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Research Paper

Origin of tatami cultivars of *Juncus decipiens*: evidence of hybridization between intraspecific lineages

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Origin of tatami cultivars of *Juncus decipiens:* evidence of hybridization between intraspecific lineages

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Juncus decipiens is a wetland plant cultivated for production of tatami mats. It is widely distributed throughout Japan and continental East Asia, and the cultivars currently in production were developed by public breeding programs from locally domesticated lineages (landraces). To clarify the origins of these landraces and cultivars, we compared the genetic structure of preserved lineages and wild populations using single nucleotide polymorphism (SNP) data generated by multiplexed ISSR genotyping by sequencing (MIG-seq). Our results

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suggest that hybridization between distinct intraspecific lineages contributed to the origin of most landraces, while landraces from Okinawa served as valuable genetic resources for modern breeding. These findings provide new insights into the domestication history of *J. decipiens* and the genetic basis of agronomically important traits.

Key words: hybridization, intraspecific lineages, *Juncus decipiens,* genetic structure, MIG-seq, origin of cultivars, Tatami material

Introduction

Plant domestication is an evolutionary process driven by human selection acting on morphological and/or physiological variation in wild populations (Doebley *et al.* 2006, Purugganan & Fuller 2009, Purugganan 2019). This process results in the fixation of genetic differences that distinguish domesticated plants from their wild progenitors (Pickersgill 2007). In some domesticated plants, such as maize (*Zea mays*) and cultivated forms of *Brassica oleracea*, morphological divergence from their wild ancestors has become so extensive that the cultivated forms are scarcely recognizable as descendants of the same species. Approximately 2,500 plant species belonging to more than 160 families have been domesticated (Zeven and de Wet 1982, Dirzo and Raven 2003, Meyer *et al.* 2012), and many molecular phylogenetic studies have been conducted to identify their wild progenitors and

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reconstruct the processes of domestication (e.g. Ohnishi and Matsuoka 1996, Ohnishi 1998, Nishino *et al.* 2023, Li *et al.* 2024, Chien *et al.* 2025).

Juncus decipiens (Buchenau) Nakai, commonly known in Japan as *igusa* (rush), is a wetland perennial plant that has long been cultivated and utilized in Japan for tatami mats, candle wicks, and traditional herbal medicine, and more recently as a raw material for processed foods and interior products (Morita 2015). Among these products, tatami is a traditional Japanese flooring material, and the oldest extant example is the "Gosho-tatami" preserved in the Shoso-in Repository, dating back to the eighth century (Nara period, 710–794) (Hirai 2020). Commercial cultivation of *J. decipiens* for tatami production is thought to have begun around 1100, during the late Heian period (794–1185) (Ikeda 2016). Since then, local varieties may have been selected from wild populations and subsequently improved by farmers, giving rise to locally domesticated lineages (landraces) (Ikeda 2016). During the Meiji (1868–1912), Taisho (1912–1926), and early Showa (1926–1945) periods, *J. decipiens* for tatami mat production was cultivated throughout Japan, from Hokkaido to Okinawa. In the latter half of the Showa period (1926–1989), however, major production areas became concentrated in western Japan, particularly in Okayama, Hiroshima, and Kumamoto (Ogo 1991, Ikeda 2016). Thereafter, cultivation gradually declined, and at present, cultivation in Japan is largely restricted to Kumamoto Prefecture in Kyushu (Agricultural Products Division, Kumamoto Prefecture 2015, Ikeda 2016, MAFF 2026:

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https://www.maff.go.jp/j/seisan/tokusan/igusa_tatami-omote.html). Despite the decline in tatami use, *J. decipiens* is expected to have applications based on its functional properties, including deodorizing and antimicrobial activities (Sekiya et al. 2022, Yahiro et al. 2025). Modern cultivars grown in Japan were developed mainly from traditional landraces (zairai) through public breeding programs. Nearly all extant cultivars and traditional landraces of *J. decipiens* are preserved as genetic lineages at the Kumamoto Prefectural Agricultural Research Center. Although several DNA markers, including single sequence repeats (SSR) and random amplified polymorphic DNA (RAPD), have been developed for cultivar identification (Iimure *et al*. 2000, Iimure 2015), no study has examined the genetic structure or intraspecific phylogenetic relationships among cultivars, landraces, and wild populations of *J. decipiens*. Understanding the origins of landraces is important not only for reconstructing the domestication history of *J. decipiens* but also for identifying genetic resources useful for future breeding. Our previous multiplexed ISSR genotyping by sequencing (MIG-seq) analysis of wild populations revealed that *J. decipiens* in Japan comprises three genetically distinct lineages (Goto *et al*. accepted). However, it remains unclear which of these lineages gave rise to the landraces and, ultimately, the cultivars. To address this question, we analyzed the genetic relationships between preserved landraces and wild populations of *J. decipiens* using MIG-seq. Our objective was to clarify the origins of the landraces and the genetic background of modern cultivars.

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Materials and methods

Plant materials

To clarify the origins and relationships of cultivars of *Juncus decipiens*, we analyzed preserved lineages and wild populations. The preserved lineages comprised 37 strains maintained at the Kumamoto Prefectural Agricultural Research Center, Agricultural System Research Institute, Yatsushiro (Table 1). These included both traditional landraces and cultivars. The landraces had been maintained by local farmers before being collected by public research institutes, and the cultivars were subsequently developed through breeding programs using these landraces as parental materials. In addition, four samples obtained from tatami products imported from China were included to compare the genetic relationships between Japanese and Chinese cultivated materials. For wild populations, we analyzed 103 individuals from 45 populations in Japan, South Korea, China, Taiwan, and Russia (Fig. 1 and Table 2). These populations were selected from the samples examined by Goto *et al.* (accepted) to represent the geographic distribution of *J. decipiens*. *Juncus polyanthemus* was used as an outgroup based on its close phylogenetic relationship with *J. decipiens* (Goto *et al.* accepted).

DNA extraction and MIG-seq

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DNA was extracted from dried stems or bracts following the method described by Goto *et al.* (accepted). Genome-wide SNPs were obtained using MIG-seq (Suyama and Matsuki 2015). Libraries were sequenced on an Illumina MiSeq platform using a MiSeq Reagent Kit v3 (150 cycle; Illumina, San Diego, CA, USA), and paired-end reads were generated. After removing primer sequences, reads shorter than 71 bp and low-quality reads (sliding-window quality score >15 over four bases, default settings) were filtered using Trimmomatic (Bolger *et al.* 2014). The filtered reads were then treated as single-end reads for SNP calling using Stacks ver. 2.55 (Catchen *et al.* 2011) with the following parameters: maximum distance allowed between stacks (M = 2), minimum depth of coverage required to create a stack (m = 5), maximum distance allowed to align secondary reads to primary stacks (N = 4), and the number of mismatches allowed between sample loci when building the catalog (n = 2). The sequence data have been deposited in the DDBJ Sequence Read Archive under accession number PRJDB35889.

Analysis of phylogenetic relationships

To infer phylogenetic relationships, all samples listed in Tables 1 and 2 were analyzed. SNP filtering was performed using the "populations" program in Stacks ver. 2.5.5 with the following parameters: maximum observed heterozygosity (max-obs-het = 0.75), minimum minor allele count (min-mac = 3), and a minimum sample coverage of 10% (R =

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115 0.1), corresponding to a maximum missing rate of 90% for each SNP. This filtering was used
116 to retain a relatively large number of SNPs for phylogenetic analysis. Potential invariant sites
117 were removed using the Python script "raxml_ascbias.py"
118 (https://github.com/btmartin721/raxml_ascbias). Maximum likelihood (ML) trees were then
119 constructed using RAxML ver 8.2.10 (Stamatakis 2014) under the GTRGAMMA model with
120 Lewis ascertainment bias correction (Leaché *et al.* 2015). Branch support (BS) was evaluated
121 by 1,000 bootstrap replicates.
122
123 *Analysis of genetic structure*
124 To investigate the genetic structure of preserved lineages and wild populations of *J.*
125 *decipiens*, Bayesian clustering analysis was performed using STRUCTURE ver. 2.3.4
126 (Pritchard *et al. 2*000). SNP filtering was performed in Stacks ver. 2.5.5 (Catchen *et al*. 2011)
127 using the following parameters: minimum stack depth (m = 3), minimum sample coverage (R
128 = 0.1), and maximum observed heterozygosity (max-obs-het = 0.75). One SNP per locus was
129 retained using the "write-random-snp" option. Using TASSEL5 (Bradbury *et al.* 2007), SNPs
130 with more than 50% missing data and SNPs with minor allele frequencies (MAF) less than
131 1% were removed. Thus, the dataset used for the genetic structure analyses had a maximum
132 missing rate of 50%, in contrast to the 90% maximum missing rate allowed in the
133 phylogenetic dataset. This more stringent filtering was applied to reduce the influence of

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missing genotypes on comparisons of genetic relationships and population structure among individuals. In addition, CH18 and all individuals from Taiwan and Russia were excluded because of their low genotyping rates. The admixture model with correlated allele frequencies was used. Analyses were performed for K = 1–10 with 10 independent runs for each K value. Each run consisted of 250,000 burn-in iterations followed by 250,000 Markov chain Monte Carlo (MCMC) iterations. The optimal number of clusters was determined based on the ΔK statistic (Evanno *et al*. 2005) and the log probability of the data [LnP(K)] using STRUCTURE Harvester ver. 2.3.4 (Earl and von Holdt 2012). Bar plots were generated using the online version of CLUMPAK (Kopelman *et al.* 2015). Pairwise genetic distances among individuals were calculated using TASSEL5, and a NeighborNet network (Bryant and Moulton 2004) was constructed with SplitsTree ver. 4.14 (Huson and Bryant 2006). Principal component analysis (PCA) was performed using GenoDive ver. 3.04 (Meirmans 2020). The same filtered SNP dataset was used for the NeighborNet, PCA, and STRUCTURE analyses.

Sequence analysis of chloroplast DNA regions and nuclear ITS

Four non-coding regions of chloroplast DNA (*psbK-psbI, trnL-trnF,* the *trnL* intron, and the *rps16* intron) and two internal transcribed spacers (ITS1 and ITS2) of nuclear ribosomal DNA were sequenced using the multiplexed phylogenetic marker sequencing

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153 (MPM-seq; Suyama *et al.* 2022) on an Illumina MiSeq platform. Primers for each region
154 were those described by Taberlet *et al*. (1991) for the *trnL-trnF* region and *trnL* intron,
155 Lahaye *et al*. (2008) for *psbK-psbI*, Oxelman *et al*. (1997) for the *rps16* intron, and Cheng *et*
156 *al*. (2016) for ITS1 and ITS2. Sequences were aligned using MAFFT ver.7 (Katoh and
157 Standley 2013) and manually refined in MEGA X (Kumar *et al.* 2018). The aligned
158 sequences were used for haplotype identification.
159
160 *Genome size comparison by flow cytometry*
161 Relative genome size was estimated by flow cytometry using a CyFlow Ploidy
162 Analyser (Sysmex Co.). *Lactuca sativa* L., which was confirmed in a preliminary analysis to
163 have a genome size distinct from that of *J. decipiens*, was used as an internal reference
164 standard. Approximately 6 cm long stem or bract tissue from *J. decipiens* and approximately
165 1 cm^2 of leaf tissue from the internal reference standard were co-chopped for about 30 s in a
166 Petri dish containing 1 mL of CyStain UV Precise P buffer (Sysmex Co.). The suspension
167 was filtered through a 30-µm disposable filter (Cell Trics; Sysmex Co.) and the DAPI
168 fluorescence intensity was measured. During measurement, the detection gain was set to 280,
169 and the fluorescence peaks were adjusted by modifying the threshold and flow rate. Relative
170 DNA count was calculated as the ratio of the median fluorescence intensity of *J. decipiens* to
171 that of the internal reference standard and was used to compare genome size among

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individuals.

Results

Phylogenetic analysis based on SNP data obtained by MIG-seq

A maximum likelihood (ML) phylogenetic tree was reconstructed using a dataset of 4,158 SNPs (genotyping rate = 40.7 %) from 41 preserved lineages including four samples obtained from Chinese tatami products and 103 individuals representing 45 wild populations (Fig. 2). Three major clades, A (BS = 91%), B (BS = 89%), and C (BS = 96%), were identified. Clade A comprised wild populations from South Korea (KR04), China (CH12, CH13, CH15), Russia (RS01, RS07, RS08, RS09), and 20 populations across Japan. Clade B included wild populations from Taiwan (TW01, TW02), China (CH04, CH05, CH06, CH09), and 10 populations from western Japan (OS02, HG01, WA01, HS01, EH01, EH02, KO01, NS01, NS02, MZ01). Clade C comprised four wild populations from South Korea (KR02, KR03, KR04, KR08) and three populations from Japan (HK01, KH16, HK18) (Fig. 1 and 2, Table 2). All preserved lineages, except for 'Tokiwa Zairai A', were included in Clade B, whereas 'Tokiwa Zairai A' was included in Clade C. An enlarged view of Clade B is shown in Fig. 3. Within Clade B, all wild populations from China, Taiwan, and western Japan, except HS01, formed a terminal subclade (B-ss; BS = 86%). Six landraces from Okinawa formed a small subclade (BS = 94%) in Clade B.

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Phylogenetic network, genetic structure, and PCA analyses

The NeighborNet, PCA, and STRUCTURE analyses were based on the same dataset of 495 SNPs (genotyping rate = 70.9%) obtained from 37 preserved lineages, three Chinese samples, and 96 individuals representing 39 wild populations from Japan, South Korea, and China. This dataset contained fewer SNPs than the phylogenetic dataset because of the more stringent filtering for missing data. In the NeighborNet network (Fig. 4), three major clusters corresponding to Clades A, B and C of the phylogenetic tree were recovered. Individuals belonging to Subclade B-ss formed a distinct cluster, within which the Okinawa landraces constituted a well-defined subgroup. In contrast, the remaining landraces and cultivars were positioned in the intermediate part between the clusters A, B-ss, and C.

The PCA likewise identified three groups of wild individuals corresponding to Clades A, B, and C of the phylogenetic tree (Fig. 5). Within Group B, the Okinawa landraces were grouped with B-ss, whereas the others were located near the Group A. 'Tokiwa Zairai A' was positioned near the center of the three groups.

Based on the highest ΔK value, the STRUCTURE analysis indicated that the most likely number of genetic clusters was $K = 3$. The bar plot for $K = 3$ showed that most individuals from wild populations in Japan, South Korea, and China were assigned almost entirely to one of the three genetic clusters (green, orange, or purple; Fig. 6). These three

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clusters corresponded to Clades A, B, and C of the phylogenetic tree, respectively. A small number of individuals from wild populations (HS01-5, CH04-06, CH13, and CH15) showed admixed ancestry. Most preserved lineages exhibited admixture between the green and orange clusters, but the Okinawa landraces were assigned almost entirely to the orange cluster. 'Tokiwa Zairai A' showed admixture of all three genetic clusters.

Haplotypes of chloroplast DNA and nuclear ITS

A total of 1,749 nucleotide sites from four chloroplast DNA regions (the *trnL* intron, *trnL-trnF*, *psbK-psbI*, and the *rps16* intron) were sequenced, and 10 haplotypes were identified. Sequence differences among haplotypes and their relationships in the haplotype network are shown in Supplemental Fig. 1. Nine haplotypes (I – IX) were detected in the wild populations, but only three haplotypes (I, IV, and X) were detected among the preserved lineages (Table 1 and 2).

A total of 624 nucleotide sites of the nuclear ribosomal ITS region was sequenced, and five haplotypes were identified. Sequence differences among the haplotypes and their relationships in the haplotype network are shown in Supplemental Fig. 2. Five haplotypes (i – v) were detected in the wild populations, but only a single haplotype (i) was detected among the preserved lineages (Table 1 and 2).

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Genome size comparison

Relative genome size was estimated by flow cytometry. The relative DNA content ranged from 0.150 to 0.168 (mean = 0.157) among the 10 preserved lineages (Table 1) and the relative DNA content ranged from 0.152 to 0.177 (mean = 0.160) among the six individuals from wild populations (Table 2). Although slight variation in relative genome size was observed, the values showed no evidence of differences in ploidy level.

Discussion

Hybrid origin of landraces and selection during domestication

The phylogenetic tree, NeighborNet, PCA, and STRUCTURE analyses consistently identified three major genetic groups within wild *J. decipiens* (Figs. 2, 4–6). All preserved lineages, except for 'Tokiwa Zairai A', were included in Clade B in the phylogenetic tree. Among them, six Okinawan landraces formed a strongly supported subclade nested in Clade B-ss, whereas most of the other preserved lineages were located near the base of Clade B (Fig. 2 and 3). Most preserved lineages occupied intermediate positions between Groups A and B in the NeighborNet and PCA analyses (Figs. 4 and 5), and individuals of these lineages showed admixture between Groups A and B in the STRUCTURE analysis (Fig. 6). Together, these results provide genetic evidence suggesting that hybridization between Groups A and B may have contributed to the origin of most preserved lineages. Group A is widely distributed

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248 throughout Japan, whereas Group B is restricted to western Honshu and Kyushu, where their
249 distributions partially overlap (Fig. 1). In the population from Hiroshima, an admixed
250 individual, HS01-5, was found to co-occur with individuals of Groups A and B. Although its
251 origin from cultivated material cannot be excluded, this finding is consistent with the
252 possibility that HS01-5 represents a naturally occurring hybrid between the two groups. The
253 genetic composition of preserved lineages was not uniform, as reflected by their varying
254 positions between Groups A and B in the NeighborNet and PCA analyses (Figs. 4 and 5),
255 suggesting that multiple hybridization events may have contributed to their formation.
256 Additionally, alternative scenarios, including post-domestication gene flow among lineages
257 or wild genetic resources, cannot be excluded.

258 The repeated selection of hybrid individuals implies that hybridization provided
259 advantageous traits favored by farmers. Long stems are one of the key traits favored in *J.*
260 *decipiens* cultivated for tatami mat production. One possible explanation is heterosis,
261 whereby hybrids exceed either parental group in plant size and are therefore more suitable for
262 tatami mat production. Another trait favored in rush cultivation is reduced flowering, because
263 stem elongation ceases after flowering and conspicuous flowering nodes reduce the usable
264 portion of the stem (Nakano 1968). Goto *et al*. (accepted) suggested that Groups A and B are
265 reproductively isolated, although the isolation is incomplete. If partial reproductive isolation
266 between Groups A and B involved postzygotic barriers affecting reproductive development,

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267 some hybrid individuals may have exhibited reduced flowering, which could have led
268 farmers to preferentially select such individuals. Although reduced fertility is
269 disadvantageous for sexual reproduction, it would not have prevented the maintenance of
270 desirable landraces because *J. decipiens* has traditionally been propagated vegetatively by
271 clump division (Nakano 1968, Sadahira 1979, Ikeda 2016). Furthermore, the nearly equal
272 genetic contributions from Groups A and B observed in most landraces raise the possibility
273 that early-generation hybrids, possibly including F_1 individuals, were preferentially
274 maintained through vegetative propagation.
275
276 *Landraces from Okinawa*
277 The six landraces from Okinawa showed a distinct genetic composition, suggesting
278 that they originated independently of other landraces. These lineages were clearly associated
279 with Group B. However, because *J. decipiens* is now rare in Okinawa, wild populations from
280 the islands were not included in this study, and their geographic origin remains uncertain.
281 Although the broad distribution of Group B in western Japan, Taiwan, and southern China
282 raises the possibility that these landraces were selected outside Okinawa and later introduced
283 to the islands, their genetic similarity to Group B alone does not allow us to distinguish this
284 scenario from an origin involving wild populations in Okinawa. Additional sampling of wild
285 populations from Okinawa will be necessary to clarify the geographic origin of these

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286 landraces. Despite their distinctive origin, the Okinawa landraces have made an important
287 contribution to modern breeding. Several cultivars, including ‘Kumamoto 3’, ‘Hinoharuka’,
288 ‘Suzukaze’, and ‘Yunagi’, show a predominance of Group B ancestry, suggesting that they
289 were derived from crosses between A–B hybrid-origin landraces and Okinawa landraces.
290 This interpretation is supported by the breeding record of ‘Suzukaze’ and ‘Yunagi’, in which
291 ‘Okinawa Futoi’ was used as one of the parental lines. Collectively, these results indicate that
292 Okinawa landraces have played an important role in the genetic improvement of tatami
293 cultivars and are likely to remain key genetic resources for future breeding efforts.
294
295 *Cultivars in China*
296 In this study, four samples (CH16–CH19) were obtained directly from tatami mats
297 imported into Japan from China. They exhibited genetic compositions indistinguishable from
298 those of Japanese preserved lineages in the STRUCTURE analysis and clustered closely with
299 them in the phylogenetic trees (Figs. 2, 3 and 6). These results indicate a close genetic
300 relationship and shared ancestry between the Chinese samples and Japanese preserved
301 lineages. According to a report by the Yano Research Institute (2022), the rush currently used
302 mainly for tatami-mat production in China was introduced from Japan after the normalization
303 of diplomatic relations between Japan and China in 1972. The report also states that Chinese
304 rush production is primarily oriented towards the Japanese market and that Japanese cultivars

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305 continue to be used to increase the value of Chinese products. The close genetic relationship
306 observed between the Chinese samples and Japanese preserved lineages is therefore
307 consistent with the reported history of Japanese cultivar introduction. However, China has a
308 long history of using rushes for woven mats and candle wicks. For example, Li (1596)
309 recorded the cultivation of *dengxin cao* (灯心草) in the Jiangnan region and its use for
310 weaving mats and rain capes. More recently, Sadahira (1979) mentioned domesticated
311 landraces of rushes in Jiangsu and Zhejiang Provinces. Although the plants referred to in this
312 historical record cannot be confidently identified as *J. decipiens*, it is possible that locally
313 selected cultivated materials existed in China before the introduction of Japanese cultivars.
314 Because only four Chinese samples were examined in the present study, further sampling of
315 cultivated materials in China is needed to clarify their genetic origins.
316
317 *A strange landrace, 'Tokiwa Zairai A'*
318 'Tokiwa Zairai A' differs markedly from all other preserved lineages in its genetic
319 composition. Although it was placed in Clade C in the phylogenetic tree, it showed admixed
320 ancestry from Groups A, B, and C in the STRUCTURE analysis and occupied an
321 intermediate position in the NeighborNet and PCA analyses (Figs. 2, 4–6). Its chloroplast
322 haplotype (haplotype X) is most similar to that of Group C individuals from Hokkaido
323 (haplotype IX), while its nuclear ITS haplotype is identical to the common haplotype shared

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by Groups A and B (haplotype i) (Tables 1 and 2, Supplemental Figs. 1 and 2). These results suggest that 'Tokiwa Zairai A' originated through hybridization between a maternal parent belonging to Group C and an A–B hybrid. At present in Japan, Group C is known only from Hokkaido (Goto *et al*. submitted). However, 'Tokiwa Zairai A' is thought to have been collected in Nagano Prefecture, where only Group A is currently known to occur. Although the origin of this unusual genetic combination remains uncertain, it is likely to reflect the artificial transportation associated with cultivation.

Conclusion

Modern cultivars of *Juncus decipiens* have been developed through the improvement of traditional landraces in Japan. Our results suggest that hybridization between genetically distinct groups contributed to the origin of many *J. decipiens* landraces. The genetic patterns observed in the preserved lineages are consistent with repeated interactions between Groups A and B, although the relative contributions of hybridization, subsequent selection, and other historical processes remain to be clarified. Western Japan, where the distributions of the two parental groups overlap, is the most likely region of origin. However, because both parental groups are also found in China, a Chinese origin cannot be excluded. Further sampling of wild populations, particularly in China, will be necessary to determine the geographic origin of the ancestral landraces.

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Author Contribution Statement

AS and KK conceived the project; AS provided materials and wrote a draft of the manuscript; NG performed the experiments, analyzed the data, and prepared figures.

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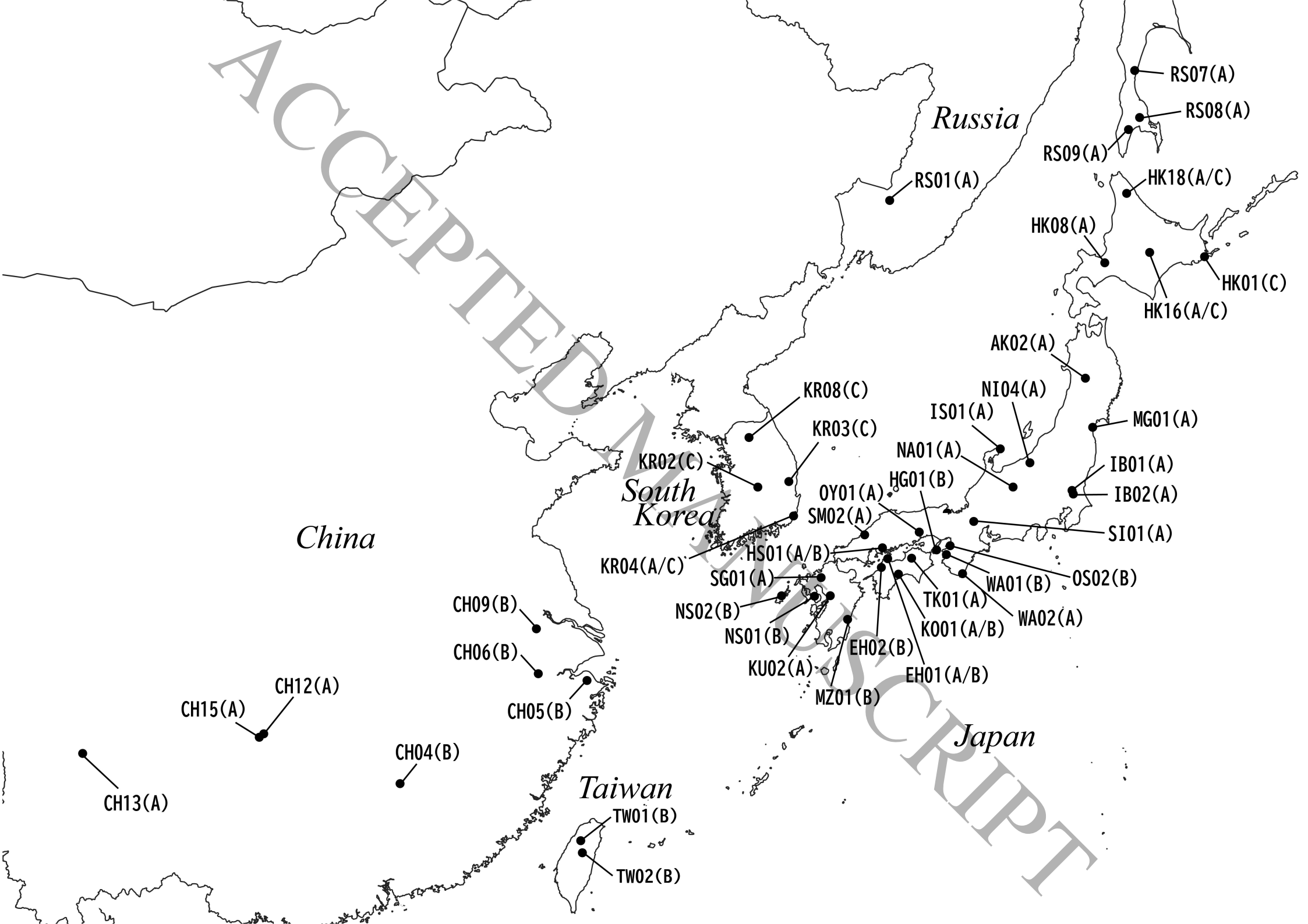
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479 Fig. 1 Map of wild populations sampled. Alphabets in parentheses indicate clades in the
480 maximum likelihood phylogenetic tree.
481 Fig. 2 Maximum likelihood phylogenetic tree of *Juncus decipiens* based on SNP data
482 obtained from MIG-seq analysis. *Juncus polyanthemus* was used as the outgroup.
483 Numbers at nodes indicate bootstrap values based on1,000 resampling. Individuals
484 from wild populations are shown in black, and preserved lineages in red.
485 Fig. 3 Enlarged view of Clade B shown in Fig. 2. Numbers at nodes indicate bootstrap
486 values based on 1,000 resampling. Individuals from wild populations are shown in
487 black, and those from preserved lineages in red.
488 Fig. 4 Phylogenetic relationships among individuals inferred by NeighborNet analysis based
489 on SNP data obtained from MIG-seq. Red dots indicate preserved lineages. A, B, C,
490 and B-ss correspond to the clades in the maximum likelihood phylogenetic tree.
491 Fig. 5 PCA result based on SNP data obtained from MIG-seq. Preserved lineages are shown
492 in red. A, B, C, and B-ss correspond to the clades in the maximum likelihood
493 phylogenetic tree.
494 Fig. 6 Bar plots showing the estimated probabilities of ancestral clusters for each sample

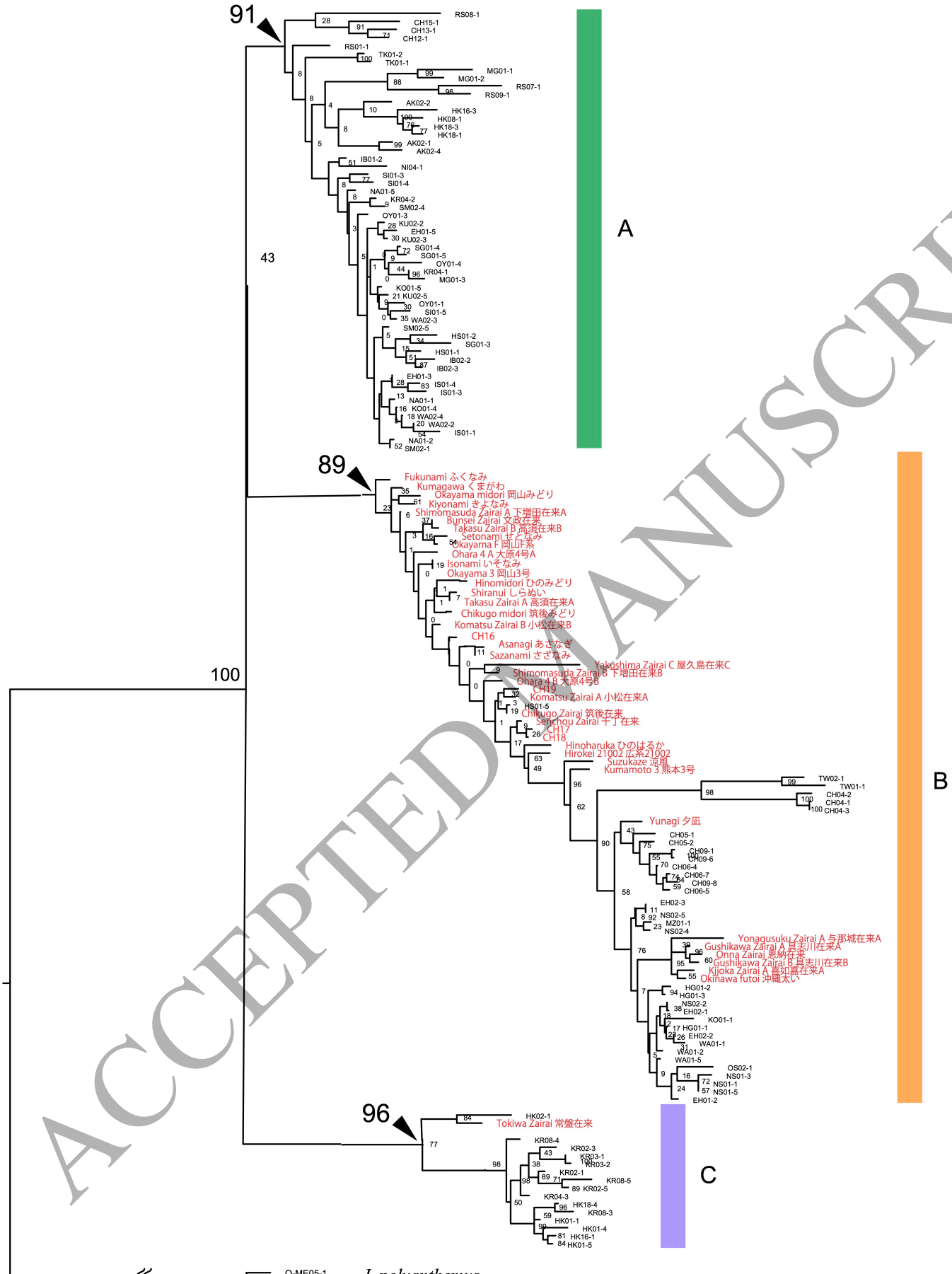
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from STRUCTURE analysis at $K = 3$. Population names are indicated below the upper bar plot. The lower bar plot provides an enlarged view of the preserved lineages, with sample names indicated.

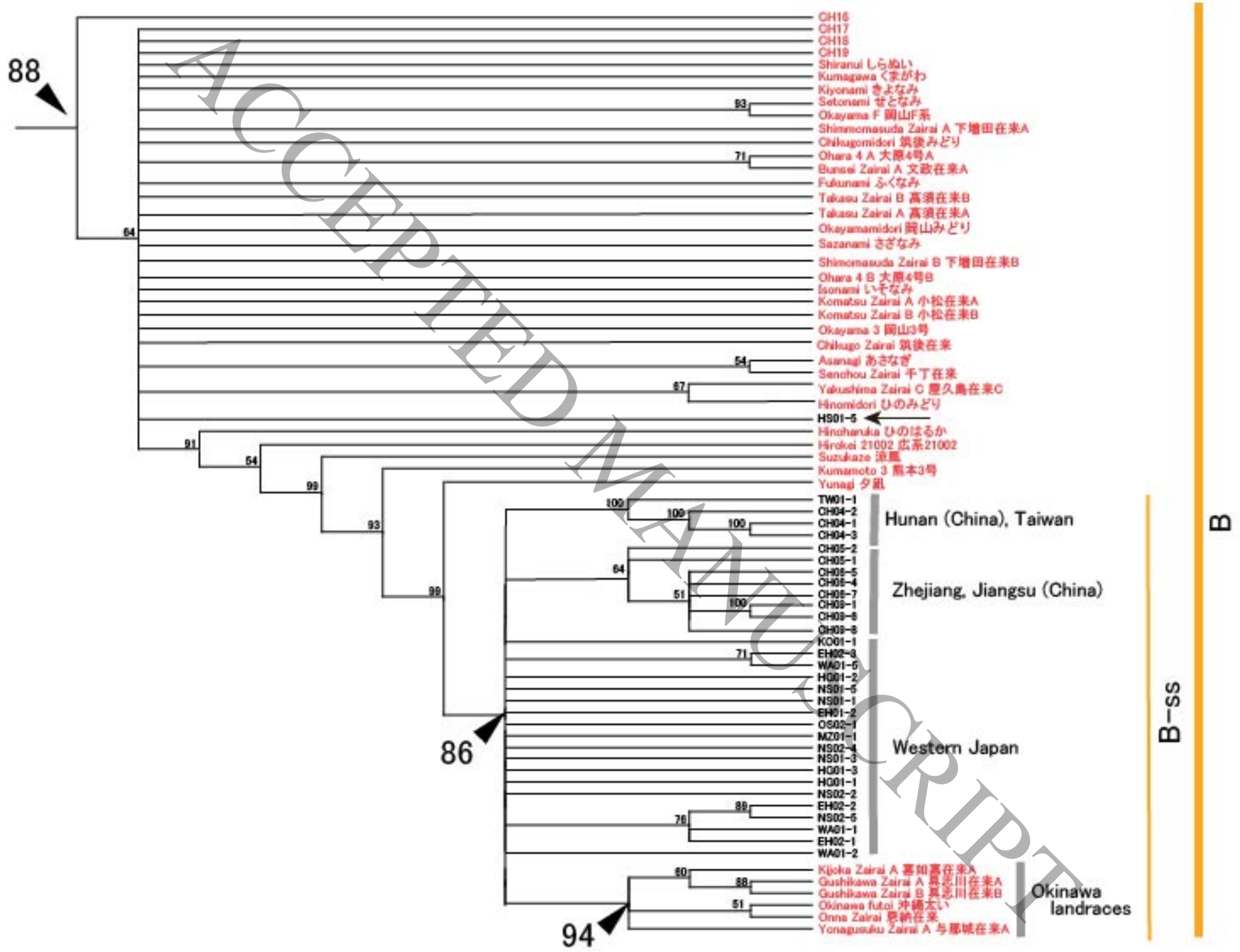
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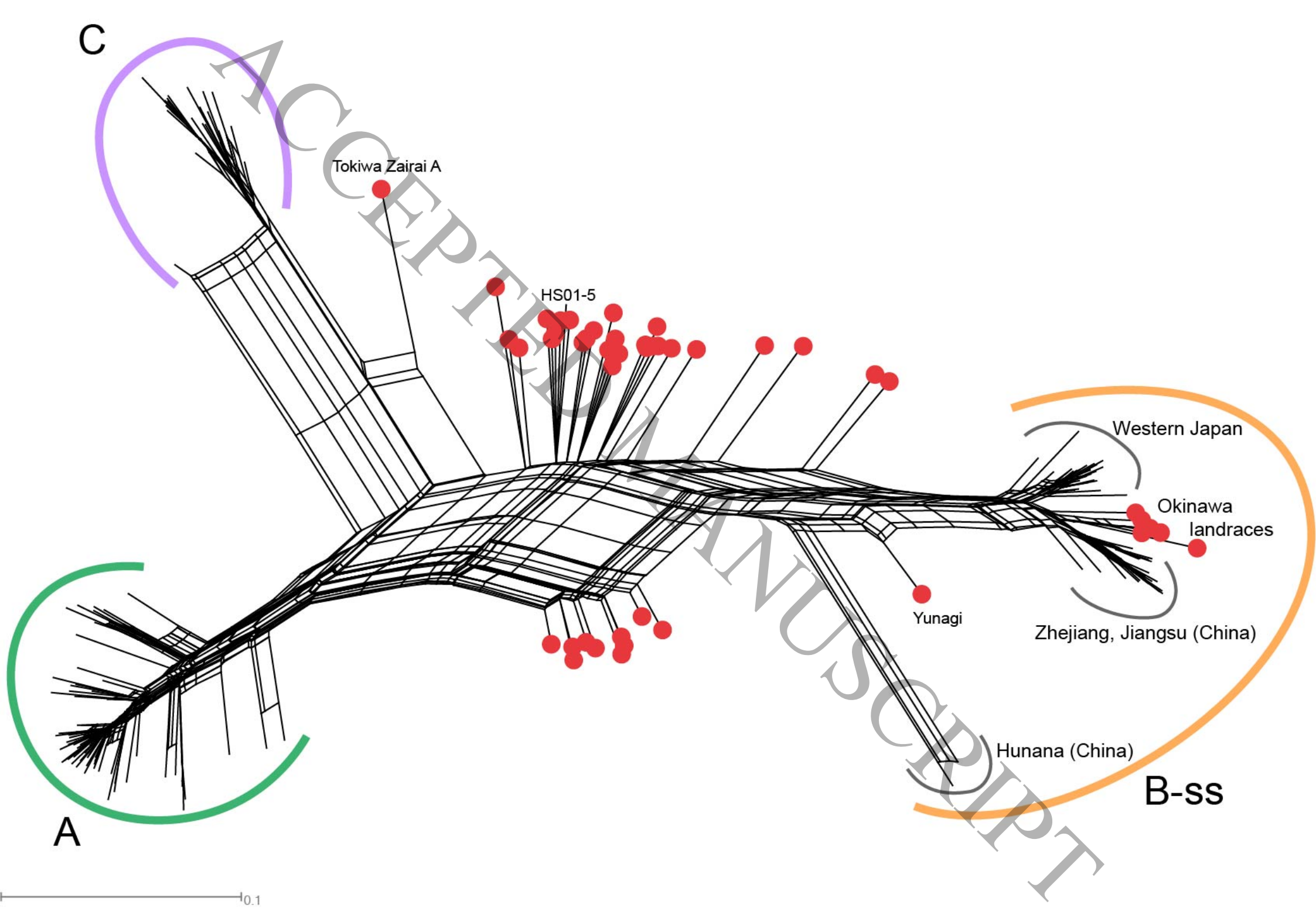
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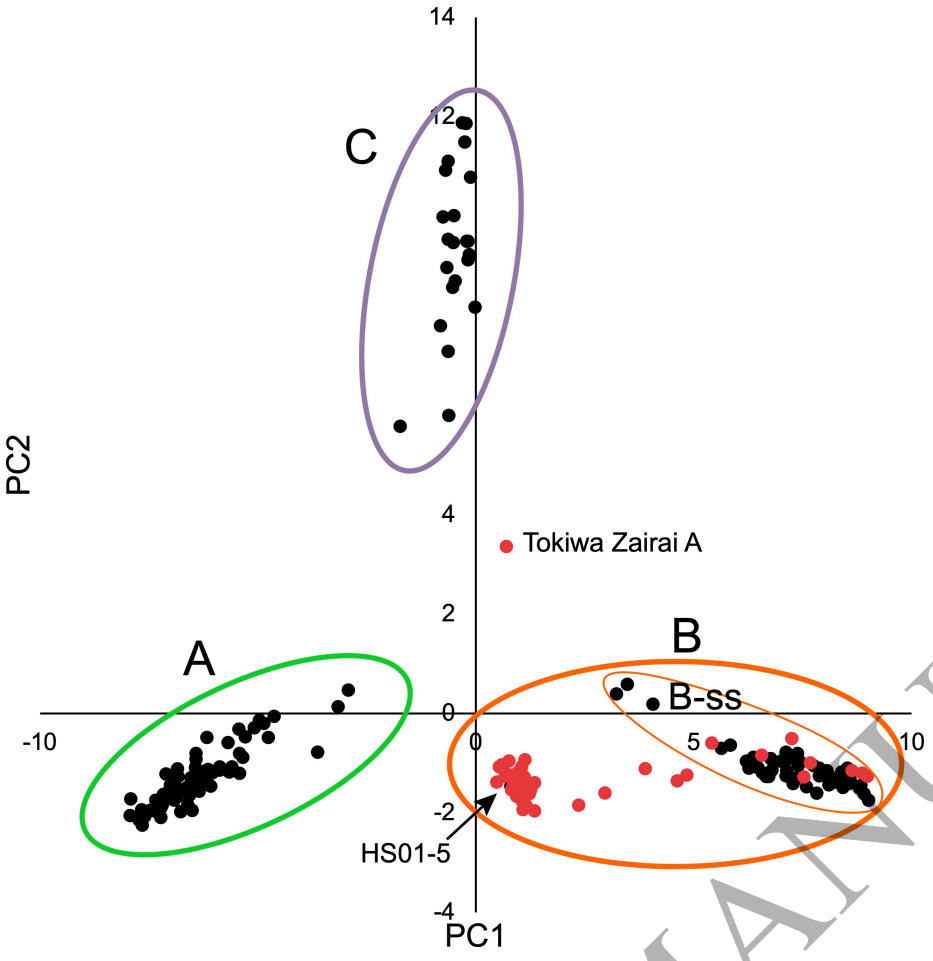
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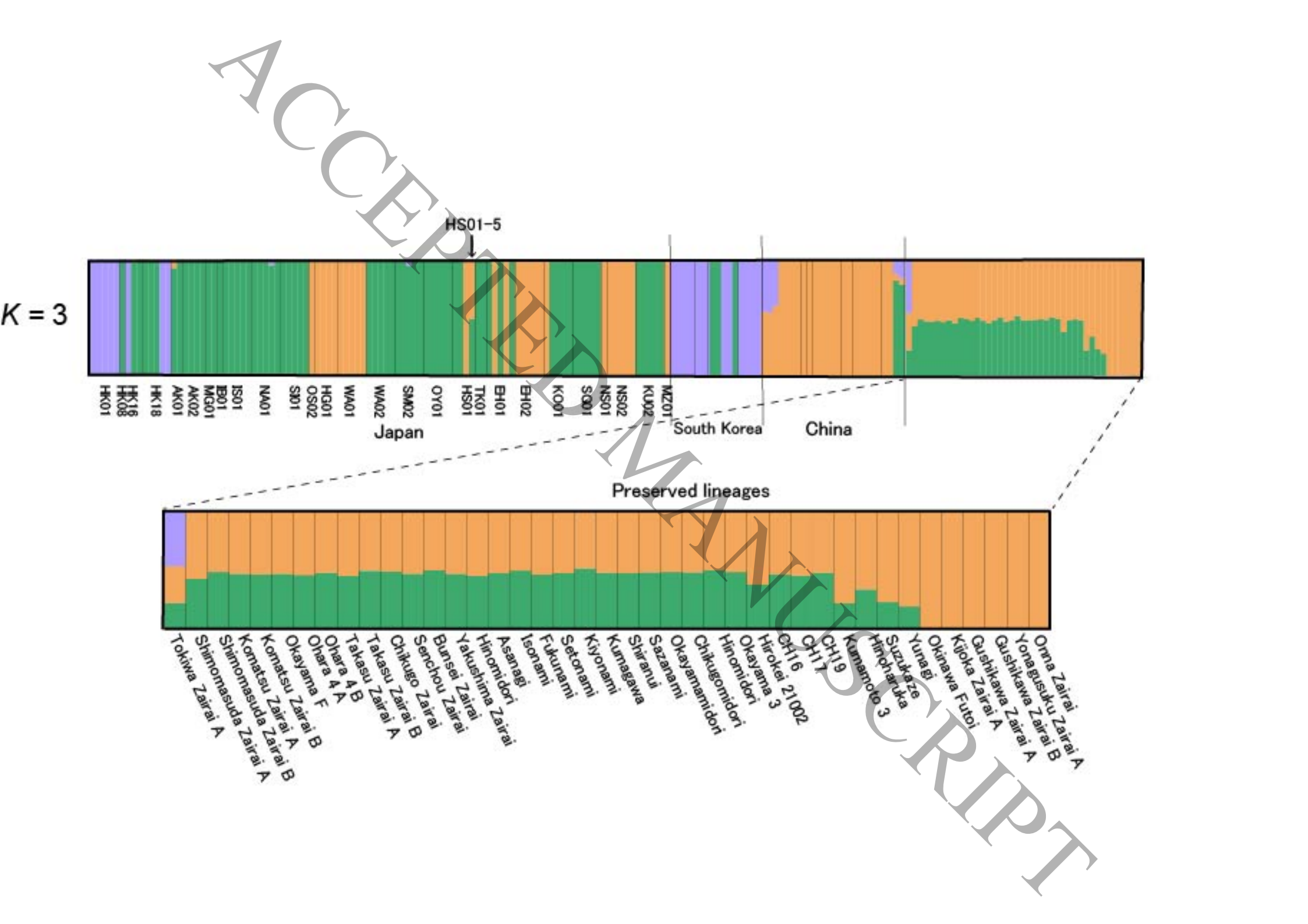
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Table 1. Landraces and cultivars of *Juncus decipiens*

Sample name	Japanese name	Origin/pedigree	cpDNA	ITS	MIG-seq clade	Genome size[a]
Landraces						
1 Shimomasuda Zairai A	下増田在来A	Miyagi Pref.	I	i	B	0.152
2 Shimomasuda Zairai B	下増田在来B	Miyagi Pref.	I	−	B	
3 Tokiwa Zairai A	常盤在来A	Nagano Pref.	X	i	C	0.156
4 Komatsu Zairai A	小松在来A	Ishikawa Pref.	−	−	B	
5 Komatsu Zairai B	小松在来B	Ishikawa Pref.	−	−	B	
6 Okayama F	岡山F系	Okayama Pref.	I	i	B	
7 Takasu Zairai A	高須在来A	Hiroshima Pref.	I	−	B	
8 Takasu Zairai B	高須在来B	Hiroshima Pref.	−	i	B	
9 Chikugo Zairai	筑後在来	Fukuoka Pref.	−	i	B	
10 Bunsei Zairai	文政在来	Kumamoto Pref.	I	i	B	
11 Ohara 4 A	大原4号A	Kumamoto Pref.	I	−	B	
12 Ohara 4 B	大原4号B	Kumamoto Pref.	−	−	B	
13 Senchou Zairai	千丁在来	Kumamoto Pref.	−	i	B	0.150
14 Yakushima Zairai C	屋久島在来C	Kagoshima Pref.	I	i	B	
15 Okinawa Futoi	沖縄太い	Okinawa Pref.	VI	−	B	0.156
16 Kijoka Zairai A	喜如嘉在来A	Okinawa Pref.	VI	i	B	
17 Gushikawa Zairai A	具志川在来A	Okinawa Pref.	−	i	B	
18 Gushikawa Zairai B	具志川在来B	Okinawa Pref.	−	−	B	
19 Yonagusuku Zairai A	与那城在来A	Okinawa Pref.	−	−	B	
20 Onna Zairai	恩納在来	Okinawa Pref.	−	−	B	
Cultivars						
21 Asanagi	あさなぎ	Clonal selection from Senchou Zairai	−	i	B	0.159
22 Isonami	いそなみ	Clonal selection from Bunsei Zairai	−	i	B	
23 Kiyonami	きよなみ	Clonal selection from Bunsei Zairai	I	i	B	
24 Kumagawa	くまがわ	Clonal selection from Okayama 3	I	i	B	
25 Sazanami	さざなみ	Clonal selection from Takasu Zairai	−	i	B	
26 Shiranui	しらぬい	Natural crossing from Sazanami	I	i	B	
27 Setonami	せとなみ	Gamma-irradiated from Asanagi	I	i	B	0.155
28 Hinoharuka	ひのはるか	Crossing between Kumamoto 3 and Hirokei 21002	−	−	B	0.154
29 Hinomidori	ひのみどり	Crossing between Shimomasuda Zairai and Setonami	−	i	B	0.168
30 Fukunami	ふくなみ	Gamma-irradiated from Asanagi	−	i	B	
31 Yunagi	夕凪	Crossing between Isonami and Okinawa Futoi	−	−	B	0.165
32 Suzukaze	涼風	Crossing between Hinomidori and Okinawa Futoi	−	−	B	0.156
33 Okayama 3	岡山3号	Clonal selection from Ohara 4	I	i	B	
34 Okayama Midori	岡山みどり	Clonal selection from Fukuda Zairai/Okayama F	I	i	B	
35 Hirokei 21002	広系21002	Selfing from Hirokei 12652	I	i	B	
36 Chikugo Midori	筑後みどり	Somatic embryogenesis from Isonami	I	i	B	
37 Kumamoto 3	熊本3号	Natural crossing from Chikugo Zairai	−	i	B	
Chinese cultivars[b]						
38 CH16		Chinese cultivar; imported product from Ningbo	−	−	B	
39 CH17		Chinese cultivar; imported product from Sichuan	−	−	B	
40 CH18		Chinese cultivar; origin unknown	−	−	B	
41 CH19		Chinese cultivar: origin unknown	−	−	B	

[a] relative DNA content: sample median/internal standard median

[b] products imported from China

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Table 2. *Juncus decipiens* from wild populations

Nation	Population	Locality	Voucher	cpDNA	ITS	MIG-seq clade	Genome size[a]
Japan							
	1 HK01	Hamanaka Town, Hokkaido	*Naito et al. 392*	IX	ii	C	
	2 HK08	Sapporo City, Hokkaido	*SAPS063021*	–	–	A	
	3 HK16	Kamishihoro Town, Hokkaido	*Shutoh 20220901*	–	–	A/C	0.152 (C1)
	4 HK18	Nakatonbetsu Town, Hokkaido	*Asai 20220916-2*	–	–	A/C	0.153 (A3), 0.152 (C2)[b]
	5 AK02	Senboku City, Akita	*S22-015*	–	–	A	
	6 MG01	Iwanuma City, Miyagi	*Asai 20200627*	I/II	i/iii	A	
	7 IB01	Tsuchiura City, Ibaraki	*M-5186*	–	–	A	
	8 IB02	Tsukuba City, Ibaraki	*M-5187*	–	–	A	
	9 NI04	Joetsu City, Niigata	*NGU 008064*	–	–	A	
	10 IS01	Suzu City, Ishikawa	*Naito et al. 496*	I	i	A	
	11 NA01	Azumino City, Nagano	*Fujii 18679*	I/II	i	A	
	12 SI01	Taga Town, Shiga	*Fujii s.n. 1509*	I	i	A	
	13 OS02	Kishiwada City, Osaka	*NGU 006871*	–	–	B	
	14 WA01	Kainan City, Wakayama	*Fujii SF0003, Fujii SF0004*	I/VI/VII	i	B	
	15 WA02	Kushimoto Town, Wakayama	*Fujii SF0005, Fujii SF0006*	I/VII	i	A	
	16 HG01	Sumoto City, Hyogo	*Soejima 1243*	I/VII	i	B	
	17 SM02	Hamada City, Shimane	*Soejima 1249*	VIII	i	A	
	18 OY01	Wake Town, Okayama	*Soejima 1238*	II	i/iv	A	
	19 HS01	Higashihiroshima City, Hiroshima	*Fujii SF0002*	I/VII	i	A/B	
	20 TK01	Higashimiyoshi Town, Tokushima	*Soejima 1256*	I	i	A	
	21 EH01	Imabari City, Ehime	*Fukuoka 01677*	–	i	A/B	
	22 EH02	Iyo City, Ehime	*Fukuoka 01676*	VII	i	B	
	23 KO01	Hidaka Village, Kochi	*Soejima 1257*	I/VI	i	A/B	
	24 SG01	Kanzaki City, Saga	*Inoue 20210722*	–	–	A	
	25 NS01	Isahaya City, Nagasaki	*S21-013*	–	i	B	
	26 NS02	Goto City, Nagasaki	*Inoue 20210731*	VII	i	B	0.156 (B1)
	27 KU02	Kumamoto City, Kumamoto	*S21-009*	I	i	A	
	28 MZ01	Miyazaki City, Miyazaki	*NGU 008154*	–	i	B	
South Korea							
	29 KR02	Sangju-si, Gyeongsangbuk-do	*Lee 20210531*	–	–	C	
	30 KR03	Jimbo-myeon, Gyeongsangbuk-do	*Lee 20210618*	–	–	C	
	31 KR04	Seosaeng-myeon, Ulsan	*Lee 20210624*	–	–	A/C	
	32 KR08	Chuncheon-si, Gangwon-do	*Lee 20211116*	–	–	C	
China							
	33 CH04	Taohuatuan, Hunan	*Shen 2295*	–	–	B	
	34 CH05	Ningbo City, Zhejiang	*Soejima 1273*	–	–	B	0.170 (B1)
	35 CH06	Hangzhou City, Zhejiang	*Soejima 1274*	–	–	B	
	36 CH09	Changzhou City, Jiangsu	*Soejima 1277*	–	–	B	0.177 (B1)
	37 CH12	Luzhou City, Sichuan	*PE01853359*	–	–	A	
	38 CH13	Liangshan, Sichuan	*PE02236426*	–	–	A	
	39 CH15	Tongren City, Guizhou	*PE02236436*	–	–	A	
Taiwan							
	40 TW01	Nanzhuang, Miaoli	*PE01047556*	–	–	B	
	41 TW02	Jinai, Nanto	*PE02236462*	–	–	B	
Russia							
	42 RS01	Khanka, Primorsky	*Tzvelev 294458*	–	–	A	
	43 RS07	Makarovsky, Sakhalin	*Probatova & Rudyka 86964*	–	–	A	
	44 RS08	Korsakov, Sakhalin	*Tolamchev 87844*	–	–	A	
	45 RS09	Anivsky, Sakhalin	*Kravchenko 87046*	–	–	A	

[a] Relative DNA content: sample median/internal standard median. Clade and the number of observed ndividuals are in the parenthes.

[b] Average of three individuals of A , and two indivuduals of B clades.

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