A natural hybrid between *Dendrocalamus pendulus* and *Gigantochloa scortechinii* (Poaceae: Bambusoideae: Bambuseae) in Peninsular Malaysia

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ABSTRACT. A natural inter-generic bamboo hybrid between *Dendrocalamus pendulus* and *Gigantochloa scortechinii* is reported for Peninsular Malaysia. The hybrid has some morphological characteristics of each parent but also shows intermediacy between the parents. We demonstrate the hybridisation using the partial Granule-Bound Starch Synthase (*GBSS*) I gene sequence. The nothogenus × *Gigantocalamus* K.M.Wong and nothospecies × *Gigantocalamus malpenensis* K.M.Wong are proposed for the hybrid. We suggest that chloroplast introgression could have occurred among the parental taxa or their ancestors and that the significance of introgressive hybridisation in the complex taxonomic relationships of woody tropical bamboos in SE Asian Bambuseae has not been fully appreciated.

Keywords. Bambuseae, bamboo, *Dendrocalamus*, × *Gigantocalamus*, *Gigantochloa*, hybridisation, introgression, Malay Peninsula

Introduction: hybridisation in bamboos

Notions of bamboo hybridisation have not had a very long history. In the Malay Peninsula and Java, *Gigantochloa* Kurz shows an interesting diversity that includes many taxa existing or known only in cultivation, which Holttum (1958) postulated could have originated from selection of the products of hybrid swarms in ancient times. The natural distribution of *Gigantochloa* is believed to be centred in the region from Myanmar to northern Malay Peninsula, and the historical migration of peoples from mainland SE Asia southwards could have brought such selected clones to Java and Peninsular Malaysia. More recently, the observations of Muller (1998) on variation among the offspring of a cultivated clump of *Gigantochloa ridleyi* Holttum in his farm at Mount Mirinjo, Queensland, and originating from Bali, provided very good corroboration for Holttum's postulation. At Mount Mirinjo, a single clump of this species flowered, so the seeds were the products of self-fertilisation. Seed set was very poor and seedling mortality, including albino forms, was significant. The confounding diversity in morphology produced among the surviving seedlings and young clumps, most of which had no close resemblance to the mother clump, was reminiscent of the multiple assortments that could be produced in the F2 generation from the selfing of a hybrid (Muller 1998).

Some degree of fertility among different subtropical bamboo taxa has been demonstrated. Zhang & Chen (1980) have reported a successful artificial hybrid, 'Cheng Ma Qing No.1', using the pollen of *Dendrocalamus latiflorus* Munro and *Bambusa pervariabilis* McClure as the seed parent, in experimental crossings. The hybrid was reported to be fast-growing and having hard culms, highly resistant to cold, and developing ornamental pale-yellow stripes on the lower internodes, making it a good cultivar for various uses. It showed intermediacy in morphological characteristics and chromosome number compared to the parental species (Zhang & Chen 1980, Zhang 1985). On the basis of chromosome numbers, morphological similarity to an experimental hybrid *B. textilis* McClure × *D. latiflorus*, and a high degree of pollen sterility, Zhang (1985) also suspected *B. stenoaurita* (W.T.Lin) T.H.Wen to be a natural F1 hybrid of the two species. Indeed, *B. stenoaurita* has proven difficult to classify and has been transferred from one genus to another without clear resolution (Xia et al. 2007, Yang et al. 2010). However, no natural bamboo hybrids in tropical Asia have been clearly demonstrated to date.

Working with temperate bamboos, Muramatsu (1981) suggested that only a weak crossing barrier exists among woody bamboos, which makes both inter-specific and inter-generic hybridisation highly possible. He arrived at this idea based on pollination and germination experiments with *Phyllostachys* Siebold & Zucc., *Pleioblastus* Nakai and *Sasa* Makino & Shibata. The monotypic *Hibanobambusa* Maruy. & H.Okamura was established as a hybrid genus (Maruyama et al. 1979) and proven to have originated from hybridisation between the distantly related *Phyllostachys* and *Sasa* (Takahashi et al. 1994).

Natural hybridisation among American bamboos was first reported by Clark et al. (1989). Their study of three natural hybrids, *Chusquea subtessellata* Hitchc. × *C. amistadensis* L.G.Clark, Davidse & R.P.Ellis, *C. subtessellata* × *C. vulcanalis* (Soderstr. & S.Calderón) L.G.Clark and *C. spencei* Ernst × *C. tessellata* Munro, also showed intermediate morphological and anatomical characteristics of their respective parental species. These hybrids displayed normal meiosis stages and high pollen fertility. Hybridisation was expected to be significant in the evolution of *Chusquea* sect. *Swallenochloa* (McClure) L.G.Clark to which these taxa conform, as some of the species exhibit a nearly continuous flowering habit, which increases the probability of hybridisation. Hybridisation was also suggested as a cause of taxonomic difficulties in the *Arundinaria* complex (McClure 1973) but this has only been investigated genetically when Triplett et al. (2010) demonstrated an F1 natural hybrid between *A. gigantea* (Walter) Muhl. and *A. tecta* (Walter) Muhl. using the Amplified Fragment Length Polymorphisms (AFLP) technique and cpDNA phylogenetic analysis. Furthermore, multiple, reciprocal hybridisation and introgression events were implicated based on the complex mosaic pattern of the genetic composition in the three hybrid individuals. Their complex origin involves not only *A. gigantea* and *A. tecta*, but also *A. appalachiana* Triplett, Weakley & L.G.Clark. It is also noteworthy that not all of these hybrid individuals showed morphological intermediacy (Triplett et al. 2010).

The present study provides molecular evidence for an inter-generic bamboo hybrid that shows a combination of morphological features of its parental species, *Dendrocalamus pendulus* Ridl. and *Gigantochloa scortechinii* Gamble. Considering that allelic heterozygosity could be a strong indication of an F1 hybrid status, we sequenced the *GBSSI* gene of the putative hybrid individuals and their parental species

to demonstrate their relationship. This approach is suitable for the current sampling scale in terms of cost- and time-effectiveness.

Materials and methods

Plant materials

A putative natural hybrid (hereafter referred to as Hybrid Tapah) was collected from among *Dendrocalamus pendulus* and *Gigantochloa scortechinii* clumps along the Tapah-Cameron Highlands road, Peninsular Malaysia, on 28 November 2001. Material raised from a rhizome offset was planted in the Bambusetum, Rimba Ilmu Botanical Garden, University of Malaya. This clone (Fig. 1) flowered in April 2007, i.e., quite soon after it grew to mature size and then died completely in July 2008. Voucher material was deposited with the Herbaria of the University of Malaya, Kuala Lumpur (KLU), Singapore Botanic Gardens (SING) and Iowa State University (ISC), and leaf material dried in silica gel was obtained for molecular studies.

A population of the same putative hybrid encountered in 2009 along the Old Gombak Road, Selangor, Peninsular Malaysia, again sympatric with *D. pendulus* and *G. scortechinii* clumps, was also studied (Fig. 2). Voucher material of two individuals (Hybrid Gombak-1 and Hybrid Gombak-2) was collected and deposited with KLU, detailed morphological observations were made, and leaf material dried in silica gel was also obtained.

Besides these three hybrid accessions, three accessions of leaf material of *D. pendulus* and five accessions of *G. scortechinii* were likewise obtained. Identification followed Wong (1995). Voucher reference numbers and collection localities are shown in Table 1.

DNA extraction and polymerase chain reaction (PCR)

Total DNA was extracted from silica-dried young leaves using Qiagen DNeasy Extraction kits following instructions by the manufacturer. Polymerase chain reaction (PCR) was run using a Perkin Elmer GeneAmp 9600 Thermocycler with the programme set at 2 min at 95.0°C; 30 cycles of 30 a at 94.0 °C, 45 s at annealing temperature, 1 min at 72.0 °C; 5 min at 72.0 °C; hold at 4.0 °C. Annealing temperatures were 59.0 °C for primers Gin (forward) and GBSS (reverse) and 55.0 °C for primers for cpDNA, *rps16-trnQ*, *trnC-rpoB*, *trnH-psbA* and *trnD-T* (Bamboo Phylogeny Group 2005). The DNA markers have been useful in resolving the phylogenetic relationships among some *Dendrocalamus* and *Gigantochloa* taxa in the analyses using both *GBSSI* and *rps16-trnQ* + *trnC-rpoB* + *trnH-psbA* + *trnD-T* (Goh et al. 2010). The PCR reaction mixture contains 1.5 mM MgCl2, 0.5 µM forward and reverse primers each, 0.2 mM of dNTPs, 1× PCR buffer and ~10 ng of DNA samples. PCR products were purified using Promega PCR Clean-up System kits following instructions by the manufacturer.

PCR cloning, haplotype-specific primer design and DNA sequencing

Purified PCR products for the partial *GBSSI* gene of the putative hybrid individuals were ligated into *pDrive* vectors and transformed into EZ competent cells following the instructions of the Qiagen PCR Cloning Plus kit. White colonies were picked to perform colony-PCR using the primers Gin (forward) and GBSS (reverse). Nine to fifteen clones for each hybrid individual were successfully amplified and sequenced.

Fig 1. The putative hybrid *Dendrocalamus pendulus* × *Gigantochloa scortechinii*. **A.** Clump habit. **B.** Culm shoot. **C.** Culm internode characteristics. **D.** Pseudospikelet cluster. Photo credits: M. Sugumaran.

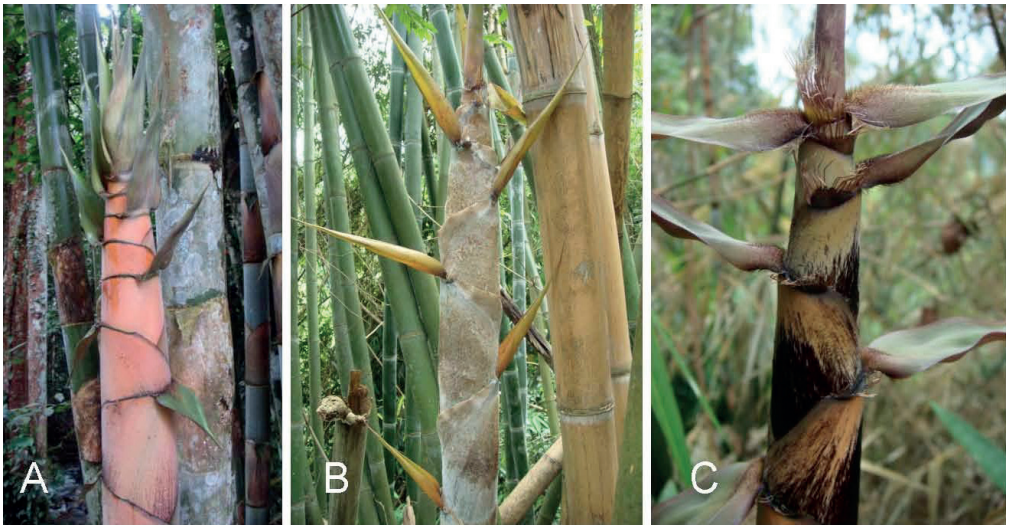

Fig 2. Culm shoots of the two parental species and their putative hybrid from clumps within the hybrid zone along the Gombak road, Selangor, Peninsular Malaysia. **A.** *Gigantochloa scortechinii*. **B.** *Dendrocalamus pendulus*. **C.** Putative hybrid. Photo credits: K.M. Wong.

The sequences of all clones were aligned. Three indel regions and a number of variable sites were observed in the DNA sequences of the clones. As some of the unique nucleotide substitutions could be possibly due to PCR or cloning errors, we designed two sets of internal primers specific for each *GBSSI* haplotype in order to obtain unambiguous DNA sequence for each allele. The location of haplotype-specific primers are shown in Fig. 3. Internal primers Gin336/1 (forward) and Gin336/2 (forward) were designed for the indel region, and Gin396/1 (reverse) and Gin396/2 (reverse) were designed for the region containing three variable sites. Primer sequences are shown in Table 2. PCR was performed using the following primer-pairs: (i) Gin–Gin396/1, (ii) Gin–Gin396/2, (iii) Gin336/1–GBSS, and (iv) Gin336/2–GBSS for each putative hybrid individual. Direct sequencing of purified PCR products was commercially done by FirstBase Laboratory Sdn. Bhd. (Malaysia). For each hybrid accession, sequences generated using the primer-pairs (i) and (iii) were merged as a haplotype, and those generated using the primer-pairs (ii) and (iv) were merged as another haplotype. All the sequences obtained were deposited in GenBank (Table 1).

DNA sequence analysis

Partial *GBSSI* gene sequences of putative hybrid individuals were aligned with those of the putative parental species using ClustalX (Thompson 1997) and manually adjusted using Bioedit v7.0.9 (Hall 1999) where necessary. Variable sites and indels were extracted and tabulated.

Phylogenetic analyses

Sequences representing the putative hybrid, *D. pendulus* and *G. scortechinii* were subjected to maximum parsimony (MP) analysis based on four cpDNA non-coding regions, *rps16-trnQ*, *trnC-rpoB*, *trnH-psbA* and *trnD-T*, and the partial nuclear *GBSSI* region. *Holttumochloa magica* (Ridl.) K.M.Wong and *Kinabaluchloa nebulosa* (Stapf.)

K.M.Wong (GenBank accession numbers given in Table 1) were used as the outgroup because of their sister relationship to the *Bambusa-Dendrocalamus-Gigantochloa* complex (BDG complex; Goh et al. 2010). *Dinochloa malayana* S.Dransfield (Genbank accession number given in Table 1), a sister-taxon of the BDG complex-*H. magica-K. nebulosa* alliance, was also included for a better polarization between the ingroup and outgroup. Maximum parsimony analysis was performed using PAUP 4.0 b10 (Swofford 2002). A strict consensus tree was reconstructed using heuristic

Table 1. List of accessions of *Dendrocalamus pendulus, Gigantochloa scortechinii*, the hybrid, and outgroup taxa, with collection localities and GenBank accession numbers.

Accession	**Collection localities / Literature reference and voucher numbers if available**	**GenBank accession numbers *rps16-trnQ*, *trnC-rpoB*, *trnH-psbA*, *trnD-T, GBSSI***
D. pendulus-1	Gombak Road, Selangor, Peninsular Malaysia	HQ697856, HQ697867, HQ697903, HQ697878, HQ697889
D. pendulus-2	Gombak Road, Selangor, Peninsular Malaysia	HQ697857, HQ697868, HQ697904, HQ697879, HQ697888
D. pendulus-3	Rimba Ilmu Botanical Garden, Univ. of Malaya, Peninsular Malaysia / GWL 6 (KLU)	HQ697855, HQ697866, HQ697902, HQ697877, HQ697890
G. scortechinii-1	Hulu Langat, Selangor, Peninsular Malaysia / GWL 2 (KLU)	HQ697861, HQ697872, HQ697908, HQ697883, HQ697897
G. scortechinii-2	Chebar, Kedah, Peninsular Malaysia	HQ697863, HQ697874, HQ697910, HQ697885, HQ697898
G. scortechinii-3	Gombak Road, Selangor, Peninsular Malaysia	HQ697864, HQ697875, HQ697911, HQ697886, HQ697899
G. scortechinii-4	Road from Kuala Kubu Baru to Fraser Hill, Peninsular Malaysia / GWL 9 (KLU)	HQ697865, HQ697876, HQ697912, HQ697887, HQ697900
G. scortechinii-5	Rimba Ilmu Botanical Garden, Univ. of Malaya, Peninsular Malaysia / Bambusetum Acc. 52	HQ697862, HQ697873, HQ697909, HQ697884, HQ697901
Hybrid Gombak-1	Gombak Road, Selangor, Peninsular Malaysia / GWL 13 (KLU)	HQ697860, HQ697871, HQ697907, HQ697882, (HQ697891, HQ697894)
Hybrid Gombak-2	Gombak Road, Selangor, Peninsular Malaysia / GWL 14 (KLU)	HQ697859, HQ697870, HQ697906, HQ697881, (HQ697892, HQ697895)
Hybrid Tapah	Tapah-Cameron Highlands road, Peninsular Malaysia / WKM 2895 (KLU, SING)	HQ697858, HQ697869, HQ697905, HQ697880, (HQ697893, HQ697896)
Dinochloa malayana (outgroup)	Goh et al. (2010)	FJ416343, GU390924, GU390951, GU390973, GU391005
Holttumochloa magica (outgroup)	Goh et al. (2010)	FJ416348, GU390931, GU391012, GU390958, GU390980
Kinabaluchloa nebulosa (outgroup)	Goh et al. (2010)	FJ416356, GU390932, GU391013, GU390959, GU390981

search with 10 random sequence additions and TBR branch swapping. 'MulTrees' was limited to 10000 trees. Bootstrap analysis was run using 1000 replicates. The potentially informative indels were scored following the Simple Indel Coding (SIC) method (Simmons & Ochoterena 2000).

The best model for the Bayesian Inference (BI) analysis was tested using MrModeltest 2.2 (Nylander 2004). BI analyses were performed in MrBayes 3.1 (Huelsenback & Ronquist 2001), using 2 runs of 4 chains each, and run for 1000000 generations with trees sampled every 100 generations. The first 2500 trees were discarded as burn-in.

Table 2. Sequences of PCR primers for the partial *GBSSI* gene.

Primer	Forward/ Reverse	Sequence (5' - 3')	Source
Gin	Forward	AAG TTT GAG CGC ATG TTC CAG AGC	Goh et al. (2010)
GBSS	Reverse	GGC GAG CGG CGC GAT CCC TCG CC	Mason-Gamer et al. (1998)
Gin336/1	Forward	GTC TTA GTC TTC TCC TTG CAG C	This contribution
Gin336/2	Forward	GTC CTA GTC TTC TTG CAG CTC	This contribution
Gin396/1	Reverse	CAA GAG TAA CGC CAT ATA TG	This contribution
Gin396/2	Reverse	CAA GAG TAA CAC CAT GTA CG	This contribution

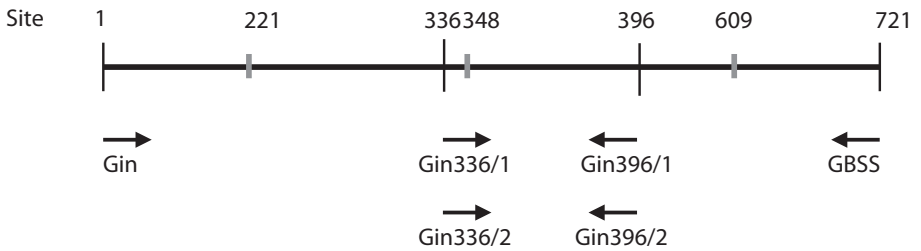

Fig. 3. Schematic diagram showing the position of haplotype-specific primers (site numbers) and indel regions (grey bars) in the partial *GBSSI* gene. Arrows indicate directions of primers.

Results

Morphological characteristics and reproductive behaviour of the hybrid

A comparison of the morphological characters of the hybrid and its parental species is shown in Table 3. As has been noted for many hybrids and hybrid derivatives (Rieseberg 1995), the morphology of the hybrid is a mixture of qualitative characters that match one or the other parental species, e.g., culm leaf auricles with bristles in the hybrid and *Dendrocalamus pendulus* (Fig. 2), and fused staminal filaments in the hybrid and *Gigantochloa scortechinii*, or are intermediate between the parents, e.g., length of the pseudospikelets. During the entire flowering period of Hybrid Tapah and up to a month afterwards, no caryopses were found in spite of careful searches.

Table 3. Some character states of the putative bamboo hybrid. Those intermediate between *Gigantochloa scortechinii* and *Dendrocalamus pendulus*, or resembling one of them, are given in bold.

Character	*Gigantochloa scortechinii*	Hybrid	*Dendrocalamus pendulus*
Culm: habit	Erect with nodding tips	**Erect, with finely drawn out, pendulous apical parts**	Flexuose, leaning on neighbouring plants, with apical parts finely drawn out and whiplike
Culm: internode waxiness	Copiously white-waxy	Not to only slightly white-waxy	Copiously white-waxy
Culm: internode hairiness	Glabrous generally except for bands of silvery brown hairs flanking each node; sparsely covered with pale hairs in juvenile clumps	Generally covered with scattered dark-brown hairs, with bands of silvery brown hairs flanking each node	Glabrous generally with bands of silvery brown hairs flanking each node
Culm leaf: sheath colour	Green at base, flushed intense orange towards the top	**Pale yellow-orange with slight tint of pink or dark purple brown**	Greenish to yellowish pink-orange near apex
Cum leaf: sheath hairs	**Dark brown to black hairs**	**Dark brown hairs**	Loose pale brown hairs
Culm leaf: sheath waxiness	Very slight waxiness	**Slight to moderately white waxy on the back**	Copious loose white wax mixed with the hairs
Culm leaf: sheath margins	Firm, not drying faster than the rest of the sheath	**Papery, drying as a thin marginal zone compared to the rest of the sheath**	**Papery, drying as a thin marginal zone compared to the rest of the sheath**
Culm leaf: auricle form	Low plane rim, 0.5–1.5 mm high, glabrous	**Rounded lobes to about 5 mm high with marginal bristles**	**Small rounded lobes, 1.5–3.0 mm high, sometimes crisped, with marginal bristles**
Culm leaf: blade colour	**Medium green and leaf-like with pink flush**	**Medium green and leaflike with pink flush**	Yellowish green to brown often with pink flush
Midculm dominant branch: habit	Dominant primary branch rigid-ascending,	**Dominant primary branch rigid ascending, tending to extend and droop at its tips**	Dominant primary branch long-flexuous, becoming pendulous-whiplike
Pseudospikelet: length	12–24 mm	**7–11 mm**	5–8 mm
Empty glumes: number	3–5	**2–3**	**2–3**
Florets: number	4–5	**2 (rarely 3)**	1–2
Terminal empty lemma: Presence	Present	**Present (but absent when there is a 3rd floret formed)**	Absent
Lemmas: hairiness	Pale-brown long-hairy all over	**Glabrous**	**Glabrous**
Staminal filaments	**Fused into a tube**	**Fused into a tube**	Free
Anther: colour	Yellow	**Pink to pale lilac**	Maroon

Sequence characteristics of the partial GBSSI gene

The haplotypes of each putative hybrid individual are called Haplotype D and Haplotype G, respectively. Haplotypes D and G were 705–706 bp in length. Multiple DNA sequence alignment of *GBSSI* haplotypes of the hybrid, *D. pendulus* and *G. scortechinii* revealed that 26 out of 35 variable/ indel sites are indicative of the parentage of the hybrid (Table 4).

Maximum parsimony (MP) and Bayesian Inference (BI) analyses

The aligned data matrix of the partial *GBSSI* gene for the ingroup consists of 707 characters, of which 26 are parsimony-informative. MP analysis resulted in four equally most parsimonious trees (shown in Fig. 4). Bayesian analysis using Model K80 generated a similar topology. All five *G. scortechinii* accessions form a clade with the G haplotypes of the hybrid accessions, whereas all three *D. pendulus* accessions form a clade with the D haplotypes of the hybrid accessions (Fig. 4).

The aligned data matrix of the combined cpDNA, *rps16-trnQ* + *trnC-rpoB* + *trnH-psbA* + *trnD-T* dataset for the ingroup consists of 3889 characters, of which 26 are parsimony-informative. MP analysis resulted in four equally most parsimonious trees (shown in Fig. 5). Bayesian analysis using Model HKY+I generated a similar topology. One of the major clades was formed by all three *D. pendulus* accessions, all three hybrid accessions, as well as three *G. scortechinii* accessions. Hybrid Tapah was at the basal node of this clade. The remaining two accessions of *G. scortechinii* form another clade, sister to the other ingroup cluster.

Table 4. The 28 variable sites and 11 indel sites of the partial *GBSSI* gene (722 bp) of the hybrid and its parental species. Dots indicate identical nucleotides compared to those in the first row. Dashes indicate the alignment gaps. Twenty six sites characterising the hybrid origin of the hybrid individuals are highlighted.

Taxon \ Site	1 8	2 8	6 8	1 4	1 9	1 2	1 6	2 2	3 3	4 0	5 0	5 1	5 2	3 6	3 7	3 8	3 9	4 0	4 2	4 8	4 9	5 6	5 7	6 0	6 1	6 2	6 3	6 5	6 6	6 7	6 8	6 9	7 0	7 1	7 2
D. pendulus-1	–	A	G	C	T	A	A	T	A	A	C	–	–	–	C	G	C	G	G	C	A	A	C	G	G	G	G	T	A	T	A	T	T	T	G	A	C	C
D. pendulus-2	A	.	.	.	.	T	.	.	.	.	.	–	–	–	T	.	.	.	.	.	T	.	.	.	.	C	.	.	.	.	.	.	.	C	.	.	.	.	.
D. pendulus-3	A	.	.	.	.	.	.	.	.	.	.	–	–	–	T	.	.	.	.	.	T	.	.	.	.	C	.	.	.	.	.	.	.	C	.	.	.	.	.
Hybrid Gombak-1 (haplotype D)	A	.	.	.	.	.	.	.	.	.	.	–	–	–	T	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.
Hybrid Gombak-2 (haplotype D)	A	.	.	.	.	.	.	.	.	.	.	–	–	–	T	.	.	.	.	.	T	–	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.
Hybrid Tapah (haplotype D)	A	.	.	.	.	T	.	.	.	.	.	–	–	–	T	.	.	.	.	.	T	–	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Hybrid Gombak-1 (haplotype G)	A	G	.	T	.	.	T	C	–	–	T	C	C	T	T	A	A	A	A	T	C	–	.	.	A	.	A	–	–	–	–	G	C	C	C	T	T	A
Hybrid Gombak-2 (haplotype G)	A	G	.	T	.	.	T	C	–	–	T	C	C	T	T	A	A	A	A	T	C	.	.	.	A	.	A	–	–	–	–	G	C	C	C	T	T	A
Hybrid Tapah (haplotype G)	A	G	A	.	.	.	.	C	–	–	T	C	C	T	T	A	A	A	A	T	C	.	.	A	A	.	A	–	–	–	–	G	C	C	C	T	T	A
G. scortechinii-1	A	G	.	.	.	.	T	C	–	–	T	C	C	T	T	A	A	A	A	T	C	.	T	.	A	.	A	–	–	–	–	G	C	C	C	T	T	A
G. scortechinii-2	A	G	.	.	C	.	T	C	–	–	T	C	C	T	T	A	A	A	A	T	C	.	.	.	A	.	A	–	–	–	–	G	C	C	C	T	T	A
G. scortechinii-3	A	G	.	T	.	.	T	C	–	–	T	C	C	T	T	A	A	A	A	T	C	.	.	.	A	.	A	–	–	–	–	G	C	C	C	T	T	A
G. scortechinii-4	A	G	.	T	.	.	T	C	–	–	T	C	C	T	T	A	A	A	A	T	C	.	.	.	A	.	A	–	–	–	–	G	C	C	C	T	T	A
G. scortechinii-5	A	G	.	.	.	.	T	C	–	–	T	C	C	T	T	A	A	A	A	T	C	.	T	.	A	.	A	–	–	–	–	G	C	C	C	T	T	A

Discussion

Indels and nucleotide substitutions observed in the partial *GBSSI* gene sequences of Hybrid Gombak-1, Hybrid Gombak-2 and Hybrid Tapah (Table 4) suggest that haplotype D is derived from *Dendrocalamus pendulus* and haplotype G is derived from *Gigantochloa scortechinii*, as expected. This hypothesis was also supported by the placement of haplotypes D and G in the *GBSSI*-based topology, where haplotypes

D form a single clade with *D. pendulus* and haplotypes G form a single clade with *G. scortechinii* (Fig. 4). From the genotypes of the hybrid and its parental species, the hybrid is reasonably interpreted as a relatively recent F1 offspring.

Assuming cpDNA is maternally inherited in the bamboos, as reported for many angiosperm taxa (Corriveau & Coleman 1988), we attempted to deduce the seed parent of the hybrid from the cpDNA-based topology. However, *D. pendulus* and *G. scortechinii* did not form distinct clades. Rather, one of the two clades consists of *D. pendulus*, *G. scortechinii* and the hybrids, and another clade consists of only *G. scortechinii* (Fig. 5). Similar inter- and intra-specific cpDNA variations have been reported for *Quercus* L. (Whittemore & Schaal 1991, Petit et al. 1997, Bordac et al. 2000, Petit et al. 2008) and such patterns were attributed to interspecific gene flow resulting from introgression (Lexer et al. 2006) or shared polymorphism (Muir & Schlötterer 2005, 2006). Other studies inferring chloroplast capture based on shared cpDNA haplotype patterns include those for Saxifragaceae (Soltis et al. 1991, Okuyama et al. 2005), Pinaceae (Watano et al. 1996, Senjo et al. 1999, Ito et al. 2008), *Phlox* L. (Ferguson et al. 2002), *Salix* L. (Hardig et al. 2000) and *Nothofagus* Blume (Acosta & Premoli 2010). Two of these studies demonstrated that this chloroplast introgression has a strong association with geographic distribution rather than with taxonomic relationships (Whittermore & Schaal 1991, Acosta & Premoli 2010). It is

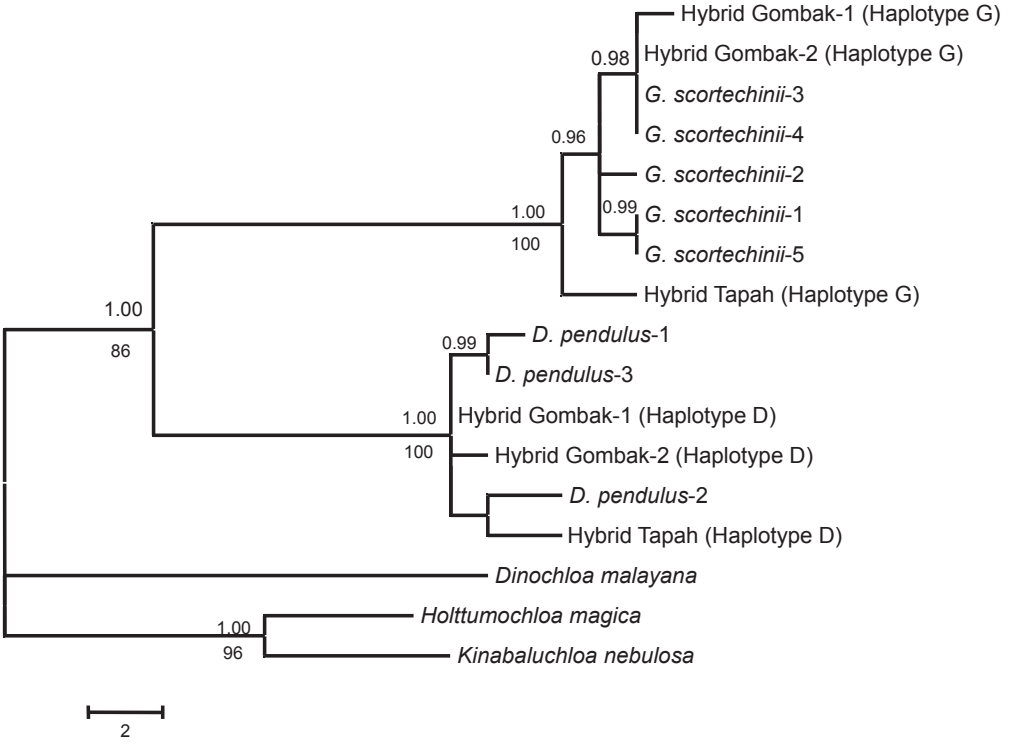

Fig. 4. One of the four most parsimonious trees from the maximum parsimony analysis based on the partial *GBSSI* region (Tree length = 67, CI = 0.9254, RI = 0.9655). Posterior probabilities >0.90 are shown above the nodes, bootstrap support values >70% below the nodes. The tree is drawn to scale, with branch lengths indicating evolutionary distances as number of base substitutions per site.

noteworthy that sharing of chloroplast DNA haplotypes was also observed in two well-defined North American bamboos, *Arundinaria tecta* and *A. appalachiana* (Triplett et al. 2010). Our cpDNA phylogeny is not feasible for determining the direction of the cross but implies that ancient chloroplast introgression has been possible between *D. pendulus* and *G. scortechinii*. However, other possible explanations for the cpDNA phylogenetic tree topology, such as reciprocal crosses followed by introgression, could not be ruled out. Extensive studies including more accessions of hybrid, more populations of *D. pendulus* and *G. scortechinii*, and perhaps more of their congeners in Peninsular Malaysia are much needed to address this problem.

Through this study, we confirm that natural inter-generic hybridisation occurs among Malaysian bamboos and suggest that the role of hybridisation in the evolution of tropical Asian bamboos could have been underestimated. There is circumstantial evidence for natural hybridisation in bamboos but well-documented instances are scarce. Some taxa appear to have a high degree of morphological variation resulting in poorer distinction among species, e.g., the Malayan-Javan *Gigantochloa* taxa (Holttum 1958). A number of taxa are cultivated for their usefulness but have never been found in the wild, e.g., the Malaysian village bamboos *Bambusa heterostachya* (Munro)

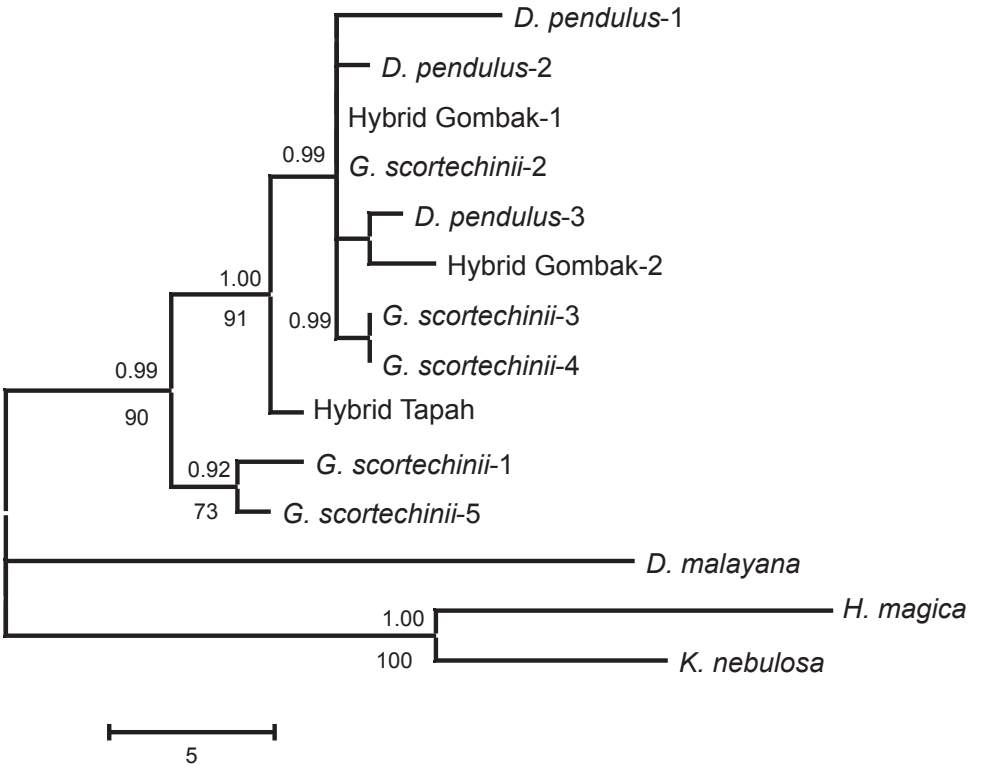

Fig. 5. One of the four most parsimonious trees from the analysis using Bayesian inference based on 4 cpDNA intergenic spacers, *rps16-trnQ*, *trnC-rpoB*, *trnH-psbA*, and *trnD-T* (Tree length = 78, CI = 0.9231, RI = 0.8868). Posterior probabilities >0.90 are shown above the nodes, bootstrap support values >70% below the nodes. The tree is drawn to scale, with branch lengths indicating evolutionary distances as number of base substitutions per site.

Holttum, *B. laxa* K.M.Wong, *D. asper* Backer and *G. thoii* K.M.Wong (Wong 1995, 2004), and many such taxa are infertile or have low fertility (Wong 1995, Ramanayake 1998, Muller 1998, Koshy & Jee 2001), as is the case with our Hybrid Tapah here. In *B. vulgaris* Schrad. (Koshy & Jee 2001), seed set failure is due to poor stigma exposure and pollen sterility resulting from meiotic irregularities; in addition, chromosomal inconstancy (mosaicism) is also found among root-tip (i.e., somatic) cells. Selfing in a reproductively isolated *G. ridleyi* clump had overwhelmingly high seed failure and the offspring that did survive were morphologically inconsistent with one another and the parent clump (Muller 1998). We suggest that introgressive hybridisation is an important source of variability and, in view of the demonstrated possibilities for hybridisation even among genera (Zhang & Chen 1980, Takahashi et al. 1994, and this study), has contributed to taxonomic complexity in the Bambusinae, particularly the closely related *Bambusa-Dendrocalamus-Gigantochloa* complex. A recent phylogenetic study of SE Asian bamboos (Goh et al. 2010) recovered incongruent elements between nuclear and chloroplast sequence-based topologies which implied that the possibility of reticulate evolution in the Bambusinae should be further investigated.

Naming and description of the new hybrid

The detected hybrid has not been previously described or named. It has been produced by hybridisation between *Gigantochloa scortechinii* and *Dendrocalamus pendulus*, both common bamboos in the foothills of the Main Range in Peninsular Malaysia, in at least two localities (Tapah and Hulu Gombak) and seems likely to occur in more localities. Although there has been a suggestion that *Dendrocalamus* and *Gigantochloa* may not be generically distinct (Soderstrom & Ellis 1987), yet currently available analyses using larger data sets (Yang et al. 2008;,Yang et al. 2010, Goh et al. 2010) have not clearly resolved this matter. On the other hand, the type species or type alliances of these genera have been recovered as distinct clades in some analyses (Goh et al. 2010), and from a morphological standpoint, these genera do at least seem to form very different extremes (as represented by their type species and other closely related species) (Holttum 1958, Wong 1995). Wong (1995, 2004) notes that there are species currently placed in *Dendrocalamus* that vary significantly from the type alliance that includes *D. pendulus*.

Here we formally name the nothogenus and nothospecies, according to the International Code of Botanical Nomenclature (McNeill et al. 2006). As the nothogeneric name should be formed from combining elements of the names of both parent genera, and *Dendrochloa* has been pre-empted by *Dendrochloa* C.E.Parkinson, we have opted for the obvious choice, *Gigantocalamus*. The species epithet is an abbreviation of "Malay Peninsula", where this hybrid was first noted.

× ***Gigantocalamus*** K.M.Wong, ***nothogenus nova***
[= *Dendrocalamus* Nees × *Gigantochloa* Kurz]
Bambusa erecta caespitosa, culmi foliorum vagina abaxialiter parum ad moderate pallide cerea, in quoque nodo ramus primarius dominans rigidus adscendens distaliter cernuus, longitudine pseudospicularum inter parentes intermedia, ut videtur seminum absentia sterilis.

× ***Gigantocalamus malpenensis*** K.M.Wong, ***nothospecies nova***
[= *Dendrocalamus pendulus* Ridl. × *Gigantochloa scortechinii* Gamble]
Hybrida naturalis Gigantochloae scortechinii similis, culmi foliorum vaginae marginibus papyraceis, auriculis lobiformibus rotundatis marginibus setosis, lemmatibus glabris differt; Dendrocalami penduli similis, culmi foliorum vagina atro-pilosa, filamentis stamineis tubo connatis differt; in characteribus ceteris inter ambo species intermedia.

TYPE: Peninsular Malaysia, Kuala Lumpur, University of Malaya, Rimba Ilmu Botanic Garden, Bambusetum accession no. 48, 13 Apr 2007 (originally collected from Perak, Tapah-Cameron Highlands road, 28 Nov 2001), *Wong et al. WKM 2895* (holo KLU; iso ISC, SING).

Clumped bamboo; ***culms*** to c. 10 m tall, at first erect, then arching outwards, diameter c. 4 cm, ***internodes*** 20–25 cm at midculm, green except for pale yellow-green stripes at the culm base, with a ring of pale matted hairs above and below each node, dark brown hairs scattered all over, not to only slightly white-waxy. ***Culm leaf sheath*** pale yellow-orange with a slight tint of pink or dark purple-brown, dark brown hairy and slightly to moderately white-waxy on the back; edge of the sheath very thin, drying as a thin papery pale-brown marginal zone; auricles rounded lobes to c. 5 mm high, dark purplish black, with pale stiff wavy bristles to over 10 mm long; ***blade*** medium green and leaflike with a pink flush, spreading to reflexed. ***Branch complement*** developing from a single bud, with one dominant primary central axis and a few smaller higher-order branches from its base, all branches ascending in habit, the dominant primary axis tending to droop at its tip. ***Branch leaf blades*** pale short-hairy on the lower side. ***Pseudospikelets*** 7–11 mm long, developing into clusters of few to many; with (from the base) 2–4 bracts subtending prophyllated buds, 2–3 empty glumes, 2 (exceptionally 3) florets and a terminal empty lemma (this terminal empty lemma sometimes replaced by a third floret). ***Lemmas*** 6–9 mm long, with a short terminal cusp, stiff and scale-like, glabrous, green to pink-flushed; ***paleas*** 5.5–8 mm long, 2-keeled, membraneous, glabrous except minutely hairy keels, 5-veined on the back, 1-veined between keel and margin; ***stamens*** 6, staminal filaments fused into a tube and extruded from the lemma when mature; anthers 3–5 mm long with an apical cusp 0.3–0.5 mm long, flushed pink to pale lilac when fresh, pale when dry; empty; ***ovary*** rounded, c. 0.5 mm long, long-hairy at the summit; ***style*** short-hairy throughout, terminating in a single plumose stigma. ***Caryopsis*** unknown, apparently not or rarely formed. (Fig. 1 A–D, 2C)

Distribution: Peninsular Malaysia, along the Tapah-Cameron Highlands road in Perak (*Wong et al. WKM 2895*) and along the old Gombak road in Selangor (*Goh et al. GWL13*, *14*).

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