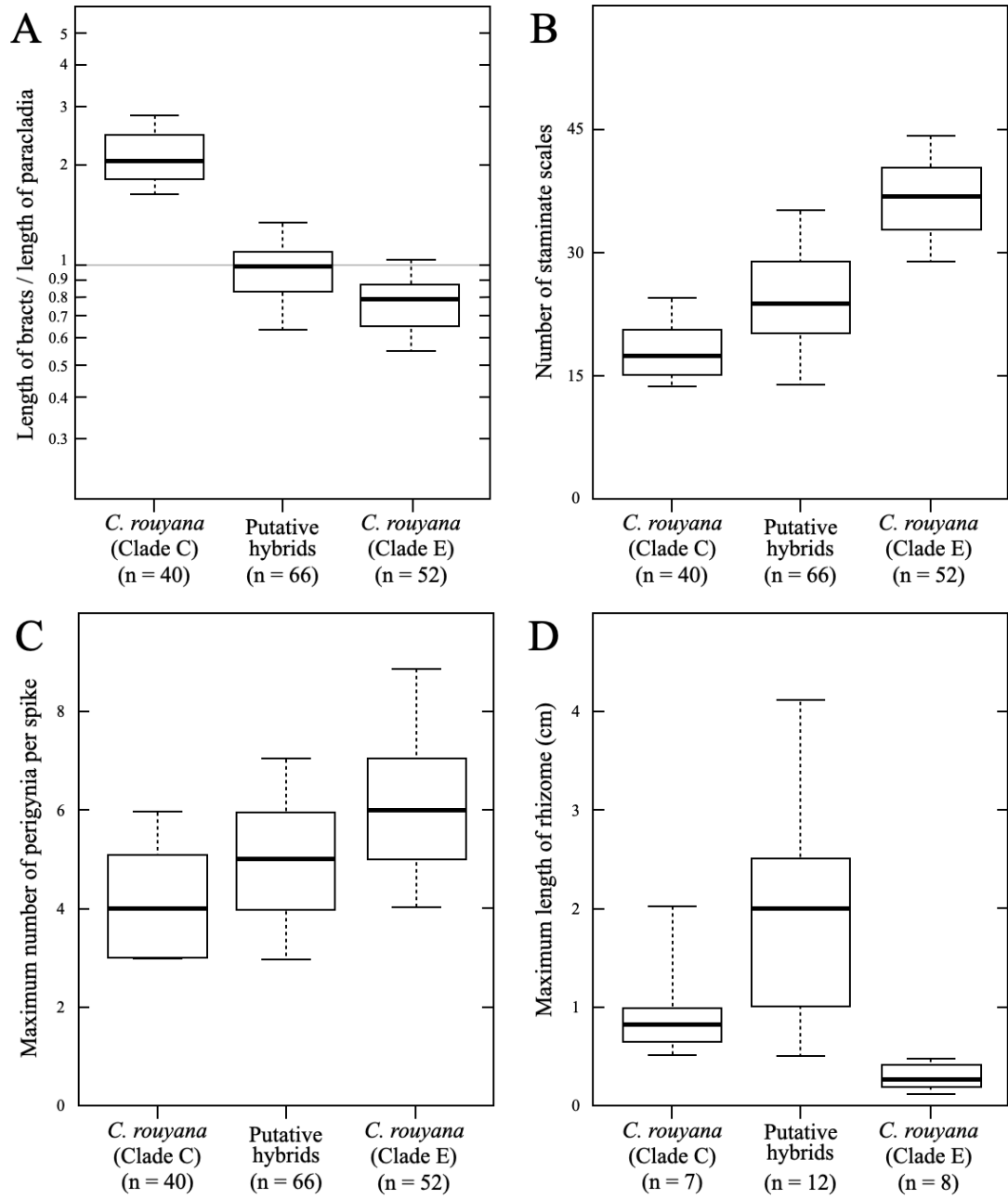


Fig. 6. Comparison of morphological characters among the two lineages of *Carex rouyana*, i.e. Clade C (*C. sohayakiensis*) and Clade E (*C. rouyana* var. *rouyana*), and their putative hybrids, i.e. Clade C $\times$ Clade E (*C. $\times$ kurogii*), using box plots. **A**, ratio of length of bracts to length of paracladia; **B**, number of staminate scales; **C**, maximum number of perigynia per spike; **D**, maximum length of rhizome. A–C were measured for each culm, and D was measured for each individual. ‘n’ in parentheses indicates number of samples measured.

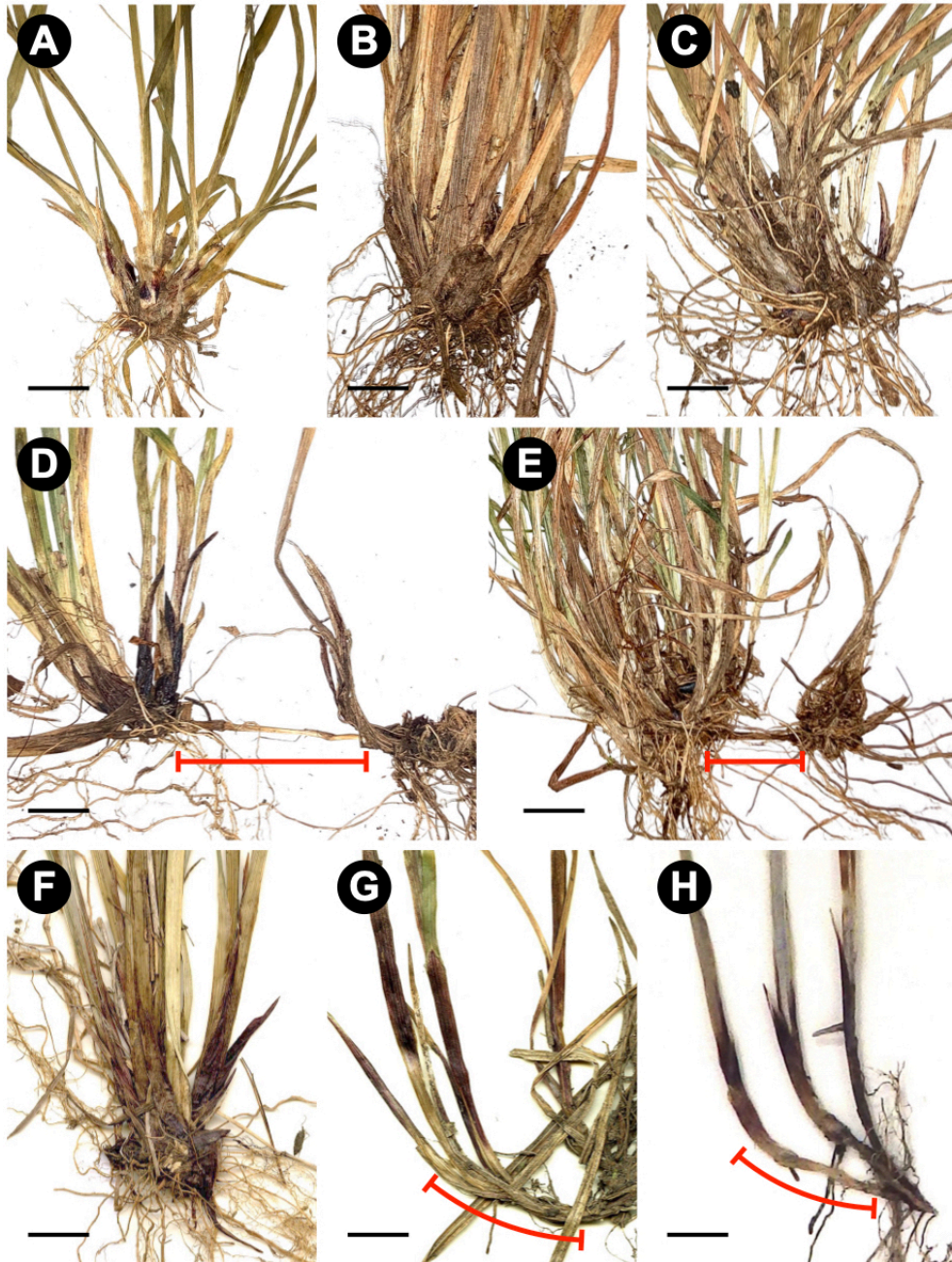


Fig. 7. Comparison of typical culms (basal part) and rhizomes among taxa in the *Carex filipes* complex. Red ranges indicate stolons. **A**, *C. filipes* var. *filipes* (sample code: B4-4) (*C. filipes* var. *tremula*); **B**, *C. rouyana* included in Clade E (E4-3) (*C. rouyana* var. *rouyana*); **C**, *C. rouyana* included in Clade C (C1-2) (*C. sohayakiensis*); **D**, *C. filipes* var. *kuzakaiensis* (B2-1) (*C. kuzakaiensis*); **E**, a hybrid (E2-2) between *C. rouyana* in Clade C and *C. rouyana* in Clade E (*C. ×kurogii*); **F**, *C. filipes* var. *sparsinux* (G3-1) (*C. sparsinux*); **G**, *C. filipes* var. *sparsinux* (G2-1) (*C. kujuzana*); **H**, *C. kujuzana* (G1-3) (*C. kujuzana*). Taxon names without parentheses indicate names initially used in floras. Taxon names in parentheses indicate names proposed here. Black bars = 1 cm. For the voucher specimen of each taxon, see information of the same sample code in Table 2.

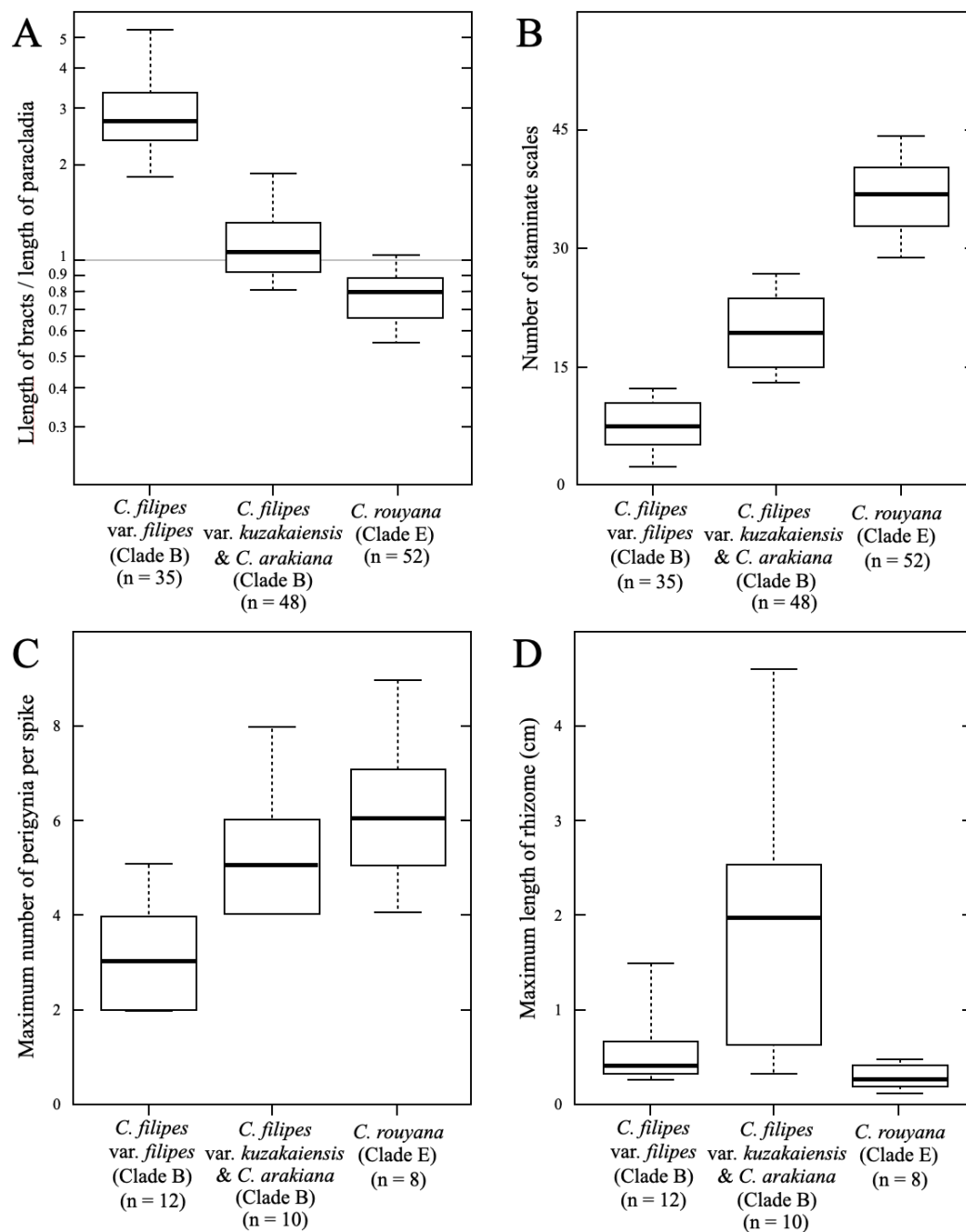


Fig. 8. Comparison of morphological characters among *Carex filipes* var. *filipes* included in Clade B (*C. filipes* var. *tremula*), *C. filipes* var. *kuzakaiensis* and *C. arakiana*, both included in Clade B (*C. kuzakaiensis*) and *C. rouyana* in Clade E (*C. rouyana* var. *rouyana*) using box plots. **A**, ratio of length of bracts to length of paracladia; **B**, number of staminate scales; **C**, maximum number of perigynia per spike; **D**, maximum length of rhizome. A–C were measured for each culm; D was measured for each individual. ‘n’ in parentheses indicates number of samples measured.

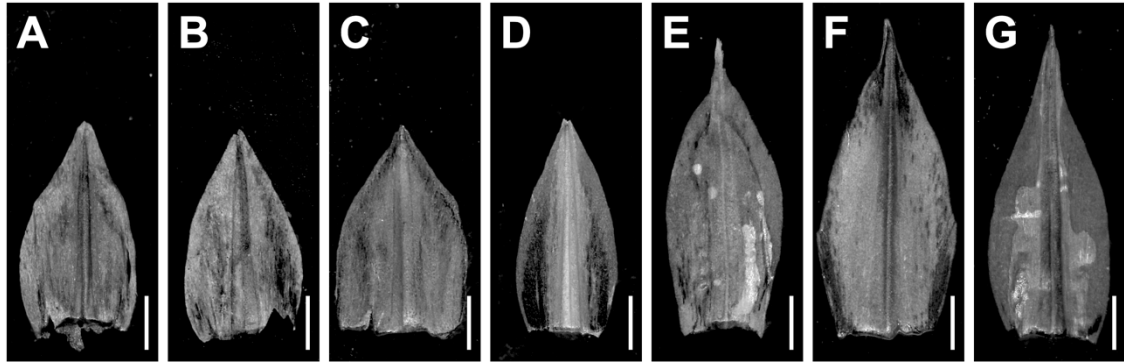


Fig. 9. Comparison of pistillate scales (abaxial surface) attached at central part of pistillate spike among taxa of *Carex filipes* complex. **A**, *C. filipes* var. *filipes* (sample code: A1-2) (*C. filipes* var. *filipes*); **B**, *C. filipes* var. *filipes* (D1-1) (*C. curtispica*); **C**, *C. rouyana* (C1-2) (*C. sohayakiensis*); **D**, *C. rouyana* (E4-3) (*C. rouyana* var. *rouyana*); **E**, *C. filipes* var. *sparsinux* (G3-1) (*C. sparsinux*); **F**, *C. filipes* var. *sparsinux* (G2-1) (*C. kujuzana*); **G**, *C. kujuzana* (G1-1) (*C. kujuzana*). Taxon names without parentheses indicate names initially used in floras. Taxon names in parentheses indicate names proposed here. Bars = 1 mm. See information for the same sample code in Table 2 for voucher specimens for each taxon.

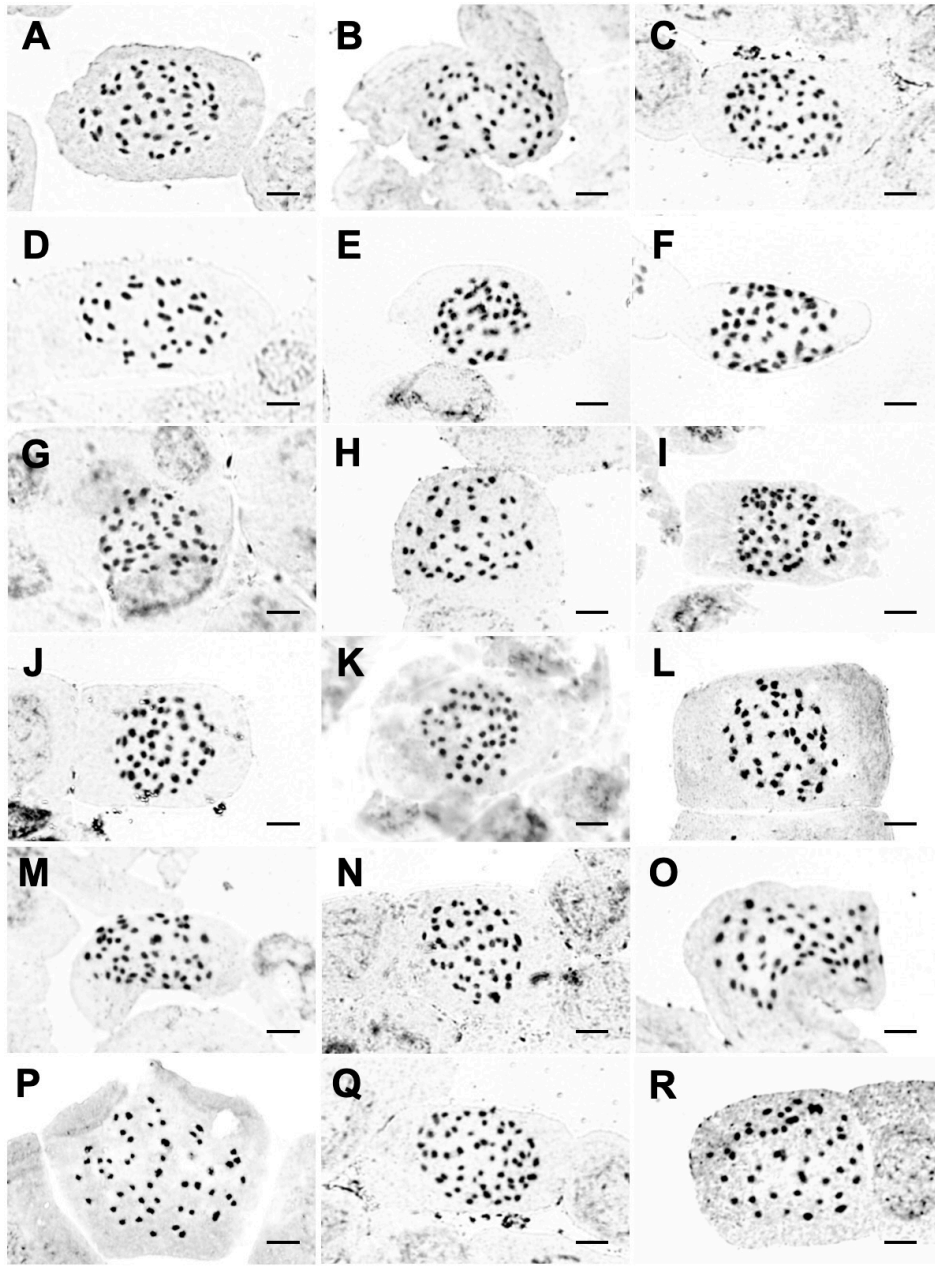


Fig. 10. Somatic metaphase chromosomes of *Carex filipes* complex. A–C, *C. filipes* var. *filipes* (*C. filipes* var. *filipes*), A, $2n = 52$ (sample code: A1-2); B, $2n = 54$ (A1-7); C, $2n = 58$ (A3-1). D–H, *C. filipes* var. *filipes* (*C. filipes* var. *tremula*), D, $2n = 40$ (B4-3); E, $2n = 42$ (B4-2); F, $2n = 44$ (B4-4); G, $2n = 46$ (B3-2); H, $2n = 50$ (B3-1). I, *C. filipes* var. *filipes* (*C. curtispica*), $2n = 60$ (D1-1). J, *C. arakiana* (*C. kuzakaiensis*), $2n = 52$ (B1-1). K, *C. filipes* var. *kuzakaiensis* (*C. kuzakaiensis*), $2n = 52$ (B2-1). L–M, *C. rouyana* (*C. rouyana* var. *rouyana*), L, $2n = 50$ (E4-3); M, $2n = 52$ (E4-1). N, *C. rouyana* (*C. sohayakiensis*), $2n = 52$ (C1-2). O, *C. rouyana* (*C. ×kurogii*), $2n = 52$ (E2-1). P, *C. nasuensis* (*C. nasuensis*), $2n = 50$ (E1-1). Q, *C. kujuzana* (*C. kujuzana*), $2n = 58$ (G1-2). R, *C. vaniotii* (*C. vaniotii*), $2n = 44$ (K3-3). Sample codes in this figure correspond to those in Table 6, Fig. 3 and Fig. 4. Taxon names without parentheses indicate names initially used in floras. Taxon names in parentheses indicate names proposed here. Bars = 5 μ m. See information for same sample code in Table 2 for voucher specimens for each cytotype.



Fig. 11. *Carex sohayakiensis* K. T. Takahashi & M. N. Tamura, sp. nov. **a**, habit; **b**, terminal staminate spike and the uppermost pistillate spike; **c**, lateral view of achene with style. All drawn from the holotype (K. T. Takahashi & J. Oda 1168, KYO).



Fig. 12. *Carex* $\times$ *kurogii* K. T. Takahashi & M. N. Tamura, hybr. nov. **a**, habit; **b**, terminal staminate spike and the uppermost pistillate spike; **c**, lateral view of achene with style. All drawn from the topotype (K. T. Takahashi & S. Kurogi 1044, KYO).



Fig. 13. *Carex curtispica* K. T. Takahashi & M. N. Tamura, sp. nov. **a**, habit; **b**, terminal staminate spike and the uppermost pistillate spike; **c**, lateral view of achene with style. All drawn from the holotype (K. T. Takahashi 1032, KYO).

Chapter III

Molecular phylogenetic analysis of the *Carex macroglossa* complex with reference to variation in morphology, chromosomal features and species delimitation

Abstract

A phylogenetic analysis of 43 individuals using five plastid and two nuclear DNA sequence regions and morphological and chromosomal observations were used to clarify the recognition and delimitation of species in the *Carex macroglossa* complex (Cyperaceae). The molecular analysis resulted in two, well-supported major clades I and II, each further consisting of two subclades A and B, and C and D, respectively. Morphologically, clade I (comprising subclades A and B) corresponded to *C. macroglossa*, subclade C to *C. parciflora*, and subclade D to *C. vaniotii*. All individuals of subclade A had high chromosome numbers ($2n = 46-48, 50$), whereas those in subclade B had lower chromosome numbers ($2n = 34, 36, 38$). The absence of intermediate chromosome numbers suggests reproductive isolation between individuals of subclades A and B. Our morphological observations further showed that individuals of subclade A have a style twisted at its base and short culms, whereas individuals of subclade B have a straight style and taller culms. Based on the molecular analysis in addition to the chromosomal and morphological observations, four species, *C. macroglossa* (subclade A), *C. tsukudensis* comb. & stat. nov. (subclade B), *C. parciflora*, and *C. vaniotii* were distinguished.

Introduction

Carex L., comprising about 1,800 species is the largest genus in Cyperaceae Juss. (Frodin 2004). The species occupy various habitats, but are mainly in wet, cold places (Goetghebeur 1998). *Carex* is characterized by unisexual flowers and a perigynium, the latter of which corresponds to an achene completely enclosed by a prophyll (Goetghebeur 1998, Starr *et al.* 2004).

Carex macroglossa Franch. & Sav. (Fig. 1), which is included in *C. sect. Paniceae* G. Don, is widely distributed in Japan (from Hokkaido to Kyushu) and in southern Korea in wet places in lowlands and mountains (Hoshino *et al.* 2020). Morphologically, *C. macroglossa* resembles *C. parciflora* Boott and *C. vaniotii* H. Lév. in having pale brown scales, pale brown base of the sheaths and erect pistillate spikes on the upper part of the plants. It differs from them in bearing grayish green leaves, fewer perigynia per spike, and shorter pistillate spikes (Hoshino *et al.* 2020). *Carex vaniotii* is distinguished from *C. parciflora* by its narrower leaves, shorter culms and perigynia oriented parallel to the inflorescence axis (Hoshino *et al.* 2020).

Ohwi (1953) included both *C. macroglossa* and *C. vaniotii* within *C. parciflora* as varieties. Akiyama (1955) and Katsuyama (2015) pointed out that the wide morphological variation of *C. macroglossa* sometimes made it indistinguishable from *C. parciflora*. Koyama (1956) described larger plants with wide, whitish leaves and marked ligules as *C. parciflora* var. *tsukudensis*. However, Hoshino *et al.* (2020) did not recognize it at any rank. Accordingly, species delimitation among *Carex macroglossa*, *C. parciflora* and *C. vaniotii*, as well as the recognition of infraspecific taxa, remains uncertain. We therefore provisionally refer to this group of species, including the infraspecific taxa, as the *C. macroglossa* complex.

Carex displays a remarkable series of aneuploid chromosome numbers among and within species due to the presence of holocentric chromosomes (Hipp *et al.* 2009). In *C. macroglossa* as well, several infraspecific aneuploids of $2n = 36, 40, 46, 48$, and 50 (Tanaka 1948), and $2n = 47$ and 50 (Hoshino 1981) have been reported. However, the systematic implication of aneuploidy has never been discussed for *C. macroglossa*.

Our study aimed to clarify whether species are distinguishable in the *C. macroglossa* complex, and if distinguishable, how many species are in the complex. For that purpose we conducted a molecular analysis and observed the chromosomes and morphology of as many individuals as possible that were assignable to the *C. macroglossa* complex. The molecular analysis was based on five plastid and two nuclear DNA regions that have often used for species level comparisons in

Carex (Oda *et al.* 2019). Observations of the chromosomes and morphology include somatic chromosome counts and measurements of the chromosomes, perigynia, leaves, and so on. Evidence obtained from those analyses was used to resolve the systematic questions underlying the *C. macroglossa* complex.

Materials and Methods

Plant materials

Forty-three individuals assignable to the *Carex macroglossa* complex were analyzed. Following Hoshino *et al.* (2020), 35 of the 43 individuals were tentatively identified as *C. macroglossa*, six as *C. parciflora*, and two as *C. vaniotii*. They are presented in Table 1 along with collection information, DNA sequence types (discussed below), chromosome and morphological data, and DDBJ accession numbers. To confirm the coherence and relationships of the three species, we added two species of *Carex* (*C. filipes* Franch. & Sav. and *C. pilosa* Scop. of *C. sect. Paniceae*) as an ingroup. We used *C. curvicolis* Franch. & Sav. of *C. sect. Racemosae* G. Don as an outgroup.

DNA extraction, PCR amplification, and DNA sequencing

Total DNA was extracted from silica gel dried leaves or from leaves removed from herbarium specimens using the modified CTAB method (Stewart & Via 1993). DNA extracted from old specimens was purified by QIAquick PCR Purification (Qiagen).

Plastid DNA regions of *atpB-rbcL*, *rpl16-rpl16-rps3*, *rps16*, *matK* and *trnL-trnL-trnF*, and nuclear DNA regions of ITS and ETS were amplified by polymerase chain reaction (PCR) using a GeneAmp PCR System 2720 or a TaKaRa PCR Thermal Cycler Dice. The amplification reaction mixture was prepared using TaKaRa Ex Taq DNA polymerase following the manufacturer's recommendations (TaKaRa Bio). We used 18 primers listed in Table 2 and the following PCR profile: a 35 cycle reaction with denaturation at 94°C for 30s, annealing at 50°C (53°C in ITS and ETS) for 30 s, and extension at 72 °C for 60– 90 s, in addition to an initial denaturation at 94 °C for 5 min

and a final extension at 72 °C for 7 min. The PCR products were purified by Exonuclease I (TaKaRa Bio) and Calf Intestine Alkaline Phosphatase (Toyobo).

Direct sequencing for each DNA region was conducted on an ABI PRISM 3130xl Genetic Analyzer (Applied Biosystems) and a BigDye Terminator v.3.1 Cycle Sequencing Kit (Applied Biosystems), according to manufacturer instructions. The DNA sequences obtained were aligned using Clustal W (Thompson *et al.*, 1994) and adjusted manually.

Phylogenetic analysis

We employed Maximum Likelihood (ML) analysis to construct a phylogenetic tree based on the combined data of all plastid and nuclear (ITS+ETS) sequences and implemented in PAUP* v.4.0a168, an update of PAUP* v.4 (Swofford 2003). Congruence between the plastid data and the nuclear data was certified with an incongruence length difference (ILD) test ($p = 0.48$) (Farris *et al.* 1994) implemented in PAUP* as a partition homogeneity test. The DNA substitution model was selected by jModelTest (Darriba *et al.* 2012). TIM1+G was selected as the best model by Akaike's Information Criterion (AIC) and Akaike's Information Criterion Corrected (AICC). The ML analysis was performed using a heuristic search option of as-is addition sequence and ML bootstrap analysis with 1000 replications.

Observations of somatic chromosomes and morphological characters

To observe the somatic chromosomes of *Carex macroglossa*, we collected root tips early in the morning from 27 individuals cultivated in pots that were not watered the day before. They were pretreated in 0.0025 M 8-hydroxyquinoline solution for 4 h at 20 °C, fixed in acetic alcohol (1:3) for 1 h at 20 °C, soaked in 1 N hydrochloric acid for 10 m at 60 °C to separate the cells, and stained with leuco-basic fuchsin for 1 h at 20°C. They were then macerated in aqueous solution of 2% (w/v) pectinase and 2% (w/v) cellulase for 40 m at 37 °C, stained again in 1% aceto-orcein, and squashed (Yamamoto *et al.* 2008).

To compare the morphology of *C. macroglossa*, *C. parciflora*, and *C. vaniotii* we examined all 43 individuals used in the molecular analyses using fresh materials or herbarium specimens (Table 1). Additionally, three collections of *C. macroglossa* from Korea were compared (Table 3). The characters examined included the number and direction of the perigynia, the shape of the base of the style, the length of the pistillate spikes, the length of the culms, and the width of the leaves (Table 1).

Results

Molecular analysis

The aligned DNA sequences of the plastid (*atpB-rbcL*, *rpl16-rpl16-rps3*, *rps16*, *matK*, and *trnL-trnL-trnF*) and nuclear (ITS and ETS) regions was 5,244 bp long, of which 164 bp were variable and 95 bp were phylogenetically informative. Comparisons of the whole DNA sequences above distinguished 15 different types among the 43 individuals examined. That is, nine types (A1–A4 and B1–B5) in the 35 individuals provisionally identified as *Carex macroglossa*, four types (C1–C4) in the six individuals of *C. parciflora*, and two types (D1, D2) in the two individuals of *C. vaniotii* (Table 1).

The ML tree (Fig. 2) obtained from the 15 DNA sequence types of the *C. macroglossa* complex as well as from DNA sequences of *C. filipes*, *C. pilosa* and *C. curvicollis* showed that the 43 individuals of the *C. macroglossa* complex formed a monophyletic clade with 100% bootstrap support (BS). That clade was split into two, well-supported major clades I (95% BS) and II (86% BS), each clade further splitting into two subclades: subclades A (100% BS) and B (95% BS), and subclades C (93% BS) and D (88% BS). The individuals of major clade I agreed with what we provisionally identified as *C. macroglossa*; those of the subclades C and D agreed with what we identified as *C. parciflora* and *C. vaniotii*, respectively.

Somatic chromosomes

Among the 27 individuals examined of *Carex macroglossa*, we found seven different chromosome numbers: $2n = 34, 36, 38, 46, 47, 48$, and 50 (Table 1 and Fig. 3). Among them, $2n = 34$ and 38 are new counts for this species. The longest chromosomes in counts of $2n = 34, 36$ and 38 were $1.9\text{--}2.4\text{ }\mu\text{m}$ long; those in counts of $2n = 46, 47, 48$ and 50 were $1.2\text{--}1.4\text{ }\mu\text{m}$ long. Chromosome numbers and the range of chromosome length within the same cell for each sample is indicated in Table 1.

Morphological characters

The results from the morphological observations are presented in Tables 1 and 3. We found morphological differences among the individuals of subclades A to D. The orientation of the perigynia was erect (directed upward parallel to the inflorescence axis) at maturity in the two individuals of subclade D, which we tentatively identified as *Carex vaniotii*. The perigynia were ascending (directed upward at an angle of 45° from the inflorescence axis) at maturity in the remaining individuals of subclades A, B and C (Table 1). The perigynia were shorter, ranging from 3.4 to 4.5 mm long in individuals of subclade C, which we tentatively identified as *Carex parciflora*. They were longer, ranging from 4.7 to 6.6 mm long in the individuals of the subclades A and B. In addition, the style is twisted at its base in individuals of subclade A, but straight in individuals of subclades B, C and D (Table 1). As for the individuals of subclades A and B, which we tentatively assigned to *C. macroglossa*, the longest culm was less than 48 cm long (mostly less than 39 cm long) in individuals of subclade A, but more than 31 cm long (mostly more than 60 cm long) in individuals of subclade B (Table 1).

The leaves were highly variable in width within individuals of each subclade and of no diagnostic value between the two subclades.

Discussion

Molecular analysis of the 43 individuals showed that the *Carex macroglossa* complex is monophyletic and consists of two, well-supported major clades, I and II, and that each major clade further consists of two subclades A and B, and C and D, respectively. The 35 individuals of subclades

A and B, the six individuals of subclade C, and the two individuals of subclade D agree with what we tentatively identified as *C. macroglossa*, *C. parciflora*, and *C. vaniotii*, respectively. As for the individuals identified as *Carex macroglossa*, differences in molecular, chromosomal and morphological characters clearly distinguished individuals of subclade A from those of subclade B. One of the morphological characteristics, the orientation of the style base (twisted vs. totally straight), has never been used diagnostically in the *C. macroglossa* complex.

Individuals of subclades A and B are widely distributed in Japan (from Hokkaido to Kyushu) and in southern Korea and their distributions overlap (Fig. 5). Furthermore, populations of different subclades are sometimes adjacent to each other (m-12 & m-28; m-24 & m-30, 31; m-23 & m-27) (Fig. 5) without any conspicuous geographical barriers, such as high mountains, between them. However, individuals of subclade A are clearly distinct from those of subclade B in having higher chromosome numbers (with $2n = 46-48, 50$) than individuals of subclade B (with $2n = 34, 36, 38$). Because of the absence of intermediate chromosome numbers between them, reproductive isolation likely exists between subclades A and B. Therefore, based on the biological species concept (Mayr 1942), it seems better to treat individuals of subclades A and B as separate species.

In the type specimen of *Carex macroglossa* (Savatier 1414, holoP!), the longest culm is short (23.5 cm long) as in individuals of the subclade A. In contrast, the longest culms of the type specimens of *C. parciflora* var. *tsukudensis* (T. Koyama 11138, holoTI!, isoTNS!) are longer (both 64 cm long), and the latter type specimens have a straight style as in individuals of subclade B. Thus, we propose that the name *C. macroglossa* be restricted to individuals of subclade A and *C. tsukudensis*, comb. & stat. nov., be applied to individuals of subclade B.

Regarding individuals from southern Korea, based on the morphological comparisons, we identified them as *C. macroglossa* s.str., while we recognize those from Jeju Island as *C. tsukudensis* (Table 3).

Taxonomy

Key to the species of *Carex macroglossa* and its close allies.

- 1a. Perigynia erect (oriented parallel to inflorescence axis) at maturity *C. vaniotii*
- 1b. Perigynia ascending (directed upward at 45° angle from inflorescence axis) at maturity 2
- 2a. Perigynia 3.4–4.5 mm long; pistillate spikes 1–3 cm long *C. parciflora*
- 2b. Perigynia 4.7–6.6 mm long; pistillate spikes 0.7–2 cm long 3
- 3a. Style totally straight; longest culm 15–39(–48) cm long *C. tsukudensis*
- 3b. Style twisted at base; longest culm (31–)60–84 cm long *C. macroglossa*

Carex macroglossa Franch. & Sav., Enum. Pl. Jap. 2: 148 (1877), 576 (1878). — *Carex jakiana* Boott subsp. *parciflora* (Boott) Kük. var. *macroglossa* (Franch. & Sav.) Kük. ex Matsum., Index Pl. Jap. 2 (1): 115 (1905); T. Koyama in Bot. Mag. Tokyo 70: 355 (1957). — *Carex parciflora* Boott var. *macroglossa* (Franch. & Sav.) Ohwi in Mem. Coll. Sci. Kyoto Imp. Univ., ser. B, Biol. 5: 290 (1930). — *Carex jakiana* Boott subsp. *macroglossa* (Franch. & Sav.) T. Koyama in Kitamura *et al.*, Col. III. Herb. Pl. Jap. 3: 292 (1964), comb. nud., excl. p. — Type: Japan, Yokosuka (Yokoska), *Savatier 1414* (holo– P!).

Japanese name. Ko-juzu-suge.

Distribution. Japan, Korea (southern Korean peninsula)

Habitat. Primarily in open, dry places.

Chromosome number. $2n = 46-48, 50$.

Carex tsukudensis (T. Koyama) K. T. Takahashi & M. N. Tamura, **comb. & stat. nov.**

Basionym: *Carex parciflora* Boott var. *tsukudensis* T. Koyama in J. Jap. Bot. 31: 286 (1956), ‘*tsukuoensis*’. — *Carex jakiana* Boott subsp. *parciflora* (Boott) Kük. var. *tsukudensis* (T. Koyama) T. Koyama in Bot. Mag. Tokyo 70: 357 (1957). — Type: Japan, Pref. Aichi, Tsukude, *T. Koyama 11138* (holo– TI!; iso– TNS!).

Carex macroglossa Franch. & Sav., Enum. Pl. Jap. 2: 148 (1877), 576 (1878), p. p.

Japanese name. Hirohano-ko-juzu-suge.

Distribution. Japan, Korea (Jeju Island).

Habitat. Shaded, swampy places.

Chromosome number. $2n = 34, 36, 38$.

Carex parciflora Boott in Mem. Amer. Acad. Arts, n. s. 6: 418 (1859). — *Carex jakiana* Boott

subsp. *parciflora* (Boott) Kük. in Pflanzenr. 38(IV-20): 638 (1909). — Type: Japan, Hokkaido,

Hakodate, *C. Wright 46* (holo— K, digital image!)

Japanese name. Guren-suge.

Distribution. Japan, Russia (Sakhalin). *Habitat.* Wet forests, swamps on mountain. *Chromosome number.* $2n = 38-40, 42, 44, 46, 48$.

Carex vaniotii H. Lév. in Bull. Acad. Int. Géogr. Bot. 11: 108 (1902). — *Carex parciflora* var.

vaniotii (H. Lév.) Ohwi in Mem. Coll. Sci. Kyoto Imp. Univ., ser. B, Biol. 5: 290 (1930), '*vanioti*'.

— *Carex jakiana* Boott subsp. *parciflora* (Boott) Kük. var. *vaniotii* (H. Lév.) T. Koyama in Bot. Mag.

Tokyo 70: 356 (1957). — Type: Japan, Mt. Iwate (Ganju), *Faurie 1701* (holo— P!; iso— KYO!).

Japanese name. Nagabono-ko-juzu-suge.

Distribution. Japan (Honshu).

Habitat. Wet forests in subalpine zone.

Chromosome number. Unknown.

Acknowledgement

We express our sincere gratitude to Mr. Tatsunari Noguchi, Mr. Takashi Ajima, Mr. Takayuki Shibata, Mr. Narihisa Matsuoka, Mr. Hidehiko Shimizu, Mr. Kazuya Yamawaki, Mr. Shuichi Kurogi and Dr. Okihito Yano for collecting samples. We also thank the directors and staff members of

GMNHJ, HYO, KPM, KYO, OKAY, OSA, P, SAPS and TNS for allowing us to use their facilities and collections. This study was supported in part by ISHIZUE 2019 of Kyoto University Research Development Program (to M. N. Tamura).

References

- Akiyama, S. 1955. Carices of the Far Eastern Region of Asia, Hokkaido Univ. Press, Sapporo (in Japanese).
- Darriba, D., G. L. Taboada, R. Doallo & D. Posada. 2012. jModelTest 2: more models, new heuristics and parallel computing. *Nature, Meth.* 9: 772.
- Farris, J. S., M. Källersjö, A. G. Kluge & C. Bult. 1994. Testing significance of incongruence. *Cladistics* 10: 315–319.
- Frodin, D. G. 2004. History and concepts of big plant genera. *Taxon* 53: 753–776.
- Goetghebeur, P. 1998. Cyperaceae. *In*: Kubitzki, K. (ed.), *The Families and Genera of Vascular Plants*, vol. IV. Flowering Plants: Monocotyledons, pp. 141–190. Springer, Berlin.
- Hipp, A. L., P. E. Rothrock & E. H. Roalson. 2009. The evolution of chromosome arrangements in *Carex* (Cyperaceae). *Bot. Rev.* 75: 96–109.
- Hoshino, T. 1981. Karyomorphological and cytogenetical studies on aneuploidy in *Carex*. *J. Sci. Hiroshima Univ., Ser. B, Div. 2, Bot.* 17: 155–238.
- Hoshino, T., T. Katsuyama, T. Masaki & M. Michikawa. 2020. *Carex* L. *In*: Iwatsuki, K., D. E. Boufford & H. Ohba (eds.), *Flora of Japan*, vol. IVa. Angiospermae: Monocotyledoneae (a), pp. 226–344. Kodansha, Tokyo.
- Katsuyama, T. 2015. *Carex* of Japan, enlarged and revised ed. Bunichi Sogo Shuppan, Tokyo (in Japanese). Koyama, T. 1956. Some novelties of Japanese Cyperaceae. *J. Jap. Bot.* 31: 286–288.
- Mayr, E. 1942. *Systematics and the Origin of Species from the Viewpoint of a zoologist*. Columbia Univ. Press, New York.
- Oda, J., S. Fuse, J. Yamashita & M. N. Tamura. 2019. Phylogeny and taxonomy of *Carex*

- (Cyperaceae) in Japan I. *C. sect. Rarae*. Acta Phytotax. Geobot. 70: 69–85. Ohwi, J. 1953. Flora of Japan. Shibundo, Tokyo (in Japanese).
- Roalson, E. H., J. T. Columbus & E. A. Friar. 2001. Phylogenetic relationships in Cariceae (Cyperaceae) based on ITS (nrDNA) and *trnT-L-F* (cpDNA) region sequences: Assessment of subgeneric and sectional relationships in *Carex* with emphasis on section *Acrocystis*. Syst. Bot. 26: 318–341.
- Shaw, J., E. B. Lickey, J. T. Beck, S. B. Farmer, W. Liu, J. Miller, K. C. Siripun, C. T. Winder, E. E. Schilling & R. L. Small. 2005. The tortoise and the hare II: relative utility of 21 noncoding chloroplast DNA sequences for phylogenetic analysis. Amer. J. Bot. 92: 142–166.
- Starr, J. R., S. A. Harris & D. A. Simpson. 2003. Potential of the 5' and 3' ends of the intergenic spacer (IGS) of rDNA in the Cyperaceae: New sequences for lower level phylogenies in sedges with an example from *Uncinia* Pers. Int. J. Pl. Sci. 164: 213–227.
- Starr, J. R., S. A. Harris & D. A. Simpson. 2004. Phylogeny of the unispicate taxa in Cyperaceae tribe Cariceae I: genetic relationship and evolutionary Scenarios. Syst. Bot. 29: 528–544.
- Starr, J. R., R. F. C. Naczi, & B. N. Chouinard. 2009. Plant DNA barcodes and species resolution in sedges (*Carex*, Cyperaceae). Molec. Ecol. Res. 9 (Suppl. 1): 151– 163.
- Stewart, C. N. Jr. & L. E. Via. 1993. A rapid CTAB DNA isolation technique useful for RAPD fingerprinting and other PCR applications. BioTechniques 14: 748– 750.
- Swofford, D. L. 2003. PAUP*: Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4. Sinauer, Sunderland.
- Taberlet, P., L. Gielly, G. Pautou & J. Bouvet. 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. Plant Molec. Biol. 17: 1105–1109.
- Tanaka, N. 1948. The problem of aneuploidy. Biological Contributions in Japan, vol. 4, pp. 1–327. Hokuryukan, Tokyo (in Japanese).

- Thompson, J. D., D. G. Higgins & T. J. Gibson. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucl. Acids Res.* 22: 4673–4680.
- Xu, D. H., J. Abe, M. Sakai, A. Kanazawa & Y. Shimamoto. 2000. Sequence variation of non-coding regions of chloroplast DNA of soybean and related wild species and its implications for the evolution of different chloroplast haplotypes. *Theor. Appl. Genet.* 101: 724–732.
- Yamamoto, N., H. Ikeda & T. Hoshino. 2008. Cytotaxonomical studies of flowering plants in Yakushima Island, Kagoshima Prefecture, Japan. Part I: dwarf taxa. *J. Phytogeogr. Taxon.* 56: 79–93.
- Yano, O., H. Ikeda & T. Hoshino. 2010. Phylogeography of the Japanese common sedge, *Carex conica* complex (Cyperaceae), based on chloroplast DNA sequence data and chromosomal variation. *Amer. J. Bot.* 97: 1365–1376.

Tables

Table 1. Source and habitat of materials, DNA sequence types, chromosome observations, results of morphological examination and accession numbers. Same DNA sequence type indicates plastid regions (*atpB-rbcL*, *rpl16-rps3*, *rps16*, *matK* and *trnL-trnF*) and nuclear regions (ITS and ETS) that have identical DNA sequences.

Code	Taxon & Locality	Voucher	Original collection(s)	Habitat	DNA sequence type	Chromosome number	Chromosome length (µm)	Length of perigynia (mm)	Number of perigynia	Direction of style & maturity	Base of style (mm)	Length of style (mm)	Length of pedicel spikes (mm)	Width of pedicel (mm)	Accession number	<i>atpB-rbcL</i>	<i>rpl16-rps3</i>	<i>rps16</i>	<i>matK</i>	<i>trnL-trnF-trnT</i>	ITS	ETS
<i>Carex macroglossa</i> Franch. & Sav.																						
m-1	Hokkaido Oshima-cho	<i>Hara 3919</i> (SAPS)	Hara	-	Al	-	-	5.3-5.9	4-6	ascending	twisted	8.1-9.8	134-203	12-1.8	LC030574	LC030620	LC030712	LC030666	LC030956	LC031002	LC031048	
m-2	Pref. Miyagi: Sendai-shi	<i>Takahashi 1134</i> (KYO)	Takahashi	open lowland	Al	2n=48	0.9-1.3	5.2-5.8	3-6	ascending	twisted	7.8-10.8	209-316	2.0-2.7	LC030575	LC030621	LC030713	LC030667	LC030957	LC031003	LC031049	
m-3	Pref. Fukushima: Fukushima-shi	<i>Takahashi 1133</i> (KYO)	Takahashi	open lowland	Al	2n=48	0.9-1.2	5.8-6.2	3-6	ascending	twisted	8.1-11.6	188-372	2.7-3.1	LC030576	LC030622	LC030714	LC030668	LC030958	LC031004	LC031050	
m-4	Pref. Niigata: Ageo-cho	<i>Hoshino & Masaki 24610</i> (OKAY)	Hoshino & Masaki	-	Al	-	-	5.7-6.2	2-4	ascending	twisted	8.1-9.7	150-189	2.0-5.8	LC030577	LC030623	LC030715	LC030669	LC030959	LC031005	LC031051	
m-5	Pref. Gunma: Kawaba-mura	<i>Ohmori 8519148</i> (GNMHI)	Ohmori	-	Al	-	-	-	2-4	-	-	-	-	-	LC030578	LC030624	LC030716	LC030670	LC030960	LC031006	LC031052	
m-6	Pref. Tochigi: Nikko-shi	<i>Takahashi 1059</i> (KYO)	Noguchi & Shibata	-	Al	2n=47	0.8-1.3	5.0-5.6	4-6	ascending	twisted	7.7-9.2	128-254	2.0-3.0	LC030579	LC030625	LC030717	LC030671	LC030961	LC031007	LC031053	
m-7	Pref. Tochigi: Kanuma-shi	<i>Takahashi 1064</i> (KYO)	Noguchi	-	Al	2n=48	0.9-1.3	-	4-7	-	-	-	-	-	LC030580	LC030626	LC030718	LC030672	LC030962	LC031008	LC031054	
m-8	Pref. Ibaraki: Hitachinaka-shi	<i>Takahashi 1030</i> (KYO)	Noguchi	-	Al	2n=48	0.9-1.3	5.0-5.5	2-4	ascending	twisted	7.3-9.1	126-184	1.6-2.7	LC030581	LC030627	LC030719	LC030673	LC030963	LC031009	LC031055	
m-9	Pref. Ibaraki: Hitachinaka-shi	<i>Takahashi 1028</i> (KYO)	Ajima	-	Al	2n=50	0.9-1.2	5.6-6.2	4-7	ascending	twisted	11.8-17.8	331-474	2.8-4.9	LC030582	LC030628	LC030720	LC030674	LC030964	LC031010	LC031056	
m-10	Pref. Toyama: Takaoka-shi	<i>Takahashi 1128</i> (KYO)	Takahashi	open lowland	Al	2n=48	0.8-1.2	5.2-5.8	4-7	ascending	twisted	8.8-12.5	237-383	2.0-2.6	LC030583	LC030629	LC030721	LC030675	LC030965	LC031011	LC031057	
m-11	Pref. Fukuoka: Asa-no-shi	<i>Takahashi 1131</i> (KYO)	Takahashi	open lowland	Al	2n=48	0.9-1.3	5.3-6.0	4-7	ascending	twisted	7.0-9.7	154-264	1.2-1.8	LC030584	LC030630	LC030722	LC030676	LC030966	LC031012	LC031058	
m-12	Pref. Gifu: Gifu-shi	<i>Takahashi 1042</i> (KYO)	Takahashi	open lowland	Al	2n=46	0.9-1.4	5.6-6.3	4-7	ascending	twisted	10.2-14.6	143-246	1.2-1.8	LC030585	LC030631	LC030723	LC030677	LC030967	LC031013	LC031059	
m-13	Pref. Mie: Komono-cho	<i>Takahashi 1119</i> (KYO)	Yamawaki	-	Al	2n=46	0.9-1.3	5.8-6.5	4-7	ascending	twisted	10.1-15.4	178-298	3.6-6.1	LC030586	LC030632	LC030724	LC030678	LC030968	LC031014	LC031060	
m-14	Pref. Mie: Komono-cho	<i>Takahashi 1120</i> (KYO)	Yamawaki	-	Al	2n=46	0.8-1.2	5.3-6.0	3-4	ascending	twisted	7.5-10.1	164-202	3.3-5.4	LC030587	LC030633	LC030725	LC030679	LC030969	LC031015	LC031061	
m-15	Pref. Nara: Ikaruga-cho	<i>Takahashi 1043</i> (KYO)	Oda	-	Al	2n=47	0.9-1.2	4.8-5.5	4-6	ascending	twisted	10.0-17.6	160-237	2.2-3.4	LC030588	LC030634	LC030726	LC030680	LC030970	LC031016	LC031062	
m-16	Pref. Osaka: Ibaraki-shi	<i>Takahashi 1118</i> (KYO)	Takahashi	open lowland	Al	2n=46	0.8-1.2	5.6-6.2	4-8	ascending	twisted	7.5-9.8	135-210	1.4-1.7	LC030589	LC030635	LC030727	LC030681	LC030971	LC031017	LC031063	
m-17	Pref. Osaka: Ibaraki-shi	<i>Takahashi 1121</i> (KYO)	Takahashi	shaded swamp	Al	2n=46	0.9-1.3	5.7-6.4	4-8	ascending	twisted	9.1-15.9	236-326	3.5-4.3	LC030590	LC030636	LC030728	LC030682	LC030972	LC031018	LC031064	
m-18	Pref. Osaka: Ibaraki-shi	<i>Takahashi 1122</i> (KYO)	Takahashi	shaded swamp	Al	2n=46	0.9-1.4	4.8-5.7	5-10	ascending	twisted	11.8-19.4	325-446	3.0-4.7	LC030591	LC030637	LC030729	LC030683	LC030973	LC031019	LC031065	
m-19	Pref. Hyogo: Kobe-shi	<i>Takahashi 1116</i> (KYO)	Takahashi	shaded lowland	Al	2n=48	0.9-1.3	-	3-5	-	-	-	-	-	LC030592	LC030638	LC030730	LC030684	LC030974	LC031020	LC031066	
m-20	Pref. Hyogo: Kobe-shi	<i>Takahashi 1117</i> (KYO)	Takahashi	open lowland	Al	2n=48	0.8-1.3	4.7-5.6	4-6	ascending	twisted	9.0-11.7	163-187	3.0-5.3	LC030593	LC030639	LC030731	LC030685	LC030975	LC031021	LC031067	
m-21	Pref. Okayama: Okuyama-shi	<i>Takahashi & Yano 1123</i> (KYO)	Takahashi & Yano	open lowland	Al	2n=48	0.9-1.2	5.2-6.0	4-6	ascending	twisted	8.2-15.2	197-332	3.3-4.2	LC030594	LC030640	LC030732	LC030686	LC030976	LC031022	LC031068	
m-22	Pref. Okayama: Kibichuo-cho	<i>Takahashi & Yano 1124</i> (KYO)	Takahashi & Yano	shaded swamp	Al	2n=48	0.8-1.2	6.2-6.6	3-5	ascending	twisted	9.0-12.3	184-265	2.9-4.1	LC030595	LC030641	LC030733	LC030687	LC030977	LC031023	LC031069	
m-23	Pref. Saga: Karatsu-shi	<i>Iwamura 24480</i> (OKAY)	Iwamura	-	Al	-	-	5.0-5.5	3-5	ascending	twisted	7.8-11.4	172-236	2.4-4.0	LC030596	LC030642	LC030734	LC030688	LC030978	LC031024	LC031070	
m-24	Pref. Fukuoka: Tsuruga-shi	<i>Takahashi 1130</i> (KYO)	Takahashi	open lowland	A2	2n=48	0.9-1.2	5.5-6.3	4-8	ascending	twisted	7.1-9.8	260-375	2.5-5.7	LC030597	LC030643	LC030735	LC030689	LC030979	LC031025	LC031071	
m-25	Pref. Akita: Yurionpo-shi	<i>Takahashi 1019</i> (KYO)	Shibata	-	A3	2n=48	0.8-1.3	6.0-6.4	4-6	ascending	twisted	10.0-12.1	248-301	2.2-2.9	LC030598	LC030644	LC030736	LC030690	LC030980	LC031026	LC031072	
m-26	Pref. Miyazaki: Nobeoka-shi	<i>Takahashi 1129</i> (KYO)	Kurogi	open lowland	A4	2n=48	0.9-1.3	5.3-5.8	3-5	ascending	twisted	7.1-9.2	161-255	2.1-2.5	LC030599	LC030645	LC030737	LC030691	LC030981	LC031027	LC031073	
m-27	Pref. Saga: Saga-shi	<i>Iwamura 24576</i> (OKAY)	Iwamura	shaded swamp	B1	-	-	5.5-6.1	3-6	ascending	straight	8.5-10.2	515-698	2.5-3.9	LC030600	LC030646	LC030738	LC030692	LC030982	LC031028	LC031074	
m-28	Pref. Gifu: Seki-shi	<i>Takahashi 1041</i> (KYO)	Takahashi	shaded swamp	B2	2n=36	0.8-2.1	4.8-5.3	4-7	ascending	straight	9.7-13.1	512-531	2.7-3.8	LC030601	LC030647	LC030739	LC030693	LC030983	LC031029	LC031075	
m-29	Pref. Aichi: Okazaki-shi	<i>Takahashi 1128</i> (KYO)	Takahashi	shaded swamp	B3	2n=38	0.9-2.0	5.3-5.9	4-8	ascending	straight	9.7-11.6	460-609	3.6-5.2	LC030602	LC030648	LC030740	LC030694	LC030984	LC031030	LC031076	
m-30	Pref. Shiga: Nagahama-shi	<i>Takahashi 1115</i> (KYO)	Takahashi	shaded swamp	B4	2n=36	0.9-2.1	5.5-6.0	2-4	ascending	straight	7.4-8.5	285-314	2.7-4.6	LC030603	LC030649	LC030741	LC030695	LC030985	LC031031	LC031077	
m-31	Pref. Shiga: Nagahama-shi	<i>Takahashi 17025</i> (KYO)	Nagasawa	-	B4	-	-	5.7-6.2	4-10	ascending	straight	9.5-16.8	555-738	2.4-3.9	LC030604	LC030650	LC030742	LC030696	LC030986	LC031032	LC031078	
m-32	Hokkaido: Tomakomai-shi	<i>Nakagawa 19009</i> (SAPS)	Nakagawa	-	B4	-	-	5.2-5.8	5-10	ascending	straight	11.9-17.7	517-635	1.9-3.0	LC030605	LC030651	LC030743	LC030697	LC030987	LC031033	LC031079	
m-33	Pref. Iwate: Morioka-shi	<i>Takano 23322</i> (OKAY)	Takano	-	B5	-	-	5.0-5.4	5-10	ascending	straight	12.3-17.5	627-715	4.5-6.8	LC030606	LC030652	LC030744	LC030698	LC030988	LC031034	LC031080	
m-34	Pref. Tochigi: Nasu-cho	<i>Takahashi 1029</i> (KYO)	Noguchi & Ajima	-	B5	2n=36	0.9-2.4	5.4-5.9	5-10	ascending	straight	10.1-19.7	228-373	2.1-3.8	LC030607	LC030653	LC030745	LC030699	LC030989	LC031035	LC031081	
m-35	Pref. Ibaraki: Shiroato-cho	<i>Takahashi 1016</i> (KYO)	Noguchi & Ajima	-	B5	2n=34	0.9-2.4	-	4-6	-	-	-	-	-	LC030608	LC030654	LC030746	LC030700	LC030990	LC031036	LC031082	
<i>C. purpurifera</i> Boott																						
p-1	Pref. Nagano: Otsu-mura	<i>Koba 1309</i> (KYO)	Koba	-	C1	-	-	3.6-4.3	6-12	ascending	straight	10.2-23.1	325-389	5.0-7.2	LC030609	LC030655	LC030747	LC030701	LC030991	LC031037	LC031083	
p-2	Pref. Hyogo: Kani-cho	<i>Takahashi 1023</i> (KYO)	Matsusaka	-	C1	-	-	3.9-4.5	4-7	ascending	straight	11.1-15.2	223-316	4.3-6.8	LC030610	LC030656	LC030748	LC030702	LC030992	LC031038	LC031084	
p-3	Pref. Hiroshima: Shobara-shi	<i>Oda 7887</i> (KYO)	Oda	-	C1	-	-	3.7-4.2	8-13	ascending	straight	10.7-17.4	387-437	4.1-6.5	LC030611	LC030657	LC030749	LC030703	LC030993	LC031039	LC031085	
p-4	Pref. Yamagata: Yuzawa-cho	<i>Hoshino et al. 21800</i> (KYO)	Hoshino et al.	-	C2	-	-	3.9-4.4	8-11	ascending	straight	13.4-17.1	566-665	2.8-4.4	LC030612	LC030658	LC030750	LC030704	LC030994	LC031040	LC031086	
p-5	Pref. Akita: Yuzawa-shi	<i>Takahashi 1020</i> (KYO)	Shibata	-	C3	-	-	3.4-4.0	10-16	ascending	straight	16.4-28.6	473-554	4.2-5.0	LC030613	LC030659	LC030751	LC030705	LC030995	LC031041	LC031087	
p-6	Hokkaido: Oyo-cho	<i>Sasaki 50118</i> (KYO)	Sasaki	-	C4	-	-	13.5-21.1	516-630	-	-	-	-	-	LC030614	LC030660	LC030752	LC030706	LC030996	LC031042	LC031088	
<i>C. variabilis</i> H. Lév.																						
v-1	Pref. Aomori: Aomori-shi	<i>Oda & Yonekura 8101</i> (KYO)	Oda & Yonekura	-	D1	-	-	4.0-4.8	4-8	erect	straight	14.0-26.1	257-405	3.0-4.2	LC030615	LC030661	LC030753	LC030707	LC030997	LC031043	LC031089	
v-2	Pref. Fukuoka: Kasuyama-shi	<i>Oda 9783</i> (KYO)	Oda	-	D2	-	-	4.1-4.9	4-7	erect	straight	10.2-18.8	203-303	3.6-5.1	LC030616	LC030662	LC030754	LC030708	LC030998	LC031044	LC031090	
<i>C. filipes</i> Franch. & Sav.																						
-	Pref. Ibaraki: Hitachinaka-shi	<i>Takahashi 1025</i> (KYO)	Ajima	-	-	-	-	-	-	-	-	-	-	-	LC030617	LC030663	LC030755	LC030709	LC030999	LC031045	LC031091	
<i>C. pilosa</i> Scop.																						
-	Hokkaido: Kushiro-shi	<i>Oda & Kato 9428</i> (KYO)	Oda & Kato	-	-	-	-	-	-	-	-	-	-	-	LC030618	LC030664	LC030756	LC030710	LC031000	LC031046	LC031092	
<i>C. curvicalis</i> Franch. & Sav.																						
-	Pref. Kyoto: Kyoto-shi	<i>Takahashi 2021</i> (KYO)	Takahashi	-	-	-	-	-	-	-	-	-	-	-	LC030619	LC030665	LC030757	LC030711	LC031001	LC031047	LC031093	

Table 2. Name, direction, nucleotide sequence and source of primers used in this study. Primers with an asterisk were used only for DNA sequencing; those without an asterisk were used for both PCR amplification and DNA sequencing.

Primer name	Direction	Sequence (5'-3')	source
[atpB-rbcL]			
f1	Forward	GTACTGGGCCAATAATTTGA	Yano <i>et al.</i> (2010)
r	Reverse	CAACACTTGCTTAGTCTCTG	Xu <i>et al.</i> (2000)
[rpl16-rpl16-rps3]			
rpL16F71	Forward	GCTATGCTTAGTGTGTGACTCGTTG	Shaw <i>et al.</i> (2005)
rpL16R1516	Reverse	CCCTTCATTCTTCCTCTATGTTG	Shaw <i>et al.</i> (2005)
rpl-03F*	Forward	CCAACCTATTGCTTCGTATTATC	Oda <i>et al.</i> (2019)
rpl-04R*	Reverse	CATAGCTTCTCTATTTCATGATTAGG	Oda <i>et al.</i> (2019)
[rps16]			
rpS16F	Forward	AAACGATGTGGTARAAAGCAAC	Shaw <i>et al.</i> (2005)
rpS16R	Reverse	AACATCWATTGCAASGATTTCGAT	Shaw <i>et al.</i> (2005)
[trnL-trnL-trnF]			
trnL-c*	Forward	CGAAATCGGTAGACGCTACG	Taberlet <i>et al.</i> (1991)
trnF-f*	Reverse	ATTTGAACTGGTGACACGAG	Taberlet <i>et al.</i> (1991)
trnL-e	Forward	GGTTCAAGTCCCTCTATCCC	Taberlet <i>et al.</i> (1991)
trnL-d	Reverse	GGGGATAGAGGGACTTGAAC	Taberlet <i>et al.</i> (1991)
[matK]			
matK-2.1f	Forward	CCTATCCATCTGGAAATCTTAG	Starr <i>et al.</i> (2009)
matK-5f	Reverse	GTTCTAGCACAAAGAAAGTCG	Starr <i>et al.</i> (2009)
[ITS]			
ITS5i	Forward	AGGTGACCTGCGGAAGGATCATT	Roalson <i>et al.</i> (2001)
ITS4i	Reverse	GGTAGTCCCGCCTGACCTGG	Roalson <i>et al.</i> (2001)
[ETS]			
ETS-1F	Forward	CTGTGGCGTCGCATGAGTTG	Starr <i>et al.</i> (2003)
18S-R	Reverse	AGACAAGCATATGACTACTGGCAGG	Starr <i>et al.</i> (2003)

Table 3. Morphological features of *Carex macroglossa* from Korea.

Locality	Voucher	Length of perigynia (mm)	Number of perigynia	Direction of perigynia at maturity	Base of style	Length of pistillate spikes (mm)	Length of culms (mm)	Width of leaves (mm)
Jeju-do, seogwipo-shi	<i>Im 121271</i> (OKAY)	5.4–6.2	4–8	patent	straight	9.4–17.5	345–603	2.0–2.6
Jeollabuk-do, Gochang-gun, asan-myeon	<i>Hoshino et al. 23447</i> (OKAY)	5.3–5.9	4–8	patent	twisted	7.8–11.8	139–208	2.0–2.7
Jeollanam-do, Guwangju-shi, Dong-gu	<i>Hoshino et al. 23794</i> (OKAY)	5.2–5.8	5–11	patent	twisted	9.1–20.6	278–332	1.6–2.3
Jeollanam-do, Guwangju-shi, Buku-gu	<i>Im & Son 132775</i> (OKAY)	5.1–6.0	4–8	patent	twisted	10.1–16.2	317–349	1.5–2.0

Figures



Fig. 1. *Carex macroglossa* from Osaka Prefecture, Ibaraki-shi (m-18 in Table 1). Left, habit; right, spikes.

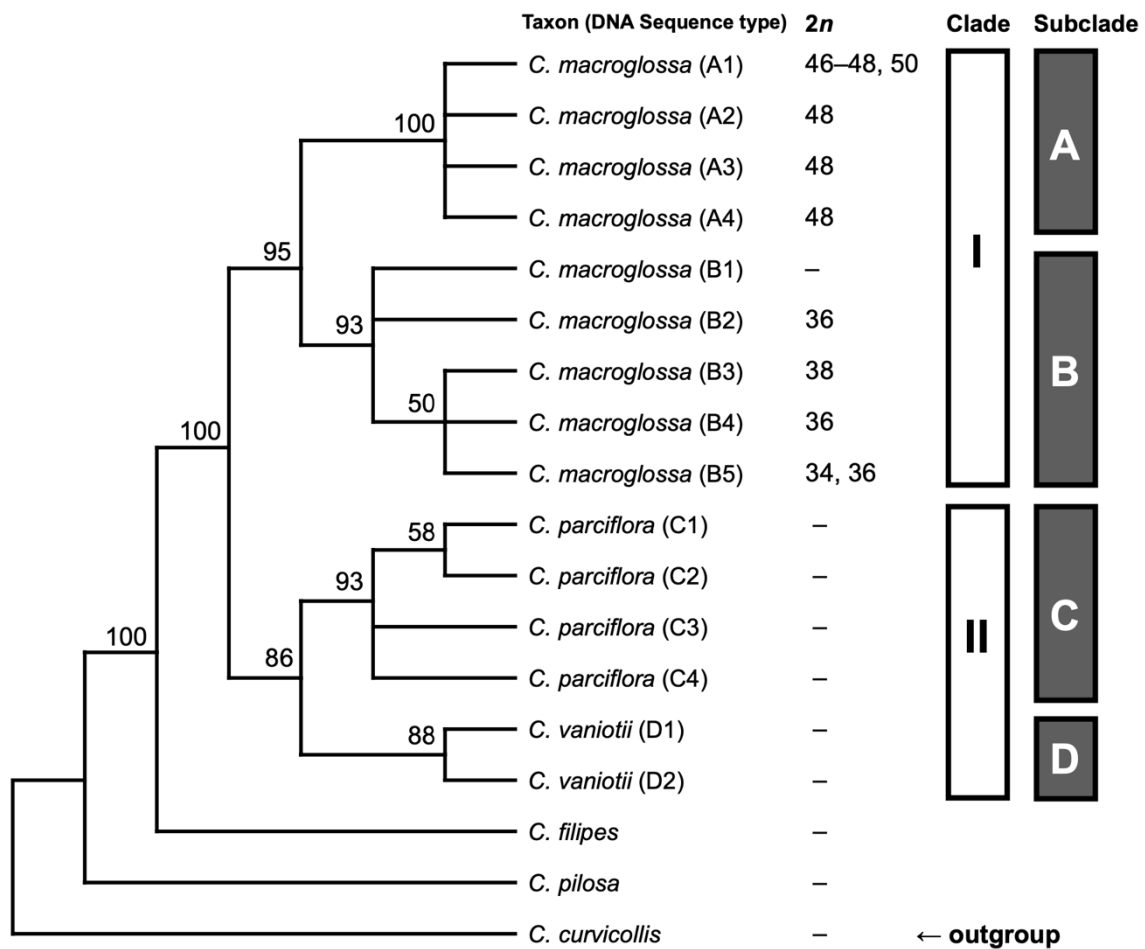


Fig. 2. Maximum likelihood tree of *Carex macroglossa*, *C. parciflora*, *C. vaniotii*, *C. filipes*, *C. pilosa* and *C. curvicollis* (outgroup) using TIM1+G model selected by jModelTest, based on combined data set of plastid regions of *atpB–rbcL*, *rpl16–rpl16–rps3*, *rps16*, *matK* and *trnL–trnL–trnF* and nuclear regions of ITS and ETS ($p = 0.48$ in ILD test). Bootstrap values (%) are shown above branches. Chromosome numbers ($2n$) are also shown. Samples of each DNA sequence type are listed in Table 1.

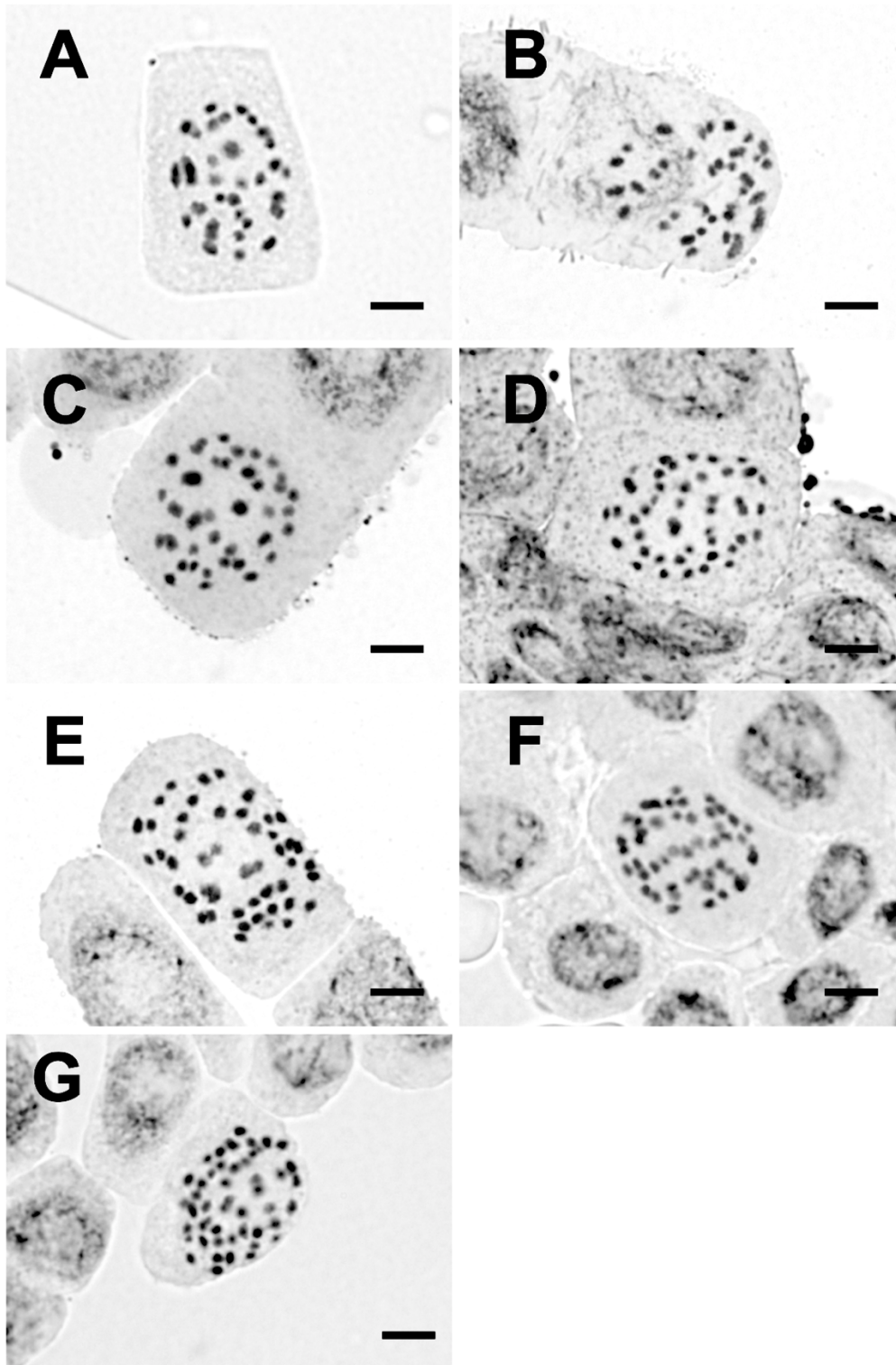


Fig. 3. Photomicrographs of somatic metaphase chromosomes of *Carex macroglossa*; A, $2n = 34$ (m-35). B, $2n = 36$ (m-34). C, $2n = 38$ (m-29). D, $2n = 46$ (m-12). E, $2n = 47$ (m-6). F, $2n = 48$ (m-25). G, $2n = 50$ (m-9). Code in parentheses corresponds to codes in Table 1. Bars = 5 μm

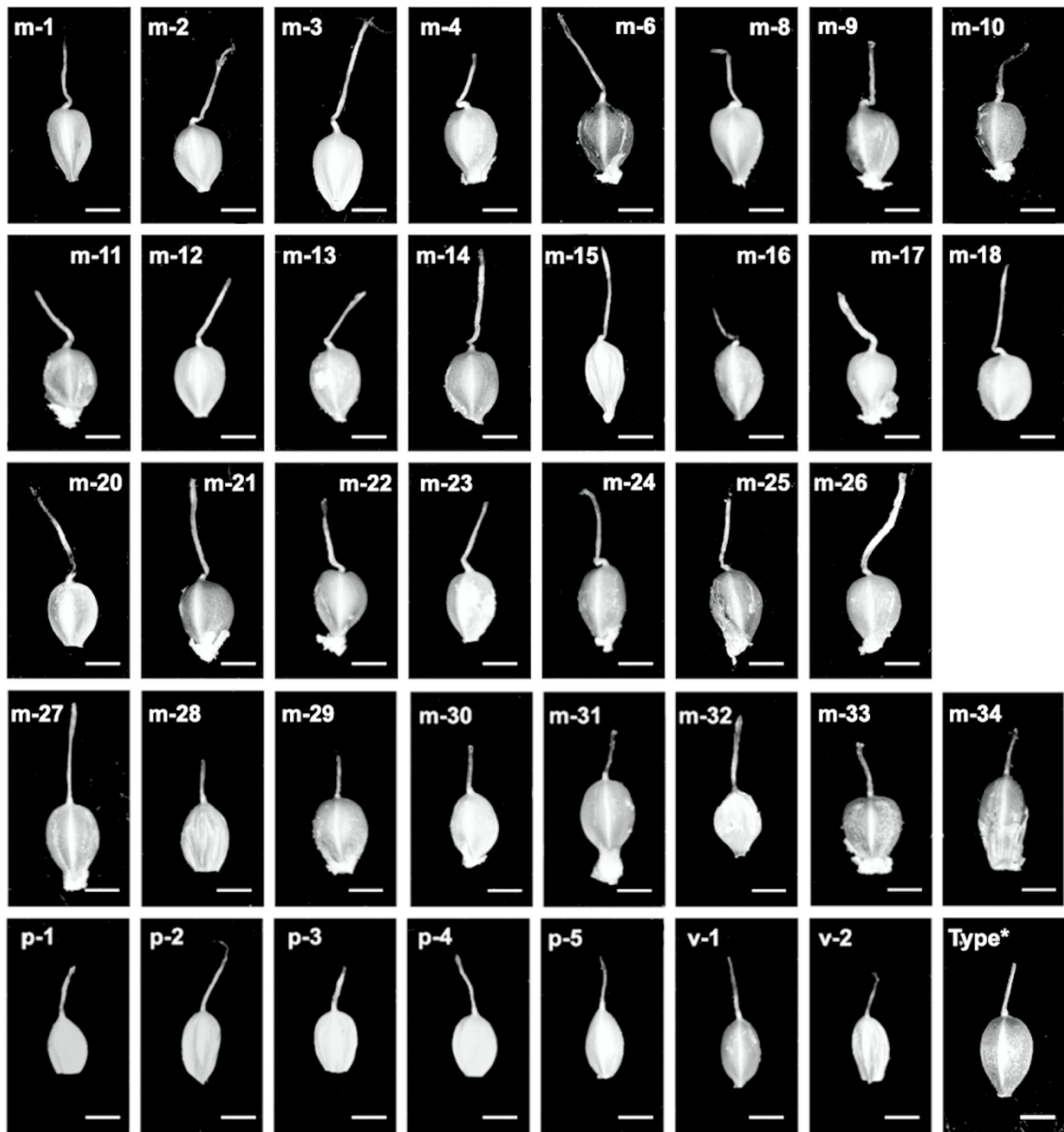


Fig. 4. Morphology of achenes and styles of *Carex macroglossa*, *C. parciflora* and *C. vaniotii*. Code for each photo corresponds to code indicated in Table 1. Type* indicates achene and style of type specimen of *C. parciflora* var. *tsukudensis* (T. Koyama 11138, TNS!). Bars = 1 mm.

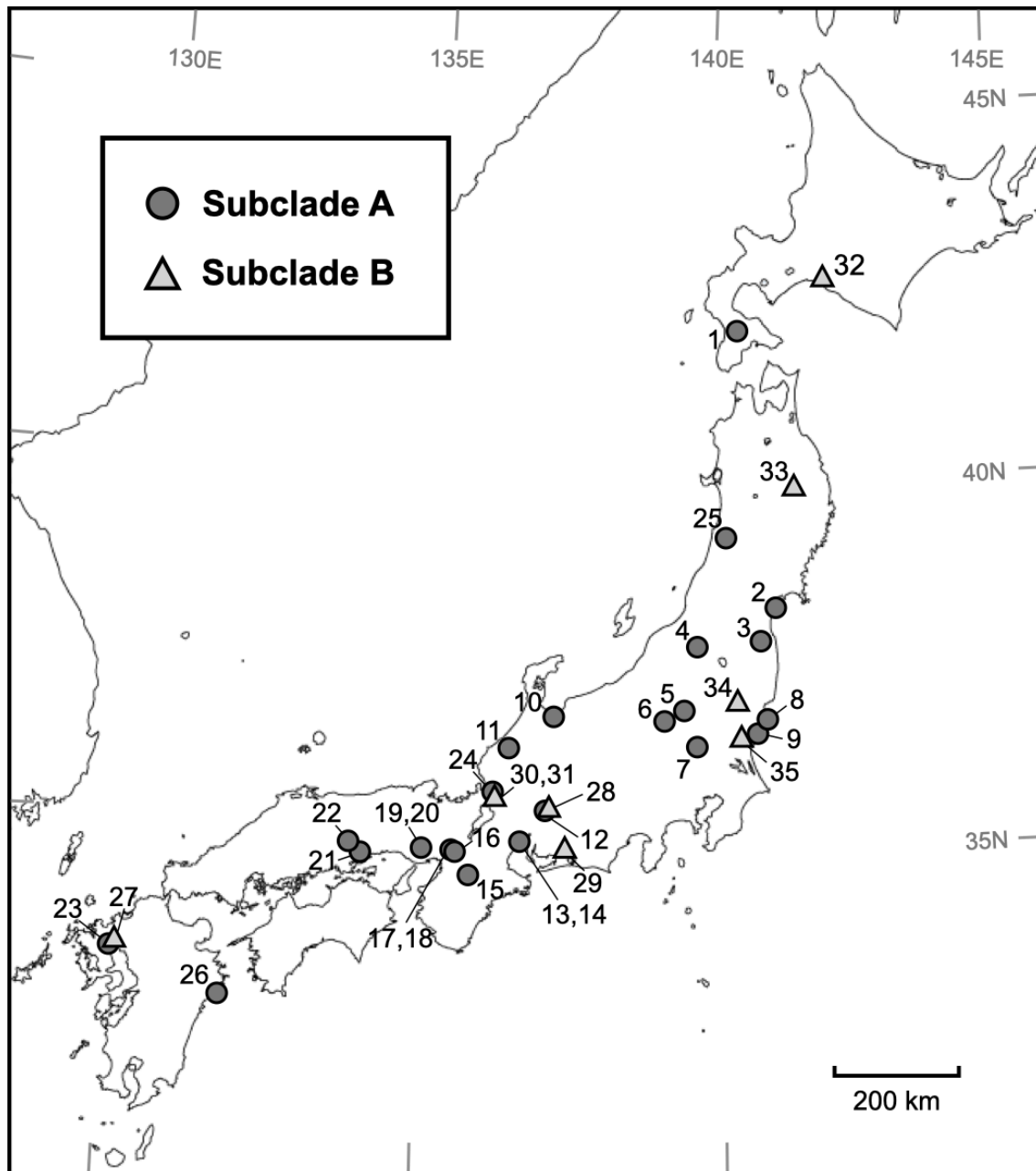


Fig. 5. Distribution of subclade A (solid circles) and subclade B (shaded triangles) of *Carex macroglossa* in Japan. Number of each symbol corresponds to code in Table 1. subclade A and subclade B correspond to subclades in Fig. 2.

Chapter IV

Carex nasuensis, a new species from Japan

Abstract

A new species of *Carex* sect. *Paniceae* (Cyperaceae) is described based on specimens from Honshu, Japan, and named *C. nasuensis*. It is similar to *C. filipes* and *C. macroglossa*, but differs from *C. filipes* in having pale green staminate scales, pale brown basal part of the sheath and perigynia tapering to the apex, and from *C. macroglossa* in having fewer (2–4) perigynia per pistillate spike, pistillate spikes more distantly located on the culm (3.6–7.5 cm between the uppermost and second uppermost pistillate spikes), flat or slightly elevated surface of the epidermal cells of the perigynium (without any conspicuous projections) and an achene epidermal cell surface with a small conical silica deposit surrounded by a marginal depression. A phylogenetic analysis based on the combined data set of plastid regions *atpB–rbcL*, *rpl16–rpl16–rps3*, *rps16*, *matK* and *trnL–trnL–trnF* and nuclear regions ITS and ETS ($p = 0.41$ in ILD test) indicated that *C. nasuensis* was distinct from both *C. filipes* and *C. macroglossa*, supporting the recognition of *C. nasuensis* as an independent species.

Introduction

Carex L., comprising about 2,000 species, is the largest genus of Cyperaceae Juss. (Roalson *et al.* 2021) and the fifth largest genus of angiosperms (Frodin 2004). *Carex* is distinguished from other genera of Cyperaceae by its unisexual flowers and a perigynium, the latter of which corresponds to a prophyll that completely encloses an achene (Goetghebeur 1998, Starr *et al.* 2004).

The late His Majesty the Emperor Hirohito discovered a questionable sedge in the Nasu region, eastern Honshu, Japan (Emperor Hirohito 1985). The sedge was questionable because it was similar

to *C. filipes* Franch. & Sav. in gross morphology, but the basal part of sheath was pale brown, instead of reddish purple as in *C. filipes*. Based on the key to species by Ohwi (1936, 1953, 1982), Akiyama (1955), Koyama (1962, 1964) and Hoshino *et al.* (2020), this sedge was identified as

C. macroglossa Franch. & Sav., primarily on the basis of the sheath color. Further, it was the most similar to *C. macroglossa* f. *subsessilis* (Ohwi) Ohwi among the infraspecific taxa of *C. macroglossa* because they share large perigynia (Ohwi 1930). It appeared to us, however, to differ from *C. macroglossa* (including f. *subsessilis*) in having more distantly positioned pistillate spikes and fewer perigynia per pistillate spike, although these quantitative morphological characters were reported to be highly variable within *C. macroglossa* (Akiyama 1955, Katsuyama 2015).

The purpose of this study was to determine whether this questionable sedge was an independent species or a variant of *C. filipes* or *C. macroglossa*. For that purpose, we observed the gross and micro-morphology of these plants and of *C. filipes* and *C. macroglossa* and conducted a molecular phylogenetic analysis of them.

Materials and Methods

Observation of gross morphology

The gross morphology of the questionable sedge, *Carex filipes* and *C. macroglossa* was observed based in herbarium specimens preserved in GMNHJ, KYO, OKAY and SAPS as well as on

living plants cultivated in the Botanical Gardens, Graduate School of Science, Kyoto University (Table 1). The characters examined in this study included the maximum number of perigynia per pistillate spike (Fig. 1A), length of perigynia, shape of the apex of the perigynia, distance between nodes of the uppermost and second uppermost pistillate spikes (Fig. 1B), color of scales on staminate spikes and color of the basal part of the sheath.

To test for significant differences between the questionable sedge and *Carex filipes* and *C. macroglossa*, we performed a Mann-Whitney *U* test, which is a non-parametric test between two

samples. The level of significance was set at $P < 0.01$. The analyses were run in R (R Development Core Team 2019).

Micro morphological observations

The materials listed in Table 1 were also used for the micro morphological observations with a scanning electron microscope (SEM). We observed the epidermal cell surface of perigynia from both fresh materials and herbarium specimens, and those of the achenes exclusively from herbarium specimens.

Fresh perigynia were initially fixed in 50% ethanol, then dehydrated through an ethanol series, subsequently critical point dried using solvent-substituted liquid CO₂, coated with gold using an ion sputtering apparatus, and observed with a Hitachi Miniscope TM-1000 (Tokyo, Japan) SEM. For the perigynia from herbarium specimens we omitted the critical point drying, and started with the gold coating.

The achenes were prepared by the acetolysis method of Oda & Nasamasu (2008), then coated with gold and observed by SEM as detailed above.

Molecular phylogenetic analysis

The materials listed in Table 1 were used also for the molecular phylogenetic analysis. To confirm that the questionable sedge was in *Carex* sect. *Paniceae*, we analyzed *C. pilosa* Scop. of sect. *Paniceae* in addition to *C. filipes* and *C. macroglossa* as an ingroup. We used *C. curvicolis* Franch. & Sav. of sect. *Racemosae* G. Don as an outgroup. DNA sequences of *C. macroglossa* f. *macroglossa*, *C. pilosa* and *C. curvicolis* were determined in Takahashi *et al.* (2021), subsequently deposited in DDBJ (DNA Data Bank of Japan), and then used in this study. The other DNA sequences were newly determined in this study.

Five plastid DNA regions of *atpB-rbcL*, *rpl16-rpl16-rps3*, *rps16*, *matK* and *trnL-trnL-trnF* and two nuclear DNA regions of ITS and ETS were analyzed in this study. Methods of DNA extraction,

PCR amplification and DNA sequencing, as well as primers used in this analysis, followed Takahashi *et al.* (2021). All DNA sequence data newly obtained in this study were deposited in DDBJ.

We employed Maximum Likelihood (ML) analysis to construct a phylogenetic tree based on the combined data of all plastid and nuclear (ITS+ETS) sequences and implemented in PAUP* v.4.0a168, an update of PAUP* v.4 (Swofford 2003). Congruence between the plastid data and the nuclear data was certified with an incongruence length difference (ILD) test ($P = 0.41$) (Farris *et al.* 1994), which was implemented in PAUP* as a partition homogeneity test. The DNA substitution model was selected by jModelTest (Darriba *et al.* 2012). TIM1+G was selected as the best model by Akaike's Information Criterion (AIC) and Akaike's Information Criterion Corrected (AICC). The ML analysis was performed using a heuristic search option of as-is addition sequence and the ML bootstrap analysis with 1,000 replications.

Results & Discussion

Gross morphological characters

Results of the morphological observations of *Carex* are presented in Table 2. In addition to the findings of the then His Majesty the Emperor Hirohito (1985) that the basal part of sheath was pale brown in the questionable sedge (not reddish purple as in *C. filipes*), we found that it also differed from *C. filipes* in the color of staminate scales and the shape of the apex of the perigynia. Staminate scales of the questionable sedge were pale green, whereas those of *C. filipes* were reddish purple. The apex of the perigynia of the questionable sedge tapered (Fig. 2A), while that of *C. filipes* was abruptly narrowed (Fig. 2B). In *C. macroglossa*, the basal part of the sheath and staminate scales were pale brown and pale green, respectively, while the apex of the perigynia tapered (Fig. 2C, D).

The perigynia of the questionable sedge ranged from 6.5 mm to 8 mm, and thus was longer than the perigynia of *Carex macroglossa* f. *macroglossa* (5–6.1 mm), but not significantly different from that of *C. macroglossa* f. *subsessilis* (6.8–8.4 mm) ($P < 0.01$; Table 2). The maximum number of

perigynia per pistillate spike of the questionable sedge ranged from 2 to 4, which was significantly ($P < 0.01$; Table 2) fewer than in *C. macroglossa* f. *macroglossa* (3–8) and f. *subsessilis* (5–8). However, some culms of *C. macroglossa* f. *macroglossa* have as few perigynia as the questionable sedge. The distance between the uppermost and the second uppermost pistillate spikes of the questionable sedge ($10.2 \text{ cm} \pm 4.1 \text{ cm}$) was significantly ($P < 0.01$; Table 2) greater than in *C. macroglossa* f. *macroglossa* ($3.8 \text{ cm} \pm 2.3 \text{ cm}$) and f. *subsessilis* ($3.3 \text{ cm} \pm 2.6 \text{ cm}$). However, the range of variation in distance between the uppermost and second uppermost pistillate spikes of the questionable sedge (3.6–17.5 cm) partially overlapped with *C. macroglossa* f. *macroglossa* (0.8–9.4 cm) and f. *subsessilis* (1.3–8 cm).

Micro morphological characters

SEM-observations showed that epidermal cell surfaces of the perigynia and achenes were similar in the questionable sedge and *C. filipes* but differed from *C. macroglossa* (including both f. *macroglossa* and f. *subsessilis*) (Table 2). The epidermal cell surface of the perigynia in the questionable sedge and *C. filipes* were flat or slightly elevated (Fig. 3A, B), whereas those of *C. macroglossa* were wholly inflated (dome-shaped) (Fig. 3C, D). We additionally observed the epidermal cell surface using dried herbarium specimens and found that they were crumpled in *C. macroglossa* (Fig. 3G, H), but not in the questionable sedge or in *C. filipes* (Fig. 3E, F). The crumpled epidermal cells of *C. macroglossa* are likely to have been due to shrinkage of the cells that were wholly inflated under natural conditions. Thus, we were able to observe the differences between *C. macroglossa* and the questionable sedge / *C. filipes*, regardless of their fresh or dried state. The conical silica deposit on the epidermal cells of the achenes was small and surrounded by a marginal depression in the questionable sedge and *C. filipes* (Fig. 4A, B), while it was large and had no marginal depression in *C. macroglossa* (Fig. 4C, D).

Molecular analysis

The aligned DNA sequences of the plastid (*atpB–rbcL*, *rpl16–rpl16–rps3*, *rps16*, *matK*, and *trnL–trnF*) and nuclear (ITS and ETS) regions were 5,244 bp long, of which 170 bp were variable and 98 bp were phylogenetically informative. When we regarded the same DNA sequences throughout 5,244 bp as the identical DNA sequence type, we distinguished 13 DNA sequence types among the 41 individuals examined. Three samples of the questionable sedge from northern Kanto District, Japan, including the Nasu region, had identical DNA sequence types. Further, both of the two individuals of *C. macroglossa* f. *subsessilis* had identical DNA sequence types, which were identical with the majority of individuals (22 of 25) of *C. macroglossa* f. *macroglossa* (DNA sequence type m1; see Table 1).

The ML tree (Fig. 5) showed that four individuals of the questionable sedge (n1 & n2) formed a clade with 100% bootstrap support. Nine individuals of *C. filipes* (f1–f5) also formed a clade (98%) sister to the clade of the questionable sedge. Twenty-eight individuals of *C. macroglossa* (m1–m4) also formed a clade (100%) sister to the clade (92%) consisting of the questionable sedge and *C. filipes*. Further, as *C. pilosa* was sister to the clade (100%) of the questionable sedge, *C. filipes* and *C. macroglossa*, *C. sect. Paniceae* analyzed here was confirmed to be monophyletic (100%), and the questionable sedge was surely included in *C. sect. Paniceae*. Based on this phylogenetic analysis, the questionable sedge was more closely related to *C. filipes* than to *C. macroglossa*. This relationship reflected the results from the micro morphological analysis base on the epidermal cell surfaces of the perigynia and achenes as discussed above.

Because the questionable sedge was determined to be morphologically and phylogenetically distinct from both *Carex filipes* and *C. macroglossa*, we treat it as a new species, *C. nasuensis* (Fig. 6).

Taxonomy

Carex nasuensis K. T. Takahashi, T. Noguchi & M. N. Tamura, **sp. nov.** — Fig. 6

Carex macroglossa auct. non. Franch. & Sav.: Ohwi in Mem. Coll. Sci. Kyoto Imp. Univ., Ser. B, Biol. 11: 426 (1936), p.p. quoad pl. ex Sawayama-mura, Hitachi.

Carex filipes auct. non Franch. & Sav.: the Emperor Hirohito, Nova Fl. Nasuensis suppl.: 76 (1985).

Carex nasuensis is similar to *C. filipes* and *C. macroglossa* but differs from *C. filipes* in having pale green staminate scales, pale brown basal part of the sheath, and perigynia tapering to the apex. From *C. macroglossa* it differs in having the surface of the epidermal cells of the perigynia flat or slightly elevated and the surface of the epidermal cells of the achene with a small conical silica deposit surrounded by a marginal depression.

Typus. Japan. Tochigi Pref.: Nasushiobara, 19 April, 2021, *T. Noguchi KT1012* (holo-KYO).

Herbs, perennial, cespitose. Rhizome 1–3 mm long. Culm 20–50 cm long, erect, trigonous, smooth. Leaves basal, 10–35 cm long, 3–10 mm broad, shorter than culm, light green, basal part of sheath pale brown. Bracts leaf-like, longer than spikes, long sheathed. Spikes 3(or 4). Terminal spike staminate, linear, 5–8 mm long, 1–1.5 mm broad, 4–8 flowered, short pedunculate, erect; staminate scales obovate, 3–4 mm long, acute, pale green, midrib green. Lateral spikes pistillate, ellipsoid to short cylindrical, 5–10 mm long, 5 mm broad, 2–4 flowered, short or long-pedunculate, slightly drooping, (3.6 –)6.1–14.3(–17.5) cm distance between uppermost and second uppermost pistillate spikes; pistillate scales obovate, 2–3(–3.5) mm long, acute, pale green, midrib green. Perigynia obtusely trigonous, 6.5–8 mm long, 1.5–2 mm broad, smooth, severalveined, long-beaked, tapering to apex. Achene rather loosely enveloped by perigynium, ellipsoid, trigonous, 2–2.5 mm long. Stigmas 3. Anthesis April to May.

Japanese name. Nasu-tamatsuri-suge. *Distribution.* Japan: Honshu (Tochigi, Ibaraki and Kyoto pref.).

Habitats. In open places at forest margins, 30–1,100 m alt.

Other specimens examined. Japan. Tochigi Pref.: Tochigi-shi, 28 April, 2019, *Takahashi 1003* (KYO); Ibaraki Pref., Shirosato-cho, 7 May, 2019, *Takahashi 1007* (KYO); Kyoto Pref., Oyamazaki-cho, 5 May, 2002, *Kurosaki 23052* (KYO).

Acknowledgement

We express our sincere gratitude to Mr. Takashi Ajima, Mr. Takayuki Shibata, Mr. Narihisa Matsuoka, Mr. Hidehiko Shimizu, Mr. Kazuya Yamawaki, Mr. Shuichi Kurogi and Dr. Okihito Yano for collecting samples. We are grateful to Dr. Hiroshi Tobe and Dr. Hiroshi Ikeda for critically reading the manuscript. Our thanks are due to Mr. Masayoshi Umebayashi for his drawings. Thanks are also due to the directors and staff members of GMNHJ, HYO, KPM, KYO, OKAY, OSA, P, SAPS and TNS for allowing us to use their facilities and collections. This study was supported in part by JSPS KAKENHI (Grants-in-Aid for Scientific Research) Grant Number JP16H05763.

References

- Akiyama, S. 1955. Carices of the Far Eastern Region of Asia, Hokkaido Univ. Press, Sapporo (in Japanese).
- Darriba, D., G. L. Taboada, R. Doallo & D. Posada. 2012. jModelTest 2: more models, new heuristics and parallel computing. *Nature, Meth.* 9: 772.
- Emperor Hirohito. 1985. Cyperaceae. *In*: Biological Laboratory, Imperial Household (ed.), *Nova Flora Nasuensis Supplementum*. pp. 74–87. Hoikusha, Osaka (in Japanese)
- Farris, J. S., M. Källersjö, A. G. Kluge & C. Bult. 1994. Testing significance of incongruence. *Cladistics* 10: 315–319.
- Frodin, D. G. 2004. History and concepts of big plant genera. *Taxon* 53: 753–776.
- Goetghebeur, P. 1998. Cyperaceae. *In*: Kubitzki, K. (ed.), *The Families and Genera of Vascular Plants*, vol. IV. Flowering Plants: Monocotyledons, pp. 141–190. Springer, Berlin.
- Hoshino, T., T. Katsuyama, T. Masaki & M. Michikawa. 2020. *Carex* L. *In*: Iwatsuki, K., D. E. Boufford & H. Ohba (eds.), *Flora of Japan*, vol. IVa. Angiospermae: Monocotyledoneae (a), pp. 226–344. Kodansha, Tokyo.

- Katsuyama, T. 2015. *Carex* of Japan enlarged and revised ed. Bun-ichi Sogo Shuppan, Tokyo (in Japanese).
- Koyama, T. 1962. Classification of the family Cyperaceae (2). J. Fac. Sci. Univ. Tokyo, Sect. 3, Bot. 8: 149–278.
- Koyama, T. 1964. Cyperaceae. In: Kitamura, S., G. Murata & T. Koyama (eds.), Color. Illus. Herb. Pl. Jap. (Monocot.), pp. 210–301. Hoikusha, Osaka (in Japanese)
- Oda, J. & H. Nagamasu. 2008. Two new species of *Carex* sect. *Capitellatae* (Cyperaceae) from Japan. Acta Phytotax. Geobot. 59: 55–66.
- Ohwi, J. 1930. Contributiones ad Caricologiam Asiae Orientalis. Mem. Coll. Sci. Kyoto Imp. Univ., Ser. B, Biol. 5: 248–292.
- Ohwi, J. 1936. Cyperaceae Japonicae I. Mem. Coll. Sci. Kyoto Imp. Univ., Ser. B, Biol. 11: 229–530.
- Ohwi, J. 1953. Flora of Japan. Shibundo, Tokyo (in Japanese).
- Ohwi, J. 1982. *Carex*. In: Satake, Y., J. Ohwi, S. Kitamura, S. Watari & T. Tominari (eds.), Wild Flowers of Japan Herbaceous plants (including dwarf subshrubs) I, pp. 145–160. Heibonsha, Tokyo (in Japanese).
- R Development Core Team. 2019. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. <[https:// www.R-project.org/](https://www.R-project.org/)>
- Roalson E. H., P. Jiménez-Mejías, A. L. Hipp, C. BenítezBenítez, L. P. Bruederle, K.S. Chung, M. Escudero, B. A. Ford, K. Ford, S. Gebauer, B. Gehrke, M. Hahn, M. Q. Hayat, M. H. Hoffmann, X.-F. Jin, S. Kim, I. Larridon, É. Lévillé-Bourret, Y.-F. Lu, M. Luceño, E. Maguilla, J. I. Márquez-Corro, S. Martín-Bravo, T. Masaki, M. Míguez, R. F. C. Naczi, A. A. Reznicek, D. Spalink, J. R. Starr, Uzma, T. Villaverde, M. J. Waterway, K. L. Wilson & S.-R. Zhang. 2021. A framework infrageneric classification of *Carex* (Cyperaceae) and its organizing principles. J. Syst. Evol. 59: 726 –762.
- Starr, J. R., S. A. Harris & D. A. Simpson. 2004. Phylogeny of the unispicate taxa in Cyperaceae tribe Cariceae I: genetic relationship and evolutionary scenarios. Syst. Bot. 29: 528–544.

Swofford, D. L. 2003. PAUP*: Phylogenetic Analysis Using Parsimony (*and Other Methods).

Version 4. Sinauer, Sunderland.

Takahashi, K. T., J. Oda, S. Fuse & M. N. Tamura. 2021. Biosystematic studies of *Carex*

(Cyperaceae) I. Molecular phylogenetic analysis of the *C. macroglossa* complex with reference to variation in morphology, chromosomal features and species delimitation. Acta Phytotax. Geobot.

72: 81–91.

Tables

Table 1. Sources of materials used in the present morphological and molecular analyses with their DNA sequence types and accession numbers. DNA sequences of *Carex macroglossa* f. *macroglossa*, *C. pilosa* and *C. curvicolis* were determined in Takahashi *et al.* (2021), subsequently deposited in DDBJ (DNA Data Bank of Japan), and then used for this molecular analysis.

Taxon & Locality	Voucher	Collector	DNA sequence type	Accession Number					ITS	ETS	
				<i>atpB-rbcL</i>	<i>rpl16-rpl116-rps3</i>	<i>rps16</i>	<i>matK</i>	<i>trnL-trnL-trnF</i>			
The questionable sedge (<i>Carex nasuensis</i> K. T. Takahashi, T. Noguchi & M. N. Tamura)											
Pref. Tochigi: Nasushiobara-shi	<i>Noguchi KT1012</i> (KYO)	Noguchi	n1	LC715306	LC715320	LC715334	LC715348	LC715371	LC715385	LC715399	
Pref. Tochigi: Tochigi-shi	<i>Takahashi 1003</i> (KYO)	Noguchi	n1	LC715304	LC715318	LC715332	LC715346	LC715369	LC715383	LC715397	
Pref. Ibaraki: Shirosato-cho	<i>Takahashi 1007</i> (KYO)	Noguchi & Ajima	n1	LC715305	LC715319	LC715333	LC715347	LC715370	LC715384	LC715398	
Pref. Kyoto: Oyamazaki-cho	<i>Kurosaki 23052</i> (KYO)	Kurosaki	n2	LC715307	LC715321	LC715335	LC715349	LC715372	LC715386	LC715400	
<i>C. filipes</i> Franch. & Sav.											
Pref. Ibaraki: Hitachiota-shi	<i>Takahashi 1025</i> (KYO)	Ajima	f1	LC630617	LC630663	LC630755	LC630709	LC630999	LC631045	LC631091	
Pref. Tochigi: Nikko-shi	<i>Takahashi 1026</i> (KYO)	Noguchi	f1	LC715304	LC715323	LC715337	LC715351	LC715374	LC715388	LC715402	
Pref. Tokyo: Hachioji-shi	<i>Takahashi 1186</i> (KYO)	Takahashi	f1	LC715309	LC715329	LC715343	LC715357	LC715380	LC715394	LC715408	
Pref. Nagano: Karuizawa-cho	<i>Takahashi 1184</i> (KYO)	Takahashi	f2	LC715315	LC715328	LC715339	LC715356	LC715379	LC715393	LC715407	
Pref. Aichi: Tahara-shi	<i>Takahashi 1183</i> (KYO)	Takahashi	f3	LC715314	LC715327	LC715342	LC715355	LC715378	LC715392	LC715406	
Pref. Gifu: Seki-shi	<i>Takahashi 1013</i> (KYO)	Shimizu	f3	LC715313	LC715322	LC715336	LC715350	LC715373	LC715387	LC715401	
Pref. Okayama: Okayama-shi	<i>Takahashi & Yano 1125</i> (KYO)	Takahashi & Yano	f4	LC715308	LC715326	LC715341	LC715354	LC715377	LC715391	LC715405	
Pref. Tokushima: Miyoshi-shi	<i>Takahashi et al. 1114</i> (KYO)	Takahashi et al.	f5	LC715312	LC715325	LC715340	LC715353	LC715376	LC715390	LC715404	
Pref. Ehime: Kumakogen-cho	<i>Takahashi 1034</i> (KYO)	Takahashi & Oda	f5	LC715311	LC715324	LC715338	LC715352	LC715375	LC715389	LC715403	
<i>C. macroglossa</i> Franch. & Sav. f. <i>macroglossa</i>											
Hokkaido: Ootobe-cho	<i>Hara 3919</i> (SAPS)	Hara	m1	LC630574	LC630620	LC630712	LC630666	LC630956	LC631002	LC631048	
Pref. Miyagi: Sendai-shi	<i>Takahashi 1134</i> (KYO)	Takahashi	m1	LC630575	LC630621	LC630713	LC630667	LC630957	LC631003	LC631049	
Pref. Fukushima: Fukushima-shi	<i>Takahashi 1133</i> (KYO)	Takahashi	m1	LC630576	LC630622	LC630714	LC630668	LC630958	LC631004	LC631050	
Pref. Niigata: Aga-cho	<i>Hoshino & Masaki 24810</i> (OKAY)	Hoshino & Masaki	m1	LC630577	LC630623	LC630715	LC630669	LC630959	LC631005	LC631051	
Pref. Gunma: Kawaba-mura	<i>Ohmori BS19348</i> (GMNHJ)	Ohmori	m1	LC630578	LC630624	LC630716	LC630670	LC630960	LC631006	LC631052	
Pref. Tochigi: Nikko-shi	<i>Takahashi 1059</i> (KYO)	Noguchi & Shibata	m1	LC630579	LC630625	LC630717	LC630671	LC630961	LC631007	LC631053	
Pref. Tochigi: Kanuma-shi	<i>Takahashi 1004</i> (KYO)	Noguchi	m1	LC630580	LC630626	LC630718	LC630672	LC630962	LC631008	LC631054	
Pref. Ibaraki: Takahagi-shi	<i>Takahashi 1030</i> (KYO)	Noguchi	m1	LC630581	LC630627	LC630719	LC630673	LC630963	LC631009	LC631055	
Pref. Ibaraki: Hitachiota-shi	<i>Takahashi 1028</i> (KYO)	Ajima	m1	LC630582	LC630628	LC630720	LC630674	LC630964	LC631010	LC631056	
Pref. Toyama: Takaoka-shi	<i>Takahashi 1132</i> (KYO)	Takahashi	m1	LC630583	LC630629	LC630721	LC630675	LC630965	LC631011	LC631057	
Pref. Fukui: Awara-shi	<i>Takahashi 1131</i> (KYO)	Takahashi	m1	LC630584	LC630630	LC630722	LC630676	LC630966	LC631012	LC631058	
Pref. Gifu: Gifu-shi	<i>Takahashi 1042</i> (KYO)	Takahashi	m1	LC630585	LC630631	LC630723	LC630677	LC630967	LC631013	LC631059	
Pref. Mie: Komono-cho	<i>Takahashi 1119</i> (KYO)	Yamawaki	m1	LC630586	LC630632	LC630724	LC630678	LC630968	LC631014	LC631060	
Pref. Mie: Komono-cho	<i>Takahashi 1120</i> (KYO)	Yamawaki	m1	LC630587	LC630633	LC630725	LC630679	LC630969	LC631015	LC631061	
Pref. Nara: heguri-cho	<i>Takahashi 1043</i> (KYO)	Oda	m1	LC630588	LC630634	LC630726	LC630680	LC630970	LC631016	LC631062	
Pref. Osaka: Ibaraki-shi	<i>Takahashi 1118</i> (KYO)	Takahashi	m1	LC630589	LC630635	LC630727	LC630681	LC630971	LC631017	LC631063	
Pref. Osaka: Ibaraki-shi	<i>Takahashi 1121</i> (KYO)	Takahashi	m1	LC630590	LC630636	LC630728	LC630682	LC630972	LC631018	LC631064	
Pref. Osaka: Ibaraki-shi	<i>Takahashi 1122</i> (KYO)	Takahashi	m1	LC630591	LC630637	LC630729	LC630683	LC630973	LC631019	LC631065	
Pref. Hyogo: Kobe-shi	<i>Takahashi 1116</i> (KYO)	Takahashi	m1	LC630592	LC630638	LC630730	LC630684	LC630974	LC631020	LC631066	
Pref. Hyogo: Kobe-shi	<i>Takahashi 1117</i> (KYO)	Takahashi	m1	LC630593	LC630639	LC630731	LC630685	LC630975	LC631021	LC631067	
Pref. Okayama: Okayama-shi	<i>Takahashi 1123</i> (KYO)	Takahashi & Yano	m1	LC630594	LC630640	LC630732	LC630686	LC630976	LC631022	LC631068	
Pref. Okayama: Kibichuo-cho	<i>Takahashi 1124</i> (KYO)	Takahashi & Yano	m1	LC630595	LC630641	LC630733	LC630687	LC630977	LC631023	LC631069	
Pref. Saga: Karatsu-shi	<i>Iwamura 24480</i> (OKAY)	Iwamura	m1	LC630596	LC630642	LC630734	LC630688	LC630978	LC631024	LC631070	
Pref. Fukui: Tsuruga-shi	<i>Takahashi 1130</i> (KYO)	Takahashi	m2	LC630597	LC630643	LC630735	LC630689	LC630979	LC631025	LC631071	
Pref. Akita: Yurihonjo-shi	<i>Takahashi 1019</i> (KYO)	Shibata	m3	LC630598	LC630644	LC630736	LC630690	LC630980	LC631026	LC631072	
Pref. Miyazaki: Nobeoka-shi	<i>Takahashi 1129</i> (KYO)	Kurogi	m4	LC630599	LC630645	LC630737	LC630691	LC630981	LC631027	LC631073	
<i>C. macroglossa</i> f. <i>subsessilis</i> (Ohwi) Ohwi											
Pref. Tokyo: Hachioji-shi	<i>Takahashi 11851</i> (KYO)	Takahashi	m1	LC715316	LC715330	LC715344	LC715358	LC715381	LC715395	LC715409	
Pref. Tokyo: Hachioji-shi	<i>Takahashi 11852</i> (KYO)	Takahashi	m1	LC715317	LC715331	LC715345	LC715359	LC715382	LC715396	LC715410	
<i>C. pilosa</i> Scop.											
Hokkaido: Kushiro-shi	<i>Takahashi 1080</i> (KYO)	Oda & Kato	-	LC630618	LC630664	LC630756	LC630710	LC631000	LC631046	LC631092	
<i>C. curvicolis</i> Franch. & Sav.											
Pref. Kyoto: Kyoto-shi	<i>Takahashi 2021</i> (KYO)	Takahashi	-	LC630619	LC630665	LC630757	LC630711	LC631001	LC631047	LC631093	

Table 2. Comparison of morphological characters of the questionable sedge (*Carex nasuensis*), *C. filipes*, *C. macroglossa* f. *macroglossa* and *C. macroglossa* f. *subsessilis*. The values of quantitative characters were measured based on the materials listed in Table 1. They are shown as mean $\pm$ SD with actual range in brackets below. Asterisks indicate significant differences from individuals of the questionable sedge (*C. nasuensis*) (Mann-Whitney *U* test, $P < 0.01$).

Taxa	The questionable sedge (<i>Carex nasuensis</i>)	<i>C. filipes</i>	<i>C. macroglossa</i> f. <i>macroglossa</i>	<i>C. macroglossa</i> f. <i>subsessilis</i>
Number of individuals examined	4	9	26	2
Number of culms (stems) examined	24	47	128	10
Gross morphological characters				
Length of perigynia (mm)	7.1 $\pm$ 0.5 (6.5–8.0)	5.6 $\pm$ 0.7* (4.8–7.0)	5.5 $\pm$ 0.4* (5.0–6.1)	7.5 $\pm$ 0.5 (6.8–8.4)
Maximum number of perigynia per pistillate spike	3.1 $\pm$ 0.7 (2–4)	2.9 $\pm$ 0.9 (2–5)	5.1 $\pm$ 1.5* (3–8)	5.7 $\pm$ 1.0* (5–8)
Shape of the apex of perigynia	tapered	abruptly narrowed	tapered	tapered
Distance between nodes of the uppermost and the second uppermost pistillate spikes (cm)	10.2 $\pm$ 4.1 (3.6–17.5)	9.0 $\pm$ 4.6 (3.9–18.9)	3.8 $\pm$ 2.3* (0.8–9.4)	3.3 $\pm$ 2.6* (1.3–8.0)
Color of scales on staminate spikes	pale green	reddish purple	pale green	pale green
Color of the basal part of sheath	pale brown	reddish purple	pale brown	pale brown
Micro morphological characters observed with SEM				
Epidermal cell surface of perigynia	flat or slightly elevated	flat or slightly elevated	wholly inflated	wholly inflated
Silica deposit on epidermal cell surface of achenes	small conical surrounded by marginal depression	small conical surrounded by marginal depression	large conical without any marginal depression	large conical without any marginal depression

Figures.

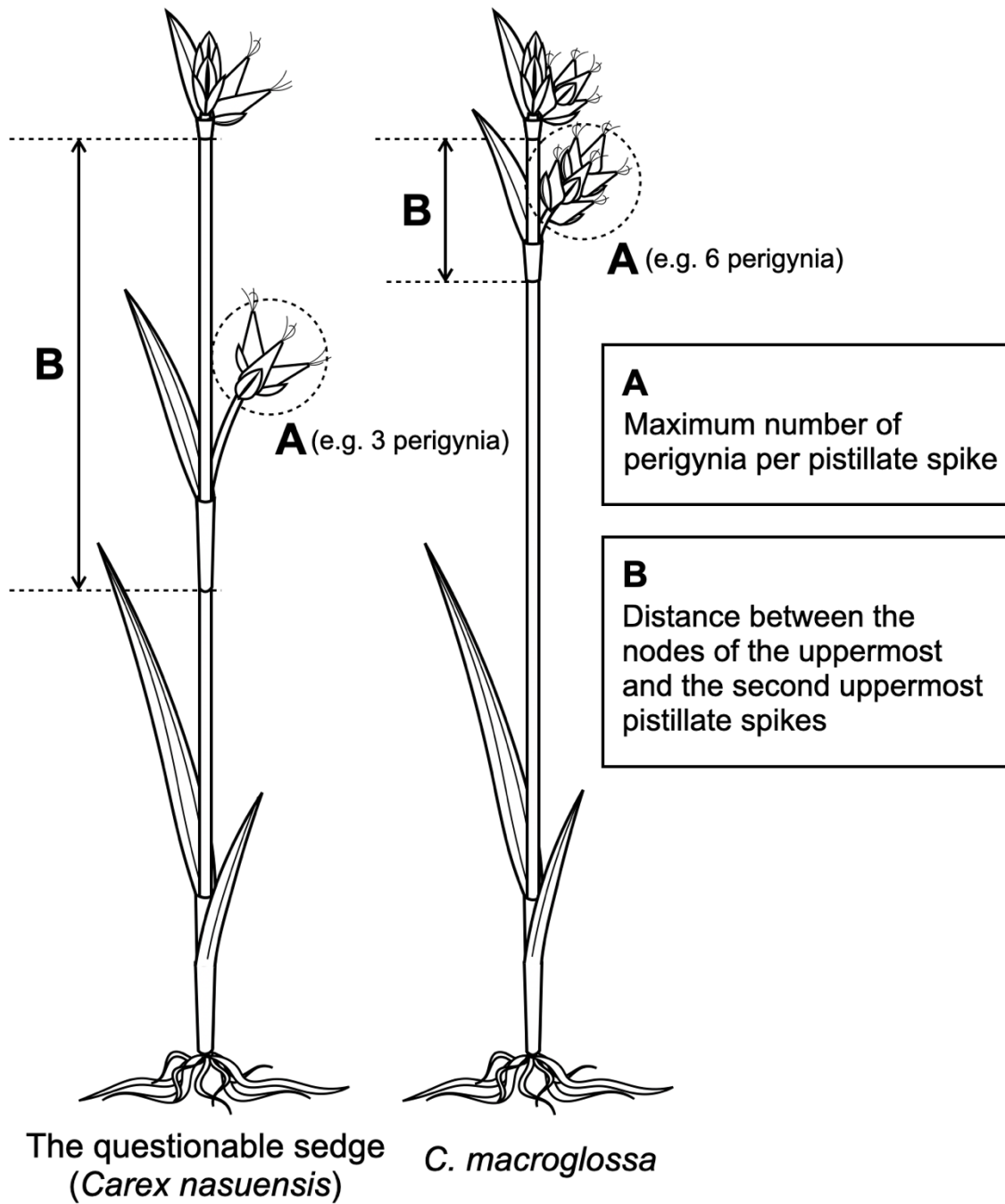


Fig. 1. Diagrams of flowering stems of the questionable sedge (*Carex nasuensis*) (left) and *C. macroglossa* (right), showing what we measured as “maximum number of perigynia per pistillate spike” (A) and as “distance between the nodes of the uppermost and the second uppermost pistillate spikes” (B).

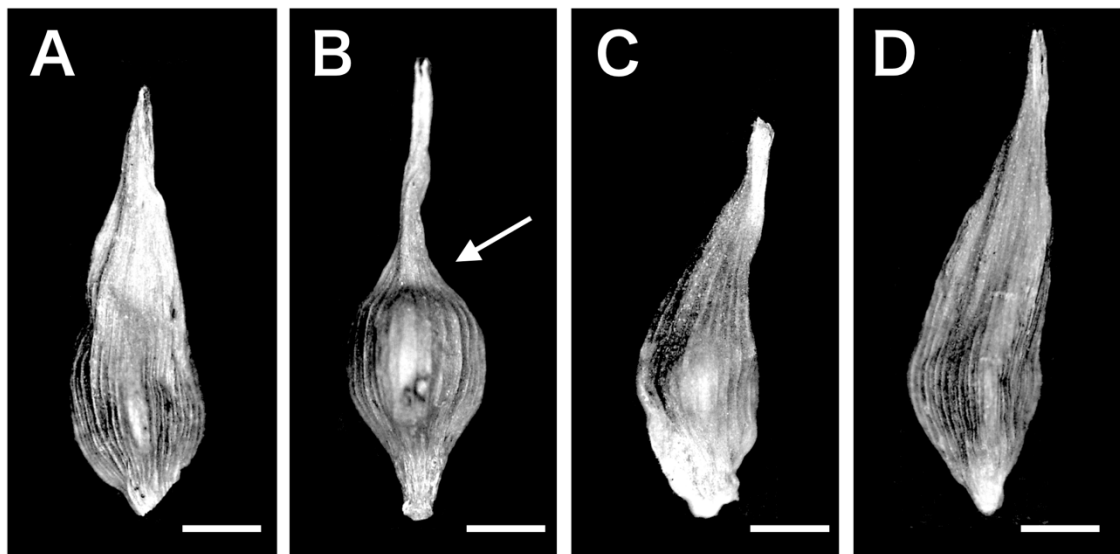


Fig. 2. Side views of perigynia of *Carex*. **A**, the questionable sedge (*C. nasuensis*) (Noguchi KT1012, KYO); **B**, *C. filipes* (Takahashi 1025, KYO); **C**, *C. macroglossa* f. *macroglossa* (Takahashi 1133, KYO); **D**, *C. macroglossa* f. *subsessilis* (Takahashi 11851, KYO). White arrow in B indicates abruptly narrowed point. Bars = 10 μ m.

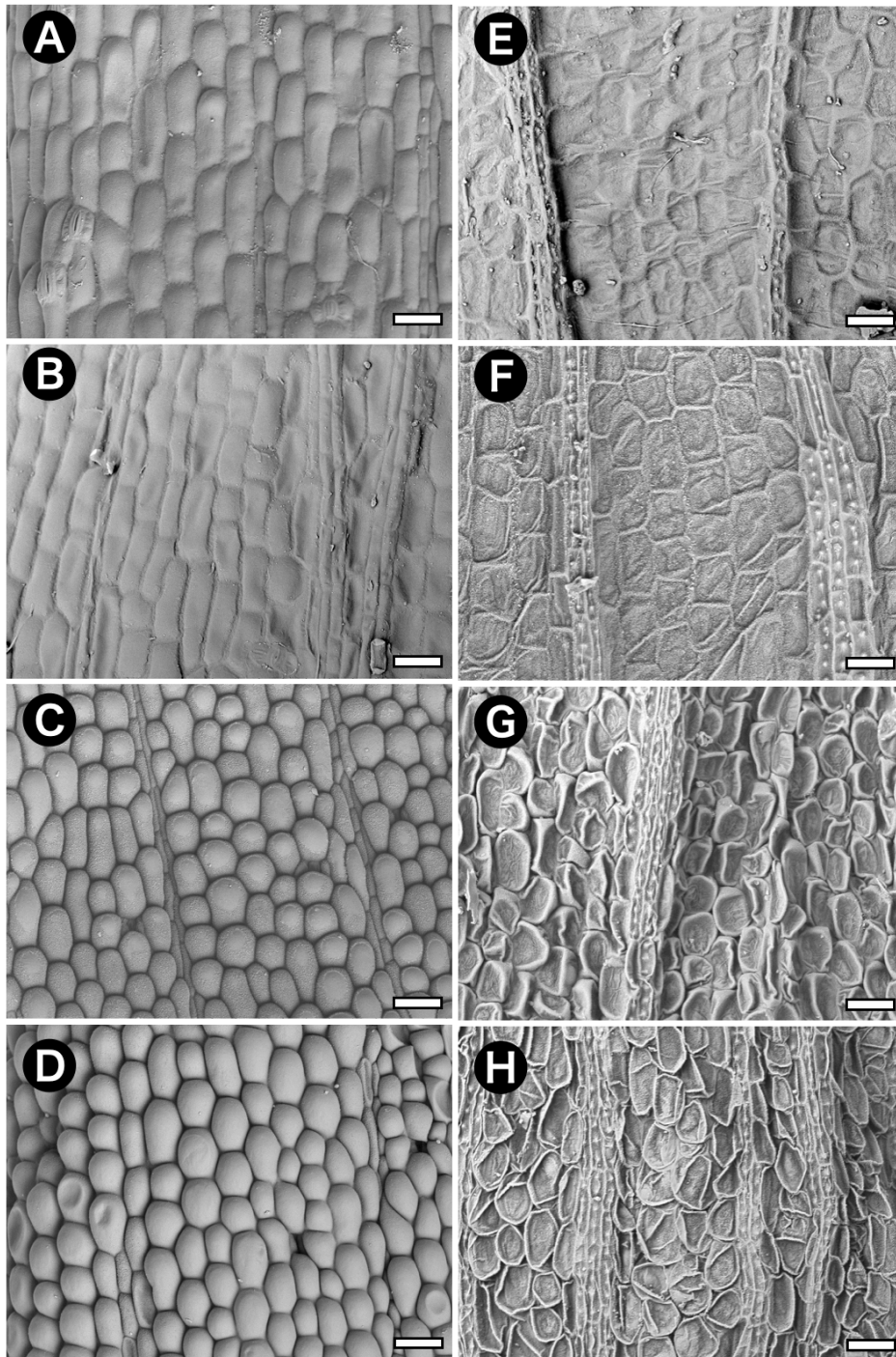


Fig. 3. SEM photographs of epidermal cell surface of *Carex* perigynia. **A–D**, perigynia taken from fresh materials, and treated with critical point drying; **E–H**, perigynia taken from dried herbarium specimens without treatments of critical point drying. **A & E**, the questionable sedge (*C. nasuensis*) (Noguchi KT1012, KYO); **B & F**, *C. filipes* (Takahashi 1025, KYO); **C & G**, *C. macroglossa* f. *macroglossa* (Takahashi 1133, KYO); **D & H**, *C. macroglossa* f. *subsessilis* (Takahashi 11851, KYO). Bars = 10 μ m.

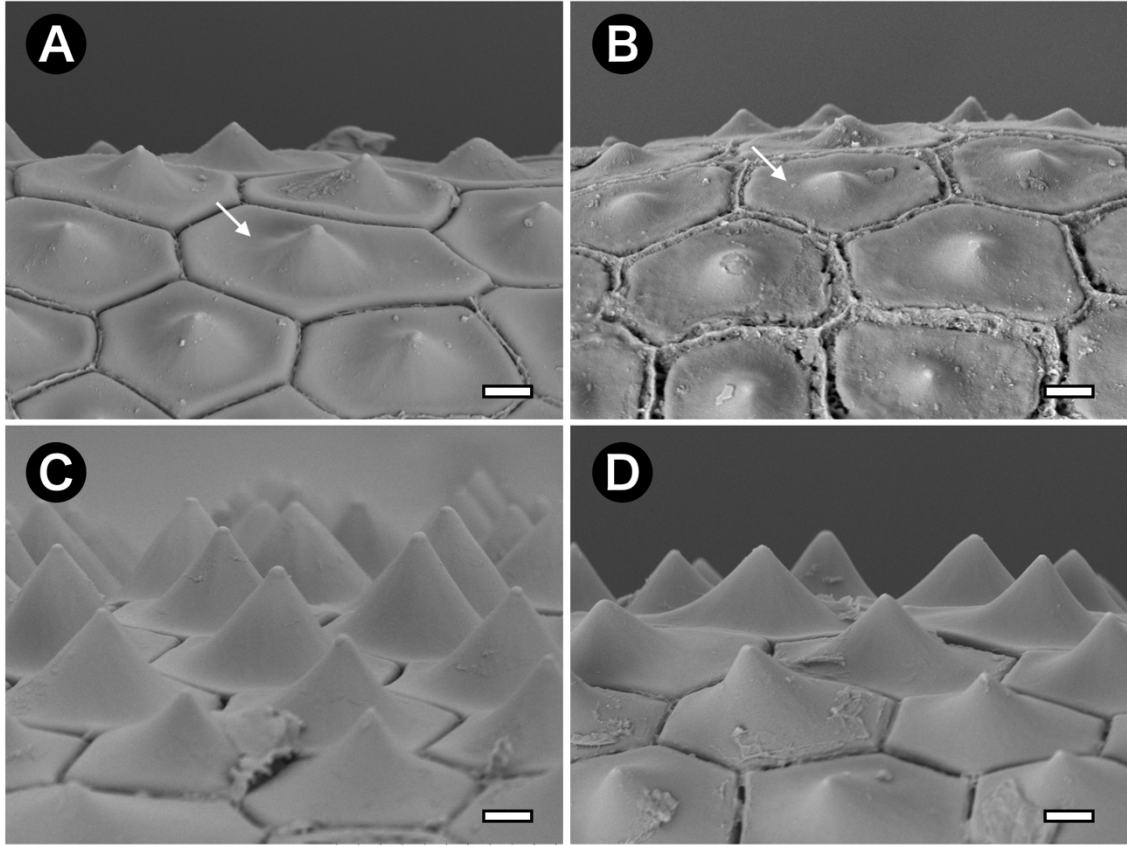


Fig. 4. SEM photographs of silica deposits on achene surface of *Carex*. **A**, the questionable sedge (*C. nasuensis*) (Noguchi KT1012, KYO); **B**, *C. filipes* (Takahashi 1025, KYO); **C**, *C. macroglossa* f. *macroglossa* (Takahashi 1133, KYO); **D**, *C. macroglossa* f. *subsessilis* (Takahashi 11851, KYO). White arrows in A & B indicate “marginal depression” surrounding a small conical silica deposit. Bars = 10 μ m.

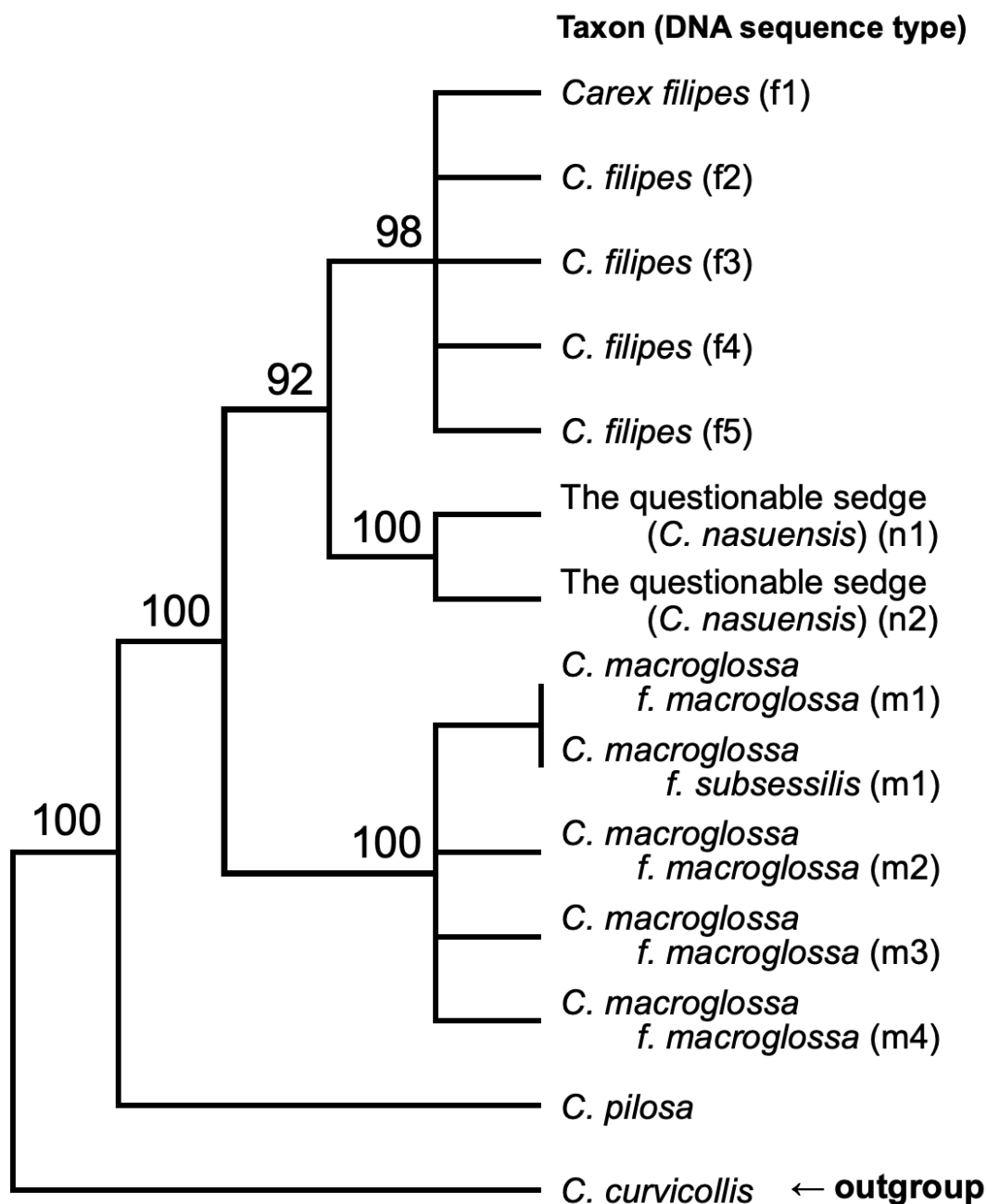


Fig. 5. Maximum likelihood tree of the questionable sedge (*Carex nasuensis*), *C. filipes*, *C. macroglossa* f. *macroglossa*, *C. macroglossa* f. *subsessilis*, *C. pilosa* and *C. curvicollis* (the latter as an outgroup) using TIM1+G model selected by jModelTest, based on the combined data set of plastid regions of *atpB-rbcL*, *rpl16-rpl16-rps3*, *rps16*, *matK* and *trnL-trnL-trnF* and nuclear regions of ITS and ETS (P = 0.41 in ILD test). Bootstrap values (%) are shown above branches. Samples of each DNA sequence type are listed in Table 1.

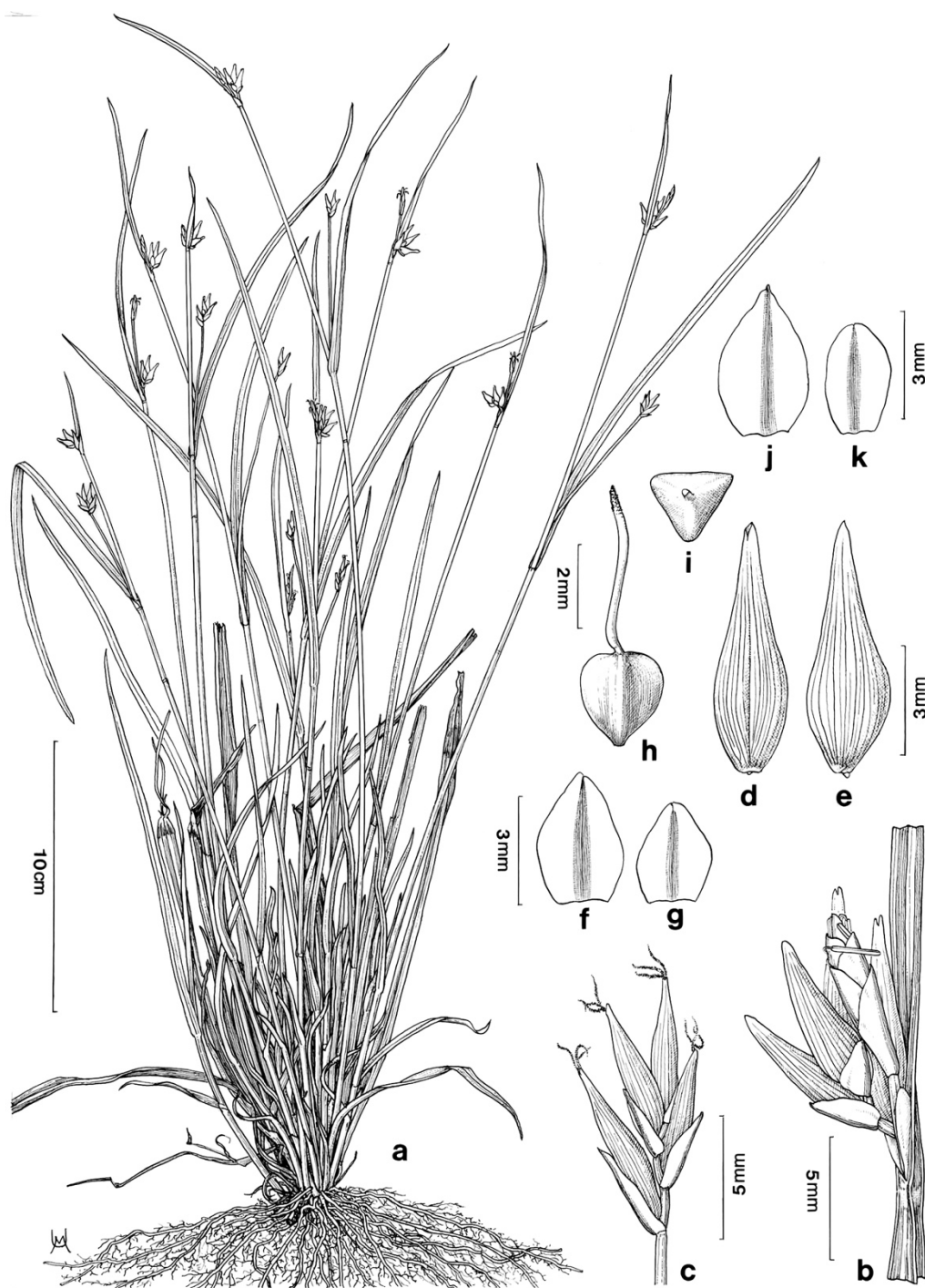


Fig. 6. *Carex nasuensis* K. T. Takahashi, T. Noguchi & M. N. Tamura, sp. nov. **a**, habit; **b**, terminal staminate spike and the uppermost pistillate spike; **c**, the second uppermost pistillate spike; **d**, abaxial side of perigynium; **e**, lateral side of perigynium; **f**, abaxial side of scale at the lowermost position of pistillate spike; **g**, abaxial side of scale at the uppermost position of pistillate spike; **h**, side view of achene with style; **i**, front view of achene lacking style; **j**, abaxial side of scale at the lowermost position of staminate spike; **k**, abaxial side of scale at the fifth lowermost position of staminate spike. **a** & **c** drawn from *Noguchi KT1012* (KYO); **b** & **d–k** from *Takahashi 1003* (KYO).

Conclusion

In Chapter I, the basal lineage of *Carex* subg. *Carex* was elucidated through phylogenetic and morphological evaluations, clarifying the previously ambiguous phylogenetic positions of *C. dissitiflora* Franch. and *C. satsumensis* Franch. et Sav.. Additionally, a new section, *C. sect. Dissitiflorae* K. T. Takahashi & M. N. Tamura, was described, and revisions to the circumscriptions of three existing sections (*C. sects. Filicinae* (C. B. Clarke) Nelm., *Indicae* (L.H.Bailey) C. B. Clarke, *Mundae* Kük.) were proposed. As a result, this study presented the first molecular phylogeny-based revision of sections within the basal lineage of *Carex* subgen. *Carex*, recognizing seven sections. These findings provided critical insights into the phylogenetic relationships within the early evolution of the subgenus *Carex*, offering a framework for understanding the evolutionary processes behind the immense species diversity of the subgenus.

In Chapters II–IV, species recognition within *Carex* sect. *Paniceae* G. Don, a highly diversified taxonomic group in East Asia, was re-evaluated. By integrating phylogenetic analyses, chromosome numbers, and morphological characters, the reproductive isolation among taxa was discussed comprehensively. This study resulted in the recognition of three new species (*C. curtispica* K. T. Takahashi & M. N. Tamura, *C. nasuensis* K. T. Takahashi, T. Noguchi & M. N. Tamura, and *C. sohayakiensis* K. T. Takahashi & M. N. Tamura), two new taxonomic ranks (*C. kuzakaiensis* (M. Kikuchi) K. T. Takahashi & M. N. Tamura, *C. tsukudensis* (T. Koyama) K. T. Takahashi & M. N. Tamura) and one new hybrid (*C. ×kurogii* K. T. Takahashi & M. N. Tamura). Consequently, the taxonomic treatment of East Asian sect. *Paniceae* was revised from 11 species and 1 variety to 15 species, 2 varieties, and 1 hybrid, revealing that the species diversity of sect. *Paniceae* in this region has been underestimated. Furthermore, ancient hybridization events were shown to have contributed to speciation within the section, while ongoing hybridization has complicated morphology-based classifications. Therefore, this study highlights the importance of examining interspecific hybridization for understanding both the taxonomy and evolutionary history of *Carex*.

Acknowledgement

I am deeply grateful to Prof. Minoru N. Tamura and Prof. Shizuka Fuse (Graduate School of Science, Kyoto University) for their invaluable guidance throughout my doctoral studies, including their constructive feedback on research plans and the first draft of this dissertation, and allowing me to join their Kyoto University Botanical Expeditions to the foreign countries.

I extend my thanks to Dr. Koji Takayama (Graduate School of Science, Kyoto University), Prof. Hidetoshi Nagamasu (Kyoto University Museum), and Prof. Emer. Hiroshi Tobe (Kyoto University) for their insightful discussions and advice on experimental design. I also appreciate the critical input and guidance on genus *Carex* studies provided by Mr. Jiro Oda, Dr. Okihito Yano (Okayama University of Science), and Prof. Xiao-Feng Jin (Zhejiang A & F University). My gratitude goes to Dr. Hiroshi Ikeda (The University of Tokyo) and Dr. Nobuko Yamamoto (Natural History Museum and Institute, Chiba) for their technical guidance on chromosome observation methods.

I am sincerely thankful to Dr. Sawita Yooprasert (BKF: The Forest Herbarium, Department of National Parks, Wildlife and Plant Conservation, Thailand), Mr. Manop Poopath (BKF), and other staff members of BKF for their support during field expeditions in Thailand. I am also grateful to the following persons for their assistance with sample collection: Mr. Tatsunari Noguchi, Mr. Takashi Ajima, Dr. Akihiro Kakezawa, Mr. Satoru Kinoshita, Mr. Shuichi Kurogi, Ms. Nobuko Kuroiwa, Mr. Narihisa Matsuoka, Mr. Nobuyuki Numakunai, Mr. Takehiro Ohmori, Ms. Tsurae Ojio, Mr. Masayuki Ozawa, Mr. Takayuki Shibata, Mr. Hidehiko Shimizu, Mr. Shigenari Takahashi, Dr. Seita T. Watanabe, and Mr. Kazuya Yamawaki. Special thanks are due to Dr. Chung Kyong-Sook (Jungwon University) for granting access to a specimen collected in Korea.

I also acknowledge the directors and staff members of the Kyoto University (KYO), Okayama University of Science (OKAY), The University of Tokyo (TI), Kanagawa Prefectural Museum of Natural History (KPM), Gunma Museum of Natural History (GMNHJ), Museum of Nature and Human Activities (HYO), Iwate University Museum (IUM), Osaka Museum of Natural History

(OSA), Hokkaido University Museum (SAPS) and the National Museum of Nature and Science (TNS) for their generous support in providing access to their facilities and collections.

Finally, I express my heartfelt appreciation to all members of the Laboratory of Plant Phylogeny and Taxonomy, Department of Botany, Graduate School of Science, Kyoto University, for their stimulating discussions and assistance with sequencing.

This research was supported in part by JSPS KAKENHI (Grant Numbers JP16H05763 and 21KK0132), JSPS-NRCT Joint Research Program (Grant Number JPJSBP120229202), ISHIZUE 2019 of the Kyoto University Research Development Program, and Kyoto University DoGS SPRING Program supported by the Japan Science and Technology Agency.