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Cryptic Hybridization in the Temperate Bamboos: Is Pleioblastus simonii a Species of Hybrid Origin?

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CRYPTIC HYBRIDIZATION IN THE TEMPERATE BAMBOOS:

IS *PLEIOBLASTUS SIMONII* A SPECIES OF HYBRID ORIGIN?

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ABSTRACT

Japanese river bamboo (*Pleioblastus simonii*, ‘medake,’‘kawadake’) is an ecologically important species of temperate bamboo native to Japan. This species is widely known and historically important in Japanese rural farm life. Based on morphological data, Japanese river bamboo is classified in *Pleioblastus* section *Medakea* (Poaceae: Bambusoideae) along with five other Japanese species, which are collectively considered to represent a phylogenetically distinct lineage. However, recent studies suggest that Japanese river bamboo may have arisen as a result of previously undetected hybridization (i.e., cryptic hybridization), while also calling into question the diversity of section *Medakea*. The role of hybridization in natural plant populations has been studied since the 1950s; however, little is known about this phenomenon in the evolution of bamboos. Species of *Pleioblastus* share an issue common to bamboo taxonomy in that they exhibit overlapping variation in leaf and stem characteristics, making them difficult to identify based on morphology alone. One potential factor contributing to, and exacerbating, this issue is cryptic hybridization. The objective of this study was to analyze molecular data, including amplified fragment length polymorphism (AFLP) and nuclear DNA (nDNA) sequence data, to test the hypothesis that *P. simonii* is a species of hybrid origin. The results provide compelling evidence in support of this hypothesis, while also suggesting that ongoing diversification has obscured bamboo ancestry. Moreover, these findings highlight the importance of using up-to-date analytical

techniques from population genetics and phylogenetics to shed light on how to navigate the complexities of bamboo taxonomy. This study provides an example of reticulate evolution in the origin of plant diversity and helps to reveal why molecular data are important tools for plant taxonomy and systematics.

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I. INTRODUCTION

In the context of evolutionary history, plant hybridization has acted as a powerful engine of morphological diversity while conserving desirable traits among plants of similar or identical species that cross-bred to produce new lineages (Stebbins 1969; Joly et al. 2009, López-Caamal and Tovar-Sánchez 2014). In grasses, the role of hybridization has been studied since as early as the 1950s, yet little is known about the role of hybridization in the evolution of bamboos (Poaceae: Bambusoideae), an important group of forest grasses (Friar and Kochert 1994; Stebbins 1956; Triplett and Clark 2010; Triplett et al. 2014; Triplett and Clark 2021). As a result of hybridization and subsequent backcrossing, also known as introgression, closely related bamboos may exhibit overlapping morphological variation and can be extremely difficult to identify. Woody bamboos especially fall into the category of being taxonomically problematic (Barkley et al. 2005; Triplett and Clark 2010). This can be largely attributed to the fact that the current mode of bamboo classification is primarily based on morphological traits. Reliance on morphological traits is a faulty method because bamboos express similar phenotypes among closely related species while also exhibiting phenotypic plasticity, rendering morphological features unpredictable and therefore unreliable for taxonomic delineation in bamboos (Lin et al. 2010). Moreover, flowering is rare and flowering schedules are often unpredictable, making the characters of reproductive organs unreliable for identification.

Hybridization in woody bamboos is not well documented and is generally considered to be rare, yet recent studies suggest that hybridization may have been a very important process shaping the phylogenetic history of bamboos (Triplett et al. 2014; Triplett and Clark 2021). Bamboos are geographically ubiquitous yet are one of the most challenging groups of plants from a taxonomic perspective. The number of species is relatively poorly known due to confusing morphological characteristics that could be attributed to hybridization. This fact is highlighted by recent evidence. For example, within the temperate bamboos, a clade that encompasses one third of all bamboos, Triplett and Clark (2021) demonstrated that several groups of bamboos were in fact intergeneric hybrids (crosses between species in different genera). Several commonly misidentified bamboos were revealed to have undergone previously undetected hybridization. For example, bamboos in the genus *Semiarundinaria* were demonstrated to be the result of crosses between plants in *Pleioblastus* and *Phyllostachys*, while *Pseudosasa japonica* (the type species of *Pseudosasa)* was revealed to be the result of a natural cross between parents in *Pleioblastus* and *Sasamorpha* (Triplett and Clark 2021). The discovery of this hidden history of hybridization events within bamboos has prompted a reevaluation of bamboo classification and nomenclature.

One group that may provide valuable information about intrageneric hybridization in temperate bamboos is the Japanese genus, *Pleioblastus*, a group of approximately 21 species (Suzuki 1978; Ohrnberger 1999; Zeng et al. 2010). *Pleioblastus* is characterized by a suite of morphological features including persistent culm leaf sheaths, extensive secondary branching, and glabrous fimbriae; however, none of these are exclusive or provide clear synapomorphies (shared derived characters). At least two types of rhizomes

are present in this genus, which served as the primary basis for intrageneric assignments. One type is described as amphipodial (leptomorph with tillering culms): the rhizomes are short and thick, with short internodes and the tips always turning upward to produce culms that are close together in a dense clump. The other type is more typical monopodial (leptomorph), with new culms arising from lateral buds at intervals along the rhizome (Suzuki 1978).

During the early twentieth century, the genus *Pleioblastus* swelled to over 100 Japanese species and as many subspecific taxa as a result of fieldwork and alpha taxonomy by botanists in Japan. The nomenclature of this complex group was revised on the basis of morphology by Suzuki (1978), who reduced it to 21 species in 3 sections: *Pleioblastus*, *Medakea*, and *Nezasa*. Section *Pleioblastus* contains many of the larger species with tillering culms, relatively long foliage leaf blades, and long inner ligules (Suzuki 1978). Sections *Medakea* and *Nezasa* contain species with monopodial rhizomes, relatively shorter foliage leaf blades, and short inner ligules; these two sections are primarily distinguished by minor differences in the upper margins of leaf sheaths, which are oblique in *Medakea* and horizontal in *Nezasa* (Suzuki 1978). Taxonomically, section *Nezasa* is the most problematic: many of its species are only known in cultivation, and several are unknown in flower. Field identification is especially challenging in this group.

In recent chloroplast DNA (cpDNA) and amplified fragment length polymorphism (AFLP) analyses (Triplett and Clark 2021), *Pleioblastus sensu stricto* (*s. s.*) was recovered as a robust lineage with subclades corresponding to the three sections defined by morphology (Suzuki 1978). Molecular data currently provide the only

synapomorphies for this genus, which is otherwise difficult to distinguish from Chinese, North American, and African species of temperate bamboos (*Arundinaria sensu lato*). The AFLP analyses supported a sister relationship between sections *Medakea* and *Nezasa* and provided resolution that was unavailable from cpDNA sequence data (Triplett and Clark 2021). Negligible cpDNA sequence variation was recovered within this group (Triplett and Clark 2021). For example, among eight sampled taxa, all except *P. chino* were distinguished from *P. simonii* (*Pleioblastus* section *Medakea*) only by a single point mutation, resulting in a weakly supported clade in phylogenetic analyses. Furthermore, data from that study revealed compelling evidence that the most widespread species of section *Medakea* (*Pleioblastus simonii,* Japanese river cane) exhibits AFLP character conflicts with species in the two other taxonomic sections of this genus (*Nezasa* and *Pleioblastus*), thus behaving like a hybrid. Triplett and Clark (2021) analyzed *Pleioblastus* using phylogenetic trees, a non-metric multidimensional scaling (NMDS) analysis plot of the AFLP variation, and NeighborNet network diagrams. However, the data failed to provide a straightforward solution to this puzzle and, instead, highlighted a complex network of relationships within *Pleioblastus*. Clearly, more work needs to be done to resolve this issue and clarify relationships and patterns of hybridization in the group.

The objective of the current study is to test the hypothesis that *Pleioblastus simonii* (Figure 1) is a cryptic hybrid between parental species in sections *Nezasa* and *Pleioblastus*. In order to test this hypothesis, data from AFLP markers and nuclear DNA (nDNA) sequences were generated and analyzed using a combination of phylogenetic analyses (PAUP, MrBayes, SplitsTree) and genotypic assignment analyses

(STRUCTURE, NewHybrids). This study provides an updated, valuable lens for understanding evolutionary relationships in this large and complex group of plants, and also provides a guide for understanding relationships in more complex groups of temperate bamboos, including the Chinese relatives of *Pleioblastus* (The Sinicae Clade; Triplett et al., 2010, Zeng et al. 2010). Through consistent results using several analyses to assess AFLP and nDNA data, this study shows that molecular data, in conjunction with morphological data, are valuable for plant identification and that it is important to use molecular analyses to study ambiguous relationships in many types of plants, not just bamboos.

II. MATERIALS AND METHODS

Taxon Sampling and DNA Extraction

Samples were obtained from natural populations in Japan and North America, and from living collections in Japan (Kyoto) and the USA (California, Tennessee, and Washington). Additional samples were obtained from colleagues. Leaf tissue was collected in the field and desiccated using silica gel (Chase and Hills 1991). Voucher specimens were obtained for all individuals and accessioned in the Jacksonville State University (JSU) Herbarium. Sampling emphasized *Pleioblastus s. s.* in Japan and putative hybrid associations within this genus based on previous molecular results and a review of the literature (Suzuki 1978). A total of 141 individual organisms representing 16 species and 3 sections of *Pleioblastus* was utilized for this study (Table 1); this represents approximately 76% of the diversity in the Japanese species of *Pleioblastus*.

Pseudosasa hindsii (basionym, *Arundinaria hindsii*; n.v., *hui zhu*) occurs in the wild in Southeast China (Wu et al. 2006). This epithet and the associated type specimen have also been applied to a plant from southern Japan (n.v., *Kanzan-chiku*) and cultivated worldwide as *Arundinaria hindsii* or *Pleioblastus hindsii* (Suzuki 1978), although morphology and cpDNA suggest that the two are not the same species (Triplett and Clark 2010). Here, we included representatives of the Japanese plants, which are herein referred to as *Pleioblastus hindsii* sensu Nakai.

Total genomic DNA was extracted from silica gel-dried samples according to the modified 2× CTAB procedure of Doyle and Doyle (1987), eluted in nuclease-free water and stored at –20º C. Nucleic acid quality was measured using a Nanodrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Waltham, Massachusetts) and DNA concentrations were standardized to 200 ng/µl for AFLP enzyme digestions and 100 ng/µl for PCR amplification, by diluting with nuclease-free water.

AFLP Data Generation and Analyses

AFLP protocols followed Vos et al. (1995) with modifications suggested by the J.F. Wendel lab at Iowa State University (Table 2). DNA was digested with restriction enzymes *EcoRI* (10 units, New England Biolabs) and *MseI* (10 units, New England Biolabs) for 2h at 37º C in a 20 µl volume, followed by ligation (20 units T4 DNA ligase [New England Biolabs] overnight at 16º C) to double-stranded *EcoRI* and *MseI* adapters. Two rounds of PCR amplification followed. First, a preselective (+1) amplification was performed using primers *MseI* +C and *EcoRI* +A in a 50 µl reaction volume, with 10 µl of undiluted template. Second, the resulting +1 product was diluted 3-fold with water, and a selective (+3) amplification was performed using one *MseI* + 3 primer and two fluorescently labeled *EcoRI* +3 primers. Six primer combinations were chosen for this study based on Triplett and Clark (2021). This follows the recommendation of Ellis et al. (1997), who suggested that at least 80% of the expected relatedness can be captured with six primer combinations. The FAM- and HEX-labeled +3 *EcoRI* primers were multiplexed in the following combinations: [1.] mCAA, eACT (FAM), eACG (HEX); [2.] mCTG, eACA (FAM), eAAC (HEX); [3.] mCTT, eACT (FAM), eACG (HEX).

Selective amplification products were separated electrophoretically at the ISU DNA Facility on a Perkin-Elmer 3100 capillary fragment analyzer (Applied Biosystems, Foster City, California) with an internal standard (GeneScan 500 Rox, ABI) and read using GeneScan software (Perkin-Elmer, Waltham, Massachusetts). To test the accuracy and repeatability of the AFLP markers, we generated replicates for a subset of samples that represented alternative DNA extractions and AFLP runs. In almost every case, the banding pattern was highly similar and produced identical results, although there was variation in PCR amplification as indicated by differences in band intensity between replicates.

Data extraction was done manually from trace files using the GeneMarker (v2.4.0) software package (SoftGenetics, State College, Pennsylvania), as described by Triplett and Clark (2021). AFLP bands were scored as present (1) or absent (0), based on the peak size of the data at each marker from the panel. Bands were hand-scored in a reiterative manner to ensure that peaks were of similar in size, shape, and intensity. Only robust, unambiguous DNA fragments ranging from 50 bp to 665 bp in size and above 200 relative fluorescent units were scored.

The AFLP data that passed the above data processing and quality control steps were subjected to phylogenetic analyses. Here, we used an iterative approach to explore the phylogenetic structure of the AFLP data. We analyzed the data with different methods in order to test for consistency and understand the cause of any observed incongruence. In particular, tree-building methods are expected to provide a poor representation of relationships that are non-bifurcating as a result of hybridization, and therefore a number

of different methods were used to detect phylogenetic signal. Selected iterations are reported below to highlight key steps in data exploration.

Relationships in *Pleioblastus* were investigated in two main stages: (1) first, analyses were run with all available AFLP samples to look for major trends; (2) and then targeted analyses were conducted on a subset of taxa. This approach allowed us to test *a priori* taxonomic assignments and to subsequently minimize noise introduced via distant relatives or genetically mosaic taxa.

The AFLP data was analyzed to look for major trends in genetic relationships among individuals, based on a method described by Triplett and Clark (2021). Pairwise genetic distances were calculated in PAUP* v4b10 (Swofford 2003) using the Nei-Li dissimilarity coefficient (Nei and Li 1979). Genetic relationships were then reconstructed from these pairwise distances using neighbor-joining (NJ) analyses (Saitou and Nei 1987) as implemented in PAUP*, with ties broken randomly. Bootstrap support for the NJ tree was estimated based on 10,000 replicates. In general, hybrid species are predicted to be positioned in intermediate positions between parent species (McDade 1992; McDade 1997; Reeves and Richards 2007).

Split-network (Bandelt and Dress 1992) analyses were conducted to further test the hypothesis of hybridization in *Pleioblastus*. Analyses were conducted using the NeighborNet algorithm (Bryant and Moulton 2004) on the Nei-Li pairwise distances matrix as implemented in SplitsTree4 (Huson and Bryant 2006). The first SplitsTree analysis was conducted using data from the sections of bamboo that contain putative parent species, *Nezasa* and *Pleioblastus*. Putative parental groups were selected based on data from the Triplett and Clark (2021) study. The second SplitsTree analysis included

sections that contain parental groups (*Nezasa* and *Pleioblastus*) plus the putative hybrid species (*Medakea*). It is predicted that the addition of a hybrid species will cause character conflicts between parental groups and rearrange the tree such that the parental species will be drawn closer to the hybrid.

Based on the results from the SplitsTree analysis, 79 individuals were selected to represent the putative parental and hybrid species for further analysis: 27 individuals of one putative parental species (*Pleioblastus chino*), 9 individuals of another putative parental species (*Pleioblastus hindsii*), and 43 individuals of the putative hybrid (*Pleioblastus simonii*). The AFLP data for the selected individuals were analyzed to investigate the genetic structure and patterns of inferred admixture between them, using the program STRUCTURE 2.3.4, which assigns individuals to groups based on their multilocus genotypes based on a Markov chain Monte Carlo (MCMC) algorithm (Pritchard et al. 2000; Falush et al. 2007). Based on the recommendations of Porras-Hurtado et al. (2013) and Triplett and Clark (2021), the following settings were used during STRUCTURE analyses: the Admixture ancestry model was applied, which assumes the genome of an individual is a mixture of genes originating from ancestral groups; POPID was set for three known population groups, where *P. chino* = population 1, *P. simonii* = population 2, and *P. hindsii* = population 3; and the USEPOPINFO selection flag was set so the putative parents were flagged as reference individuals (1) where the individuals' ancestry is well defined, and the putative hybrid was flagged as having unknown ancestry (0). STRUCTURE will estimate the proportion of each individual's genome that is derived from *K* amount of ancestorial populations. Further settings used were: $K = 1–20$ possible ancestorial groups, with each value of *K* evaluated

using 5 independent MCMC replicates, a burn-in of 50,000 iterations followed by a run of 250,000 iterations. We predicted that a hybrid would exhibit 50/50 mixed ancestry between the putative parent species, due to sharing equal proportions of DNA with each parent.

Analyses were also conducted on the AFLP data using the program NewHybrids (Anderson 2003), which computes an MCMC simulation that predicts the probability that individuals fall into a particular hybrid category (F_1, F_2, or backcross generations), given the data for the putative hybrid and the putative parents. To avoid over- or under-representation of species in this analysis, a randomized subset of the data was created that included 20 representative individuals from each species (*P. chino*, *P. hindsii*, and *P. simonii*) for a total of 60 individuals. The settings in NewHybrids, based on recommendations by Anderson (2003), were: the default genotype frequency classes, no prior information, 600 and 900 for random number seeds, and 40,000 sweeps. Furthermore, the *z* option was used to indicate that knowledge about the data was known beforehand (the *s* option was not used), where there are two parental groups, labeled z0 (*P. chino*) and z1 (*P. hindsii*), and a hybrid species (*P. simonii*) of unknown genetic relation to the other species (no *z* option). We predicted that the putative hybrid individuals would be recovered as a hybrid cross between the parental species.

nDNA Data Generation and Analyses

Two nuclear genes were investigated in this study based on previous work on the temperate bamboos (Triplett et al. 2014). These were *cellulase1 α* (*pvcel1 α*) and *cellulase1* β (*pvcel1* β). These two represent homologs of the *cellulase1* gene and were

shown in a previous study to be the product of an ancestral whole-genome duplication event (Triplett et al. 2014). Thus, we assumed that both of these gene regions provide independent estimates of phylogeny.

Most of the sequences used in this analysis were downloaded from GenBank. We supplemented the available sequences with two samples of *Pleioblastus simonii* (JT 296 and JT 410) for which we had DNA extractions. The primers used for PCR and sequencing reactions are based on Triplett et al. 2014 (Table 3). PCR amplification used the following protocol: initial denaturation phase of 95° C for 5 min, 35 cycles of amplification at 95° C for 30 sec, primer-specific annealing temperature for 45 sec, 72° C elongation for 1 min 20 sec, followed by a final elongation phase of 72° C for 15 min. PCR reactions were conducted in a 25 mL volume of Taq polymerase buffer, 100–500 ng total genomic DNA, 2.0 mM MgCl 2, 0.4 mM of both forward and reverse primers, 1.00 mM dNTPs (0.25 mM each dNTP), and 2 units of GoTaq Flexi DNA polymerase (Promega, Madison, WI, USA).

PCR products were purified using a Qiagen Gel Extraction Kit (QIAGEN, Valencia, CA, USA) and cloned using the TOPO TA Cloning Kit (Life Technologies, Grand Island, NY, USA) following the manufacturer's protocol, except that all reaction volumes were quartered (12.5 μl). To assess PCR errors and allelic sequences, 8–24 colonies were selected from each accession. Transformed colonies were used for PCR with primers M13 F-20 (5'-GTA AAA CGA CGG CCA G-3') and M13 R (5'-CAG GAA ACA GCT ATG AC-3'), cleaned using the ExoSAP-IT™ method, and sequenced following the ABI-Prism Big Dye Terminator sequencing method (version 3.1; Applied Biosystems, Foster City, CA, USA). Sequence reactions were run on an Applied

Biosystems ABI Hitachi 3730XL DNA Analyzer at the Laboratories of Analytical Biology at the Smithsonian Institution.

Vector sequences and ambiguous bases from the ends of both forward and reverse reads were removed manually. Clone sequences were imported and manually inspected with MEGA-X (Kumar et al. 2018). Ambiguous bases in each clone sequence were corrected manually by comparing sequence quality from trace files. Corrected clones were assembled into accession-specific files and aligned with MEGA. Consensus sequences for each sequence type per individual were constructed to minimize the inclusion of sequencing errors. In general, a substitution that appeared in a single sequence was considered to be PCR error. Sequences with two or more nucleotide differences were interpreted as different alleles. Potentially informative indels located in regions of unambiguous alignment were scored following the "simple indel coding" method (Simmons and Ochoterena 2000) and added to the matrix as binary presence/absence characters. All data matrices are available from the author upon request.

Separate analyses were run for both loci. Each data set was analyzed using two methods: Bayesian inference (BI) analysis with MrBayes 3.2 (Ronquist et al. 2012) and parsimony analyses (MP) using PAUP* 4.0b10 (Swofford 2003). The BI analysis was conducted using a partitioned GTR + I + G model for reasons outlined by Huelsenbeck and Rannala (2004), with all parameter values estimated during analysis. A Dirichlet prior was used for base frequencies and the rate matrix. A uniform prior was used for the shape parameter (α), proportion of invariable sites (I), and topology. Branch lengths were unconstrained. Partitions were designated for each data set and for the microstructural

characters and all parameters were unlinked across partitions. Four separate MCMC runs were initiated, each with 10,000,000 generations. Runs were started from a random tree; the topology was sampled every 1,000 generations of the MCMC chain. Performance of individual runs was assessed in MrBayes and phylogenies compared between runs. Majority rule (50%) consensus trees were constructed after removing the first 10% of sampled trees ("burn-in").

Maximum parsimony (MP) analyses used 1,000 random addition sequences, tree bisection-reconnection (TBR) branch swapping, and Multrees on. Full heuristic bootstraps were performed for MP with 1,000 bootstrap replicates.

Branch support was assessed according to a 70% bootstrap criterion for MP and a 0.95 posterior probability measure for BI (Mason-Gamer and Kellogg 1996; Wilcox et al. 2002).

III. RESULTS

AFLP Data

A total of 2,897 markers was scored for the six AFLP primer combinations. Scored fragments represent six size classes: 50–150 (607; 21%), 151–250 (723; 25%), 251–350 (607; 21%), 351–450 (445; 15.4%), 451–550 (270; 9%), and 551–665 (245; 9%). The average number of scored bands per primer pair was 348, with a range of 305 to 407.

AFLP Phylogeny

Results of the NJ analysis of *Pleioblastus s. s.* are presented in Figure 2. Three clusters were recovered in this analysis, corresponding to *Pleioblastus* sections *Nezasa*, *Medakea*, and *Pleioblastus*. Samples of *Pleioblastus simonii* formed a cluster between species in sections *Nezasa* and *Pleioblastus*.

Hybrid Tests Using Split Networks

NeighborNet diagrams were produced to test for character conflict in the AFLP data. First, we assembled a core taxon subset that included all samples in sections *Nezasa* and *Pleioblastus* and excluded the putative hybrid, and then used this as a basis of comparison with an additional subset that included the core taxon subset plus the putative hybrid, *P. simonii*. The resulting network diagrams are presented in Figures 3 and 4. The

split network revealed substantial character conflict between two divergent clusters (Sections *Nezasa* and *Pleioblastus*) as a result of re-including the putative hybrid (*P. simonii*). Furthermore, the inclusion of *P. simonii* repositioned particular species in the other two sections (Figure 4). Specifically, *P. chino* and *P. hindsii* were repositioned to be closer to *P. simonii*. Based on this observation, further analyses were conducted on putative parental and hybrid species.

Structure Analysis

The STRUCTURE analysis of the complete sample set revealed $K = 3$ ancestral clusters as the most likely model for ancestry in this group, based on an assessment made through the program STRUCTURE HARVESTER (Earl and vonHoldt 2012) (Figure 5). Three ancestral populations were recovered based on ancestral clustering: *Nezasa* individuals were revealed to have ancestry from one ancestral cluster, *Pleioblastus* species were attributed to a second ancestral cluster, and individuals of *P. simonii* were revealed to have ancestry from both of these two ancestral groups, with marginal contributions from a third ancestral population.

Based on the previous analysis, another STRUCTURE analysis was conducted on a subset of the data containing the putative parental and hybrid individuals. This analysis revealed $K = 3$ ancestral clusters, with two ancestral populations contributing to the largest portion of the ancestry analysis and the remaining population attributed to miniscule proportions (Figure 6). Of the two main ancestral groups, one group accounted for 97.9% of all Population 1 individuals (*Nezasa*) and 58.1% of all Population 2 individuals (*P. simonii*). The other ancestor group accounted for 99.8% of all Population

3 individuals (*P. hindsii*) and 38.4% of all Population 2 individuals (*P. simonii*). Thus, all individuals of Population 2 (*P. simonii*) exhibited admixture from major ancestral clusters belonging to each putative parent population.

NewHybridsAnalysis

Results of the MCMC simulation in New Hybrids are presented in Figure 7. This analysis included a randomly sampled subset of taxa for each major category: Putative Parent 1 (*P. chino*), Putative Parent 2 (*P. hindsii*), and Putative Hybrid (*P. simonii*). For each species, 20 representatives were randomly selected. When putative parents were predetermined and the putative hybrid was specified as unknown, the program recovered *P. simonii* (individuals 21 – 40) as the products of an F1 cross between *P. chino* and *P. hindsii*, with 99% probability.

nDNA Data

The nDNA gene *pVCel1* α was analyzed for 8 species of bamboos in the genus *Pleioblastus* (Figure 8), with representatives of *Arundinaria* and *Sasamorpha* as outgroups. This analysis also included haplotypes of *Pseudosasa japonica*, which was previously determined to represent an intergeneric hybrid. Topologies of the strict consensus of the equally most parsimonious trees (45 parsimony informative characters; 1192 trees of 114 steps; CI = 0.7018; RI = 0.8502) and the BI phylogeny were highly congruent. The analysis revealed that *P. simonii* possesses two alleles for the gene: one that clusters with bamboos in section *Nezasa* and another that clusters with bamboos in section *Pleioblastus* (Figure 8). Similarly, the gene phylogeny for *PvCel1* β revealed the

same condition for *P. simonii*: two haplotypes (alleles) were recovered from both of the sampled individuals (JT 296 and JT 410): one that clusters with bamboos in section *Nezasa* and another that clusters with bamboos in section *Pleioblastus* (Figure 9; 36 parsimony informative characters; 2 trees of 70 steps; CI = 0.9143; RI = 0.9653).

IV. CONCLUSIONS

The Triplett and Clark study (2021) provided compelling evidence that *P. simonii* is of hybrid origin. While that study was not specifically about the origin of *P. simonii*, their clustering and tree-based phylogenetic analyses revealed preliminary evidence that *P. simonii* arose via hybridization between parent species in sections *Nezasa* and *Pleioblastus*. Given this evidence, the current study aimed to analyze molecular data from *P. simonii* and other bamboo within the genus *Pleioblastus* to test the hypothesis that *P. simonii* is in fact a cryptic hybrid. Moreover, this study aimed to identify the likely parental species.

Pleioblastus section *Medakea* encompasses six species that are distinguished primarily on the basis of foliage vestiture and culm coloration, including one widespread species (*P. simonii*) and five that are narrow endemics. A total of 37 wild accessions of *P. simonii* from throughout Japan plus five cultivars in the United States and Japan was included in the current AFLP study, and these had virtually identical AFLP genotypes. The lack of genetic variation within this species is striking, but reminiscent of the low genetic diversity observed among North American populations of *A. gigantea* throughout its broad distribution in a previous study by Triplett et al. (2010). Interestingly, both species are known by similar common names (river cane, *kawa-dake* in Japanese), and their wide distribution and correlated low diversity could be connected with the

successful exploitation of a habitat favorable to clonal growth. Alternatively, anthropogenic interaction could account for the genetic structure of *P. simonii*; historically, this species was widely used for thatched roofing and other purposes in rural Japan, and current populations may represent widespread clones from an original source population.

Although our sampling is incomplete with respect to the full geographical distribution of the species, the current analysis calls into question the taxonomic diversity of section *Medakea*. Plants matching the description of *P. kodzumae* were collected from a number of localities in Japan, mostly correlated with harsh or disturbed habitats including river basins and exposed hillsides. All of these had AFLP genotypes matching those of *P. simonii*. Plants collected from the type locality of *P. kodzumae* (Japan: Kyushu; Makino 1928) were morphologically and genetically consistent with *P. simonii*. Populations of *Pleioblastus kodzumae* putatively occur in Izu Peninsula, western Shikoku, central and southern Kyushu, and in several isolated localities on the northern side of Honshu. For such a broad distribution, this species would have been widespread in the past, with subsequent habitat fragmentation. However, none of the plants collected from putative *P. kodzumae* localities were genetically different from *P. simonii*, in spite of morphological variation. Thus, the current data and results support the recognition of *P. kodzumae* as an ecotype of *P. simonii*.

Four additional species from section *Medakea* could not be located in the wild. *Pleioblastus nabeshimanus*, *P. matsunoi*, and *P. higoensis* are each considered relatively rare, and most are locally endemic. These are distinguished from *P. simonii* primarily on vestiture (i.e., culm leaf sheaths puberulous or pilose vs. glabrous; foliage leaf sheaths

puberulous or pilose vs. glabrous). *Pleioblastus simonii* was common in the vicinity of the type localities and other reported locations of some of these species. One other member of section *Medakea, P. pseudosasaoides,* is only known from two locations (Japan: Niigata and Fukushima Prefectures, central Honshu), and has not been studied in molecular or morphological analyses. The type specimen reveals a very distinctive plant with a single branch per node and other morphological features suggesting *Sasa* and *Sasamorpha* (J. Triplett, pers. observation). Thus, it is possible that this species represents a distinctive hybrid association; hybrid links have been established between *Sasamorpha* and *Pleioblastus* sect. *Pleioblastus* (i.e., *Pseudosasa*) and between *Sasa* and *Pleioblastus* sect. *Nezasa* (i.e., *Sasaella*), but none are currently documented between sect. *Medakea* (*P. simonii*) and *Sasa* or *Sasamorpha*.

Clearly, identification based on morphological features causes complications within section *Medakea*. Evidence from Triplett and Clark (2021) alludes to the fact that the current species in section *Medakea* are most likely synonymous with *P. simonii*. If true, this would reduce the diversity in section *Medakea* from six species to one.

In the current study, split-network analyses show how the addition of *P. simonii* repositions species in sections *Nezasa* and *Pleioblastus* closer to the putative hybrid. Specifically, *P. chino* and *P. hindsii* are drawn near *P. simonii*, and this strongly suggests that *P. simonii* shares a combination of diagnostic AFLP bands with the other two species from two different sections. Our STRUCTURE analysis recovered the most likely ancestral populations for *P. chino, P. hindsii,* and *P. simonii* groups, revealing that *P. simonii* has ancestry that is almost a 50% split between the two putative parents. The AFLP data was further analyzed in NewHybrids to test if the data would be consistent

with our findings that strongly point to *P. simonii* being of hybrid origin. Findings show that when *P. chino* and *P. hindsii* are identified as parents, *P. simonii* is recovered as an F_1 hybrid, a direct cross between the putative parent species. Lastly, the analysis of nuclear DNA sequence data reveals the occurrence of divergent alleles in *P. simonii*, one of which tracks *P. chino* and allies, and one that tracks *P. hindsii* and allies. Thus, *P. simonii* demonstrates heterozygosity consistent with the hypothesis of hybridization. Collectively, these findings consistently support the hypothesis that *Pleioblastus simonii* is a species of hybrid origin.

Although the findings of this study strongly point to *P. simonii* as a cryptic hybrid of *P. chino* and *P. hindsii*, an alternative hypothesis to explain these results would be that incomplete lineage sorting could be the reason that *P. simonii* possesses similar DNA to the proposed parent species (Joly et al. 2009). This would be a reasonable alternative hypothesis if the data set concerned only a few loci; however, AFLP data represent numerous presumed independent nuclear loci (in this case, over 2800 loci). However, lineage sorting cannot be completely ruled out. Moreover, the SplitsTree results (Figure 4) reveal that *P. simonii* individuals are clustered on a long branch. This strongly-divergent branching pattern may indicate subsequent diversification since the origin of this species, or it may be an artifact of clonal history of *P. simonii*. This ongoing diversification may also be the reason that *P. simonii* has been characterized as a distinct species in section *Medakea*. Nevertheless, the analyses presented in this study strongly support the hypothesis of hybridization. Further research is needed to characterize this ecologically and economically important species and its allies within section *Medakea*. For example, next-generation sequencing approaches, such as ddRAD-seq, could be

conducted using the AFLP data for further genotyping. Moreover, analyses could be done to approximate the timing of the initial hybridization event for this species. This group provides an excellent model system for ongoing research on the evolutionary and genetic impacts of hybridization in natural populations of temperate bamboos.

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APPENDIX A: TABLES

TABLE 1.

List of all samples used in the current study. Species are organized alphabetically. Native geographic regions are indicated for each genus. Samples with unclear species identity are referred to as "aff. [species name]" (see text for additional information). Vouchers are deposited at JSU herbarium unless otherwise indicated. Samples with AFLP patterns identical to another sample of the same taxon are indicated in the notes; these redundant samples were excluded from most analyses.

Arundinaria sensu stricto **(North America):** ***Arundinaria appalachiana*** Triplett, Weakley & L.G. Clark, *Triplett 99*, Alabama, USA; ***Arundinaria gigantea*** (Walt.) Muhl., *Triplett 197*, Indiana, USA*;* ***Arundinaria tecta*** (Walt.) Muhl., *Triplett 24*, North Carolina, USA; ***Pleioblastus*** **(China and Japan):** ***Pleioblastus argenteostriatus*** (Regel) Nakai, *Triplett 31*, Cult. (Washington, USA); *Triplett 64*, Cult. (Kyoto, Japan)*; Triplett 204*, Cult. (California, USA); ***Pleioblastus aff. argenteostriatus***, *Triplett 353*, Shizuoka Prefecture, Japan; *Triplett 359*, Mie Prefecture, Japan; *Triplett 378*, Ehime Prefecture, Japan; ***Pleioblastus argenteostriatus f. glaber*** (Makino) Murata, *Triplett 66*, Cult. (Kyoto, Japan); *Triplett 67*, Cult. (Kyoto, Japan), AFLP = *Triplett 66*; ***Pleioblastus chino*** (Franchet & Savatier) Makino, *Triplett 11*, Cult. (Georgia, USA); *Triplett 14*, Cult. (Georgia, USA); *Triplett 73*, Cult. (Kyoto, Japan); *Triplett 297*, Kanagawa Prefecture, Japan; *Triplett 301*, Kanagawa Prefecture, Japan; *Triplett 302*, Kanagawa Prefecture, Japan; *Triplett 304*, Kanagawa Prefecture, Japan; *Triplett 305*, Kanagawa Prefecture, Japan; *Triplett 307*, Tokyo Prefecture, Japan; *Triplett 309*, Ibaraki Prefecture, Japan; *Triplett 310*, Ibaraki Prefecture, Japan; *Triplett 313*, Ibaraki Prefecture, Japan; *Triplett 315*, Ibaraki Prefecture, Japan; *Triplett 316*, Ibaraki Prefecture, Japan; ***Pleioblastus aff. chino***, *Triplett 373*, Kochi Prefecture, Japan; *Triplett 402*, Kumamoto Prefecture, Japan; *Triplett 412*, Kagoshima Prefecture, Japan; ***Pleioblastus chino*** **'Gracilis'**, *Triplett 49*,

Cult. (Kyoto, Japan); ***Pleioblastus chino* 'Kimmei'**, *Triplett 40*, Cult. (Washington, USA); ***Pleioblastus chino* 'Murakamianus'**, *Triplett 32*, Cult. (Washington, USA); ***Pleioblastus chino* 'Variegatus'**, *Triplett 129*, Cult. (Tennessee, USA); *Triplett 236*, Cult. (California, USA); ***Pleioblastus chino f. elegantissimus*** (Makino ex Tsuboi) Muroi & H. Okamura, *Triplett 57*, Cult. (Kyoto, Japan), AFLP = *Triplett 236*; ***Pleioblastus aff. chino f. villosus*** S. Suzuki, *Triplett 398*, Kumamoto Prefecture, Japan; ***Pleioblastus chino var. vaginatus*** (Hack.) S. Suzuki, *Triplett 415*, Shizuoka Prefecture, Japan; *Triplett 419*, Shizuoka Prefecture, Japan; *Triplett 420*, Shizuoka Prefecture, Japan; ***Pleioblastus gauntlettii*** (As known in cult.; possibly *Pleioblastus humilis*), *Triplett 145*, Cult. (Tennessee, USA); ***Pleioblastus gozadakensis*** Nakai, *Triplett 337*, Iriomote Island, Japan; *Triplett 338*, Iriomote Island, Japan; *Triplett 342*, Ishigaki Island, Japan; *Triplett 343*, Ishigaki Island, Japan; *Triplett 344*, Ishigaki Island, Japan; ***Pleioblastus gramineus*** (Bean) Nakai, *Triplett 35*, Cult. (Washington, USA); *Triplett 36*, Cult. (Washington, USA); *Triplett 58*, Cult. (Kyoto, Japan); *Triplett 59*, Cult. (Kyoto, Japan); *Triplett 327*, Iriomote Island, Japan, AFLP = *Triplett 329*; *Triplett 329*, Iriomote Island, Japan; *Triplett 330*, Iriomote Island, Japan, AFLP = *Triplett 348*; *Triplett 334*, Iriomote Island, Japan, AFLP = *Triplett 348*; *Triplett 336*, Iriomote Island, Japan, AFLP = *Triplett 348*; *Triplett 340*, Iriomote Island, Japan, AFLP = *Triplett 348*; *Triplett 347*, Ishigaki Island, Japan, AFLP = *Triplett 348*; *Triplett 348*, Ishigaki Island, Japan; Zhang 06157 (KUN), Cult. (Zhejiang, China); ***Pleioblastus hindsii*** (Munro) Nakai, *Triplett 39*, Cult. (Washington, USA); *Triplett 65*, Cult. (Kyoto, Japan); *Triplett 229*, Cult. (California, USA); *Triplett 326*, Iriomote Island, Japan, AFLP = *Triplett 331*; *Triplett 331*, Iriomote Island, Japan;

Triplett 333, Iriomote Island, Japan, AFLP = *Triplett 331*; *Triplett 335*, Iriomote Island, Japan; *Triplett 339*, Iriomote Island, Japan; *Triplett 341*, Iriomote Island, Japan; *Triplett 346*, Ishigaki Island, Japan; *Triplett 408*, Kagoshima Prefecture, Japan; *Triplett 411*, Kagoshima Prefecture, Japan; ***Pleioblastus humilis*** (Mitford) Nakai, *Triplett 41*, Cult. (Washington, USA); *Triplett 158*, Cult. (Tennessee, USA); *Triplett 219*, Cult. (California, USA); ***Pleioblastus kongosanensis*** Makino, *Triplett 68*, Cult. (Kyoto, Japan); *Triplett 74*, Cult. (Kyoto, Japan); *Triplett 366*, Nara Prefecture, Japan; ***Pleioblastus aff. kongosanensis***, *Triplett 364*, Mie Prefecture, Japan; *Triplett 367*, Osaka Prefecture, Japan; *Triplett 370*, Kochi Prefecture, Japan; ***Pleioblastus kongosanensis* 'Aureostriatus'**, *Triplett 46*, Cult. (Kyoto, Japan); *Triplett 144*, Cult. (Tennessee, USA), AFLP = *Triplett 46*; ***Pleioblastus linearis*** (Hackel) Nakai, *Triplett 328*, Iriomote Island, Japan; *Triplett 349*, Okinawa Island, Japan; *Triplett 350*, Okinawa Island, Japan; *Triplett 351*, Okinawa Island, Japan; *Triplett 352*, Okinawa Island, Japan; ***Pleioblastus linearis* 'Nana'**, *Triplett 157*, Cult. (Tennessee, USA); ***Pleioblastus nagashima*** (Mitford) Nakai, *Triplett 54*, Cult. (Kyoto, Japan); *Triplett 62*, Cult. (Kyoto, Japan); *Triplett 75*, Cult. (Kyoto, Japan); *Triplett 123*, Cult. (Tennessee, USA); ***Pleioblastus pygmaeus*** (Miquel) Nakai, *Triplett 17*, Cult. (Georgia, USA); *Triplett 28*, Cult. (Washington, USA); *Triplett 45*, Cult. (Kyoto, Japan); *Triplett 127*, Cult. (Tennessee, USA); ***Pleioblastus pygmaeus* 'Distichus'**, *Triplett 12*, Cult. (Georgia, USA); ***Pleioblastus shibuyanus f. tsuboi*** (Makino) S. Suzuki, *Triplett 30*, Cult. (Washington, USA); *Triplett 61*, Cult. (Kyoto, Japan); ***Pleioblastus simonii*** (Carrière) Nakai, *Triplett 9*, Cult. (Georgia, USA); *Triplett 42*, Cult. (Washington, USA); Triplett *232*, Cult. (California, USA); *Triplett 292*,

Shizuoka Prefecture, Japan, AFLP = *Triplett 42*; *Triplett 293*, Shizuoka Prefecture, Japan; *Triplett 295*, Shizuoka Prefecture, Japan, AFLP = *Triplett 42*; *Triplett 296*, Shizuoka Prefecture, Japan; *Triplett 299*, Kanagawa Prefecture, Japan; *Triplett 322*, Chiba Prefecture, Japan, AFLP = *Triplett 399*; *Triplett 324*, Chiba Prefecture, Japan, AFLP = *Triplett 399*; *Triplett 325*, Chiba Prefecture, Japan, AFLP = *Triplett 399*; *Triplett 354*, Shizuoka Prefecture, Japan, AFLP = *Triplett 399*; *Triplett 355*, Aichi Prefecture, Japan, AFLP = *Triplett 399*; *Triplett 356*, Mie Prefecture, Japan, AFLP = *Triplett 399*; *Triplett 357*, Mie Prefecture, Japan, AFLP = *Triplett 42*; *Triplett 358*, Mie Prefecture, Japan; *Triplett 363*, Mie Prefecture, Japan, AFLP = *Triplett 42*; *Triplett 368*, Kochi Prefecture, Japan, AFLP = *Triplett 42*; *Triplett 372*, Kochi Prefecture, Japan, AFLP = *Triplett 377*; *Triplett 374*, Kochi Prefecture, Japan, AFLP = *Triplett 42*; *Triplett 376*, Ehime Prefecture, Japan, AFLP = *Triplett 42*; *Triplett 377*, Ehime Prefecture, Japan; *Triplett 379*, Ehime Prefecture, Japan; *Triplett 380*, Ehime Prefecture, Japan, AFLP = *Triplett 399*; Triplett *384*, Kagawa Prefecture, Japan, AFLP = *Triplett 399*; *Triplett 389*, Yamaguchi Prefecture, Japan; *Triplett 391*, Fukuoka Prefecture, Japan, AFLP = *Triplett 377*; *Triplett 390*, Yamaguchi Prefecture, Japan; Triplett *394*, Fukuoka Prefecture, Japan, AFLP = *Triplett 399*; *Triplett 395*, Fukuoka Prefecture, Japan, AFLP = *Triplett 377*; *Triplett 396*, Fukuoka Prefecture, Japan, AFLP = *Triplett 399*; *Triplett 399*, Kumamoto Prefecture, Japan; *Triplett 400*, Kumamoto Prefecture, Japan; *Triplett 401*, Kumamoto Prefecture, Japan; *Triplett 406*, Kumamoto Prefecture, Japan, AFLP = *Triplett 377*; *Triplett 409*, Kagoshima Prefecture, Japan; *Triplett 410*, Kagoshima Prefecture, Japan, AFLP = *Triplett 389*; *Triplett 413*, Miyazaki Prefecture, Japan, AFLP = *Triplett 377*;

Pleioblastus aff. simonii, *Triplett 323*, Chiba Prefecture, Japan; *Triplett 382*, Ehime Prefecture, Japan; *Triplett 383*, Ehime Prefecture, Japan; ***Pleioblastus simonii*** **'Heterophyllus'**, *Triplett 53*, Cult. (Kyoto, Japan); ***Pleioblastus simonii*** **'Kishima'**, *Triplett 56*, Cult. (Kyoto, Japan), AFLP = *Triplett 296*; ***Pleioblastus aff. variegatus*** (Siebold ex Miquel) Makino, *Fukuda s.n.* Cult. (Japan); *Triplett 361*, Mie Prefecture, Japan; *Triplett 375*, Kochi Prefecture, Japan; *Triplett 385*, Kagawa Prefecture, Japan; *Triplett 386*, Hiroshima Prefecture, Japan; *Triplett 387*, Yamaguchi Prefecture, Japan; Fukuda, s.n., Ibaraki Prefecture, Japan; ***Pleioblastus variegatus*** **'Fortunei'** (Siebold ex Miq.) Makino, *Triplett 37*, Cult. (Washington, USA), AFLP = *Triplett 63*; *Triplett 63*, Cult. (Kyoto, Japan); ***Pleioblastus variegatus 'humilis'***, Cult. (Kyoto, Japan); ***Pleioblastus viridistriatus*** (Regel) Makino, *Triplett 29*, Cult. (Washington, USA); ***Pleioblastus aff. viridistriatus***, *Triplett 414*, Kyoto Prefecture, Japan; ***Pleioblastus viridistriatus*** **'Chrysophyllus'**, *Triplett 154*, Cult. (Tennessee, USA); ***Pseudosasa*** **(China and Japan):** ***Pseudosasa japonica*** (Siebold & Zuccarini ex Steudel) Makino ex Nakai, = *Triplett 320*, Fukuoka Prefecture, Japan; *Triplett 369*, Tokushima Prefecture, Japan; *Triplett 403*, Kumamoto Prefecture, Japan; ***Pseudosasa owatarii*** (Makino) Makino ex Nakai, *Triplett 33*, Cult. (Washington, USA); *Triplett 47*, Cult. (Kyoto, Japan), AFLP = *Triplett 33*; *Triplett 48*, Cult. (Kyoto, Japan). ***Sasamorpha*** **(Japan):** ***Sasamorpha borealis*** (Hackel) Nakai, *Triplett 294*, Shizuoka Prefecture, Japan; *Triplett 311*, Ibaraki Prefecture, Japan; *Triplett 407*, Kumamoto Prefecture, Japan; *Triplett 692*, Hokkaido Prefecture, Japan.

TABLE 2.
Primers for AFLP reactions.

A. Ligation Adapters

EcoRI forward	5′- CTC GTA GAC TGC GTA CC -3′
EcoRI reverse	5′- AAT TGG TAC GCA GTC -3′
MseI forward	5′- GAC GAT GAG TCC TGA G -3′
MseI reverse	5′- TAC TCA GGA CTC AT -3′

B. Pre-selective (+1) Primers

EcoRI +1	5′- GAC TGC GTA CCA ATT CA -3′
MseI +1	5′- GAC GAT GAG TCC TGA GTA AC -3′

C. Selective (+3) Primers

EcoRI +3 (eACT FAM)	5′- ACT GCG TAC CAA TTC ACT -3′ FAM
EcoRI +3 (eACG HEX)	5′- ACT GCG TAC CAA TTC ACG -3′ HEX
EcoRI +3 (eACA FAM)	5′- ACT GCG TAC CAA TTC ACA -3′ FAM
EcoRI +3 (eACC HEX)	5′- ACT GCG TAC CAA TTC ANN -3′ HEX
MseI +3 (mCAA)	5′- GAC GAT GAG TCC TGA GTA ACA A -3′
MseI +3 (mCTG)	5′- GAC GAT GAG TCC TGA GTA ACT G -3′
MseI +3 (mCTT)	5′- GAC GAT GAG TCC TGA GTA ACT T -3′

TABLE 3.
Primers for nDNA region *pvcel1*.

pvcel1_for:	5′- GCC AAC ATG GTT CAG TTG G -3′
pvcel1_rev:	5′- CGC CCC TCT GTG GTG TAC -3′

APPENDIX B: FIGURES

FIGURE 1.
Pleioblastus simonii, Miyazaki Prefecture, Kyushu, Japan. Photograph by Jimmy Triplett.

FIGURE 2.
Results of Neighbor Joining analysis of the AFLP data for Sections *Nezasa*, *Medakea*, and *Pleioblastus*. All clusters received bootstrap support of 100%.

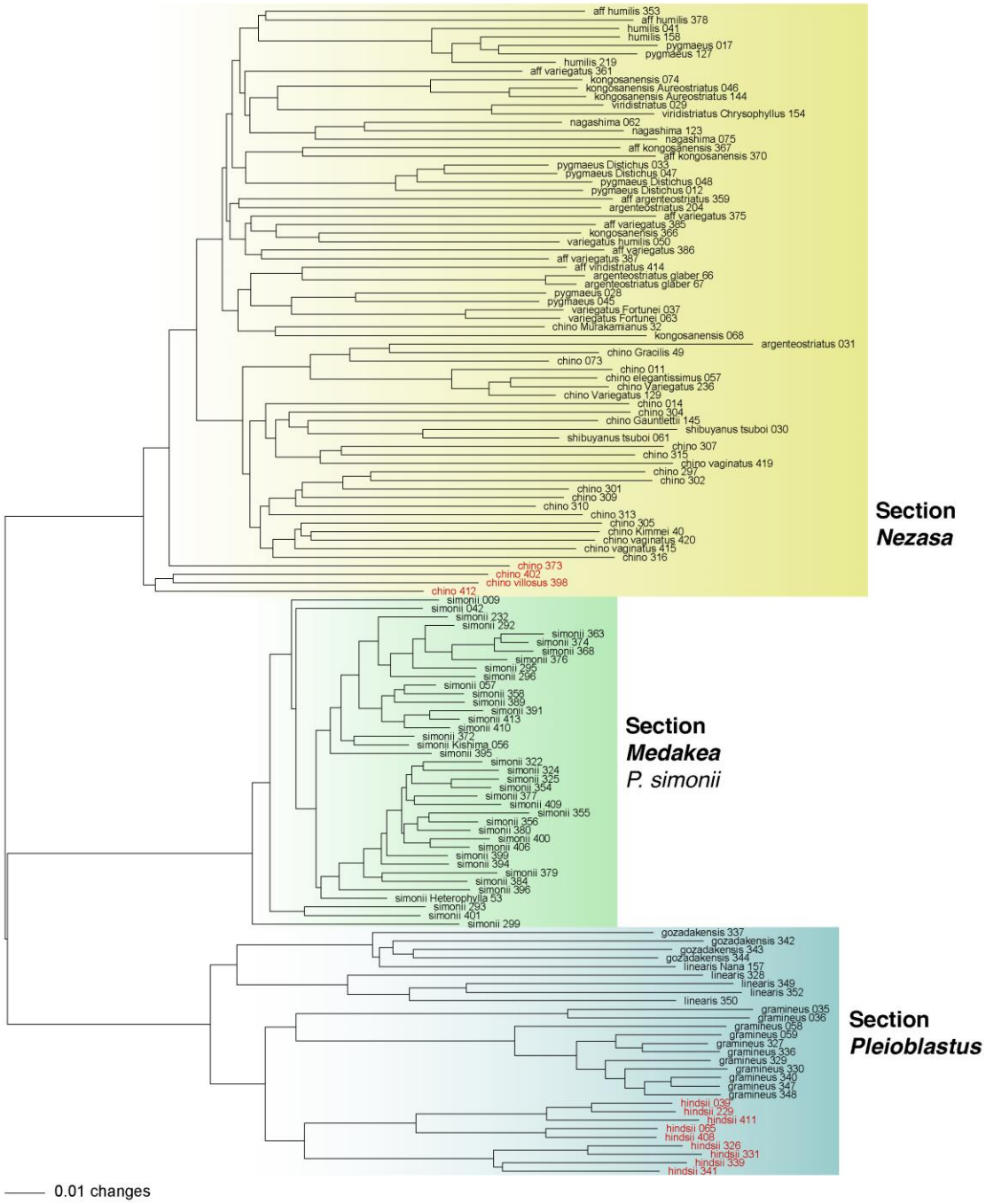


FIGURE 3.
Results of split-network Analysis of the AFLP data for Sections *Nezasa* and *Pleioblastus.*

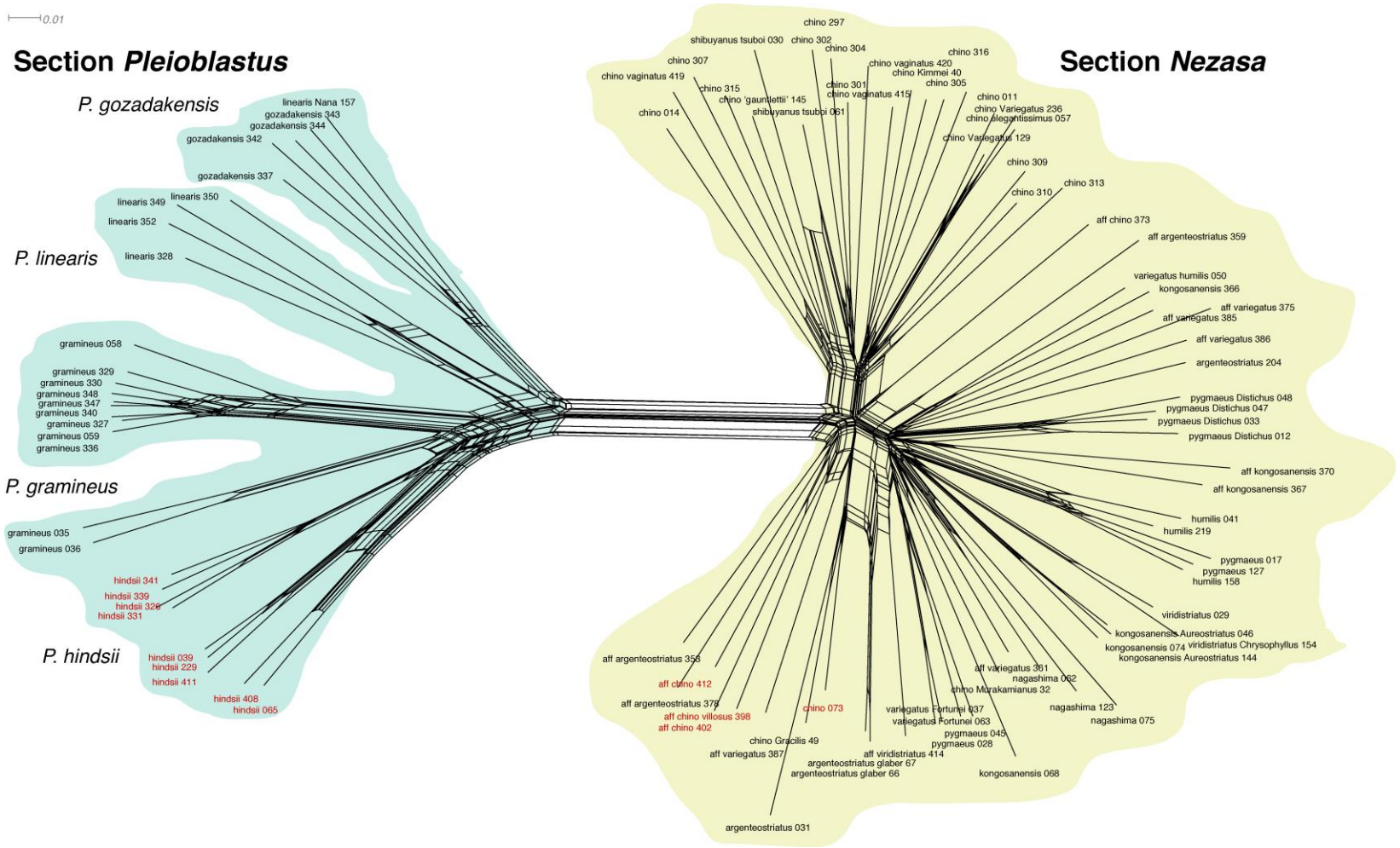


FIGURE 4.
Results of the split-network Analysis of the AFLP data for Sections *Nezasa*, *Medakea*, and *Pleioblastus*.

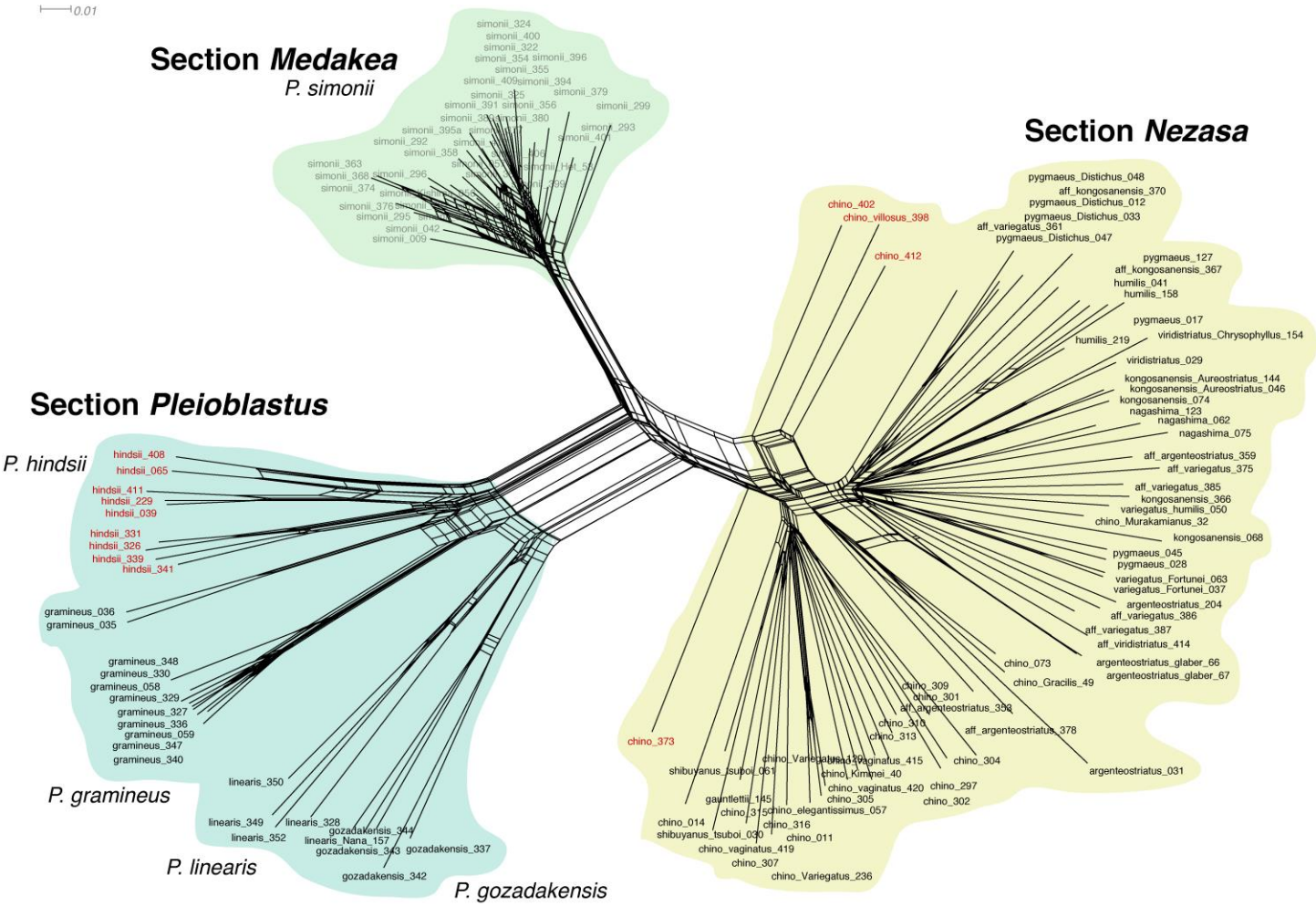


FIGURE 5.
Results of the STRUCTURE analysis of the total species for section *Nezasa* (1 – 69), *Medakea* (70 – 112), and *Pleioblastus* (113 – 141). Ancestral cluster (*K*) values ranged from 1 – 20, with the best value inferred to be *K* = 3. The allele are frequencies independent. *N* = 141.

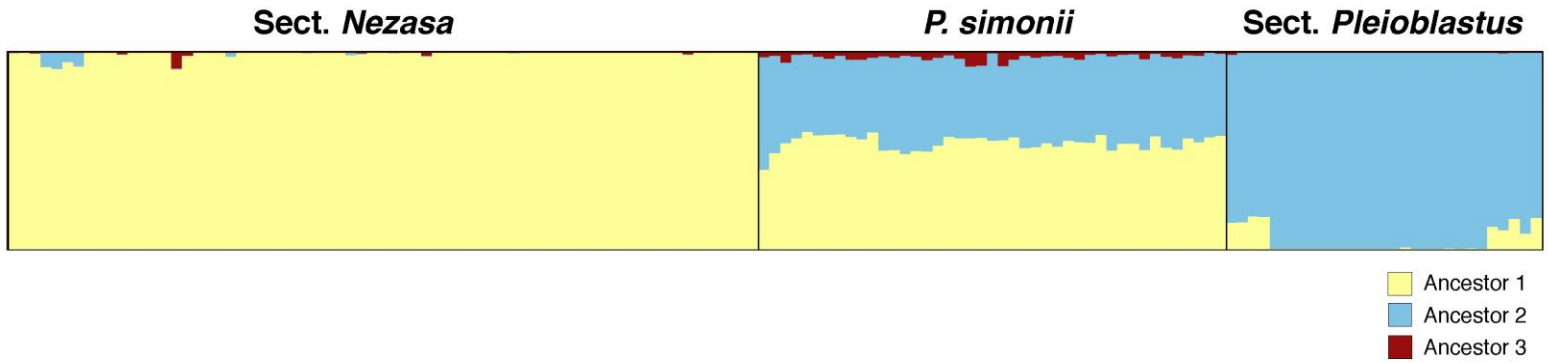


FIGURE 6.
STRUCTURE analysis of a subset of parental and hybrid species (N = 79): *Pleioblastus chino* (1 – 27), *Pleioblastus simonii* (28 - 69), and *Pleioblastus hindsii* (70 – 79). Ancestral cluster (*K*) values ranged from 1–20, with the best value being *K* = 3. The allele frequencies are independent.

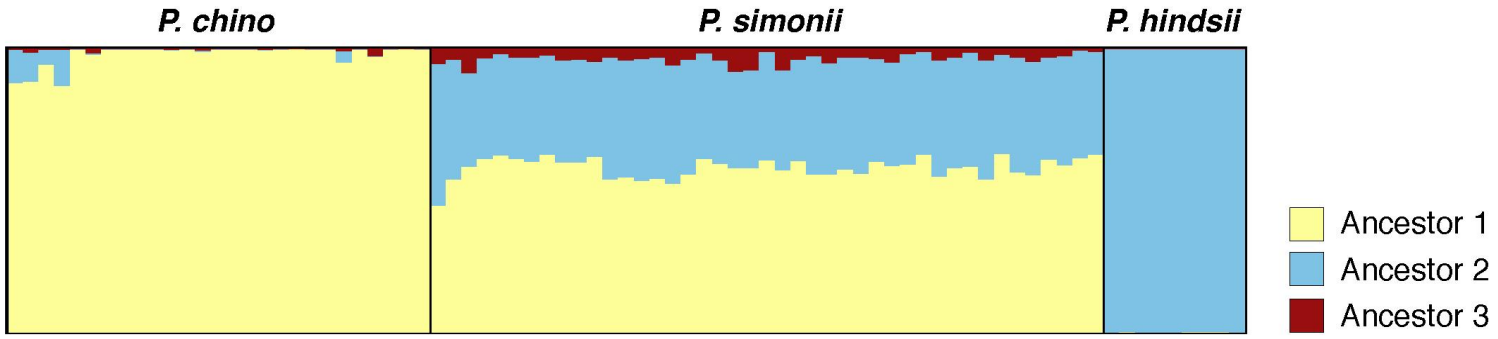
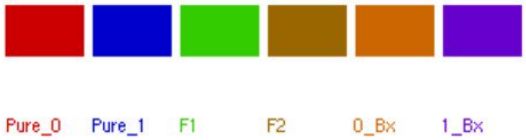


FIGURE 7.
NewHybrids analysis of AFLP data. A randomized simulation of a subset of 20 individuals for each the putative parental and hybrid species were utilized, with *z* options: *Pleioblastus chino* (Pure_0; z = 0; red), *Pleioblastus hindsii* (Pure_1; z = 1; blue), and *Pleioblastus simonii* (F1; no z; green).



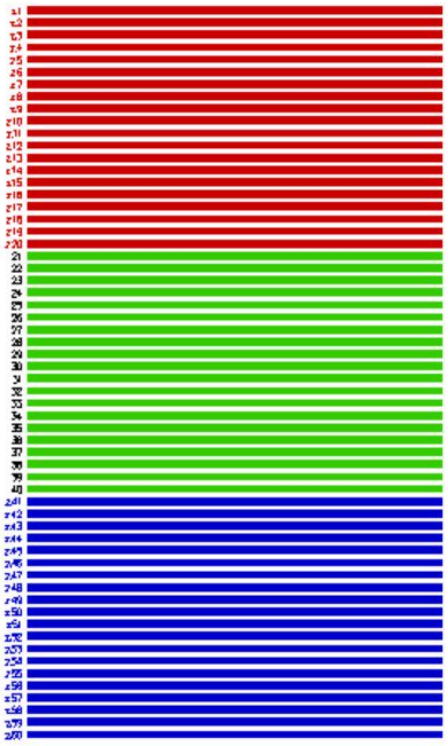


FIGURE 8.
Results of the phylogenetic analysis of nuclear gene *pvcel*1 α, highlighting haplotypes of *Pleioblastus simonii* and an intergeneric hybrid (*Pseudosasa japonica*). Phylogram of the majority rule consensus tree from the Bayesian analysis in MrBayes; Bayesian posterior probabilities ≥ 0.95 are given above the branches, while maximum parsimony (MP) bootstrap values ≥ 70% are given below the branches.

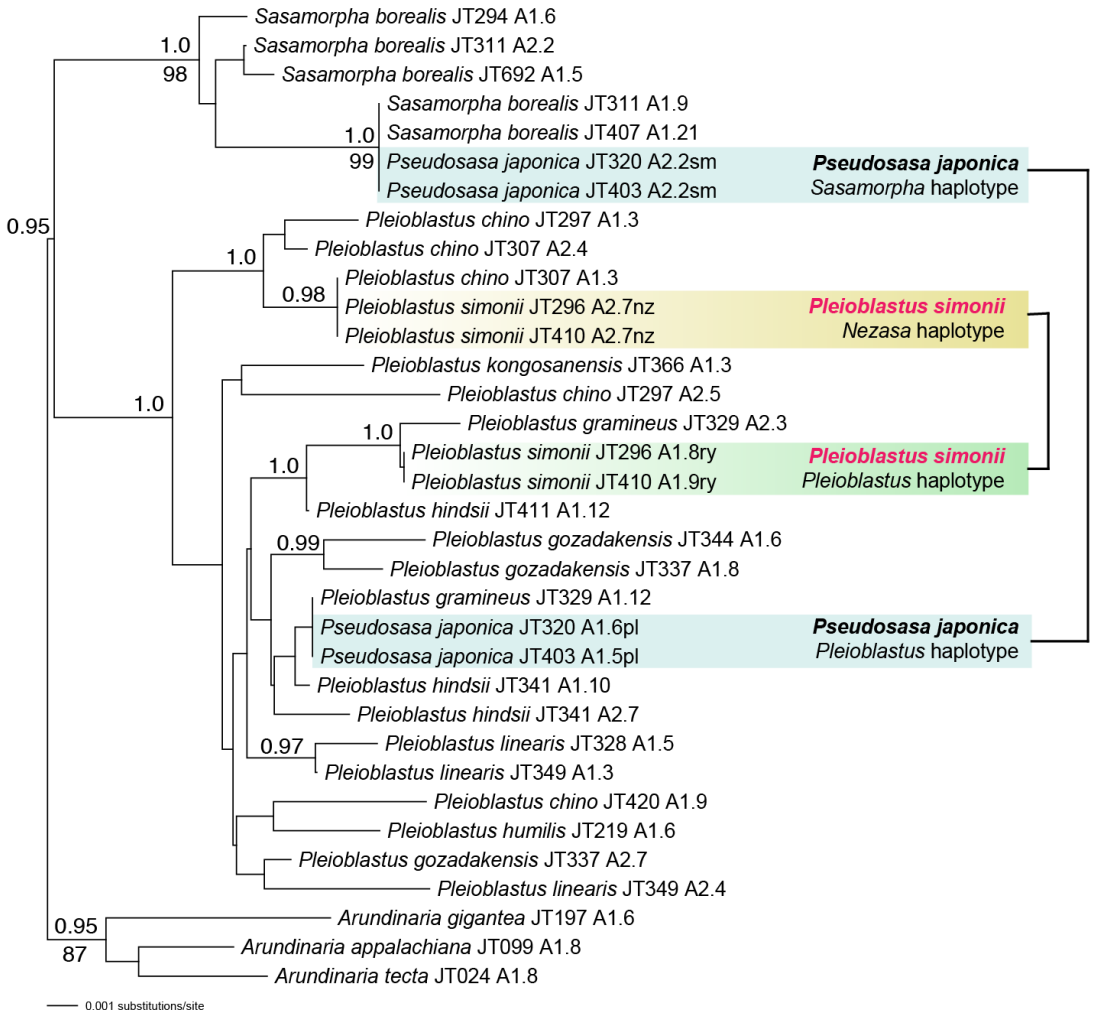


FIGURE 9.
Results of the phylogenetic analysis of the nuclear gene *pvcel*1 β, highlighting haplotypes of *Pleioblastus simonii* and an intergeneric hybrid (*Pseudosasa japonica*). Phylogram of the majority rule consensus tree from the Bayesian analysis; posterior probabilities ≥ 0.95 above branches, maximum parsimony bootstrap values ≥ 70% below branches.