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Research Article

Preliminary molecular phylogeny of Bornean *Plagiostachys* (Zingiberaceae) based on DNA sequence data of internal transcribed spacer (ITS)

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ABSTRACT
A molecular phylogenetic analysis based on DNA sequence data of internal transcribed spacer region (ITS 1, ITS 2) and 5.8S gene and re-evaluation of morphological characters were performed in order to examine the relationships of *Plagiostachys* and related genera, and to elucidate the previous informal grouping of Bornean *Plagiostachys*. A total of 111 taxa, including 25 taxa of *Plagiostachys* were included in the analysis. The strict consensus tree (length = 1094; CI = 0.482; HI = 0.518) showed that *Plagiostachys* consisted a strong supported (BS = 96%) clade with some *Alpinia* species that belong to section *Alpinia*. However, species of *Plagiostachys* comprised three subclades (A, B and C) and each subclade was moderately to strongly supported with relatively high bootstrap values. The three subclades of *Plagiostachys* were also recognized morphologically by the combination of inflorescence and capsule characters. Poor resolved tree prevent us to conclude phylogenetic status of the genus *Plagiostachys*, but at the moment we propose this genus remain an independent genus and wait for further analysis. Previous informal grouping of Bornean *Plagiostachys* was not supported from both molecular and morphological analyses.

Keywords: Bornean, phylogeny, *Plagiostachys*, Zingiberaceae

INTRODUCTION

Plagiostachys Ridl., is relatively small but complex genus in Zingiberaceae. It is distributed mainly in the Malesian region with the centre of diversity in Borneo, where currently 10 species of *Plagiostachys* are known. The genus is distinguished from the other genera in Zingiberaceae by the tightly congested, apparently lateral inflorescence, which is, in fact, terminal on the short stem of the leafy shoots, and usually breaks through the leaf sheaths just above ground level or sometimes in the middle (Smith, 1990). The flower is subtended by a usually tubular bracteole, and the labellum is small and rather fleshy, with diverged venation of petaloid in some species. The bracteoles and flowers are very mucilaginous and tend to decay within one day in some species. Such a mucilaginous nature makes herbarium works difficult, therefore relatively few studies have been done in this genus (Smith, 1985; Cowley, 1999; Sakai & Nagamasu, 2003; Gobilik *et al*., 2005). In order to study *Plagiostachys*, collecting sufficient number of spirit specimens of flowers, inflorescences and observations of morphological characters in the field or a living status are vital.

Smith (1985) divided the Bornean *Plagiostachys* into two informal groups, Group 1 and Group 2 (Table 1). Species belonging to Group 1 have bilobed ligule, mucilaginous inflorescence, distinctly tubular and early decaying bracteoles, yellow and white with some pink flowers, fleshy and soon-decaying calyx, style that adnate to corolla wall, and oblong-pyriforms capsules. On the other hand, species of Group 2 have truncate or emarginate ligule, tubular at base or open bracteoles, pinkish-red with yellow labellum flowers, papery calyx, style that free from the corolla wall, and globose capsules. The grouping was, however, disputed by Cowley (1999) and Sakai & Nagamasu (2003) as the characters used for the grouping did not fit with any species described recently. Cowley (1999) mentioned that the characters of the ligule, style and capsule of *P. parva* J. Cowley belonged to Smith's Group 1, whereas characters of the calyx, bracteole and non-mucilaginous nature of the inflorescence belonged to Group 2. Another species, *P. breviramosa* J. Cowley differed from Smith's Group 2 by its adnated style to the corolla tube, non-globose capsule and bilobed ligule. Similarly, the species described recently by Gobilik *et al*. (2005), i.e. *P. lasiophylla* Gobilik & A. L. Lamb and *P. oblanceolata* Gobilik & A. L. Lamb also belong to neither Smith's groups. Although Gobilik *et al*. (2005) placed their two new species in Smith's Group 2 on the basis of the not mucilaginous inflorescences, but the characters of the style, capsule shape and ligule

Table 1: Characters used for the classification of the genus *Plagiostachys* (after Smith, 1985)

Character	Group 1	Group 2
1. Inflorescence	Mucilaginous	Non-mucilaginous
2. Bracteole	Distinctly tubular, early decaying, only the very basal part remaining	Tubular at base or open, partially decaying or persistent at fruiting stage
3. Calyx	Fleshy, at least decaying in upper part	Calyx not fleshy, not decaying
4. Style	Sometimes adnate to the wall of the corolla tube above the epigynous glands	Usually free from the wall of the corolla-tube at the base
5. Capsule	Oblong-pyriform, angled, rarely globose	Globose
6. Ligule	Bilobed	Truncate or emarginate, rarely bilobed
7. Floral colour	Yellow and white with some pink	Pinkish-red with yellow labellum

of these two species belong to Smith's Group 1. Thus, the reliability of Smith's classification became doubtful. Re-evaluation of these morphological characters used for the informal grouping and grouping itself should be needed.

Recent molecular phylogenetic analyses based on DNA sequence data (plastid *matK* and nuclear rDNA ITS) done by Kress *et al*. (2002, 2005 & 2007) and Pedersen (2004) confirmed that *Plagiostachys* and *Alpinia* were sister group. Kress *et al*. (2002, 2005 & 2007) also showed that *Amomum* and *Alpinia* were polyphyletic groups and *Plagiostachys* was embedded within *Alpinia*. *Plagiostachys* was moderately to strongly supported (BS>70%) as a monophyletic group. Unfortunately only two species of the genus were included in their analyses, making the phylogenetic position of *Plagiostachys* within Alpinioideae remained inconclusive.

In this preliminary study, we examined morphological characters of *Plagiostachys* used for classification and analyzed DNA sequences of the internal transcribed spacer (ITS) from an expanded taxon sampling of the genus *Plagiostachys*, together with related genera of the Alpinioideae previously used in the investigation of Kress *et al*. (2005) in order to (1) resolve the position of *Plagiostachys* within Alpinioideae, (2) evaluate the informal grouping within *Plagiostachys* as proposed by Smith (1985) and (3) evaluate the morphological characters used in classification of the genus with respect to our phylogenetic results.

MATERIALS AND METHODS

Morphological Study

Plant collection and observation were mainly made during December 2003 to July 2004, late February to early March and from November to December 2005 in Sabah, Malaysia. These specimens were deposited at BORH, HYO and SAN. Additional materials, i.e. specimens and digital images of specimens of *Plagiostachys* (including type specimens) from BO, E, Fl, G, IBSC, K, NHM and PR were also consulted. Morphological attributes used in Smith's informal grouping were examined for the 17 taxa of *Plagiostachys*: ligule, bracteole, floral colour, calyx, style and capsule.

Taxon Sampling

The sources of plant material with voucher and accession numbers are represented in Table 2. The 19 taxa of *Plagiostachys* and three taxa of *Alpinia* were newly sequenced. The expanded sampling of Bornean *Plagiostachys* is well represented by both Smith's Group 1 and Group 2. In addition, 89 accessions including 58 species of *Alpinia* representing all six major clades of Kress *et al*. (2005) were downloaded from GenBank. In total 111 taxa were used for the analysis. The tribe Riedelieae represented by four genera (*Burbidgea*, *Pleuranthodium*, *Riedelia* and *Siamanthus*) and the *incertae sedis Siliquamomum* were selected as outgroups.

Genomic DNA Extraction, PCR Amplification and Sequencing

Fresh or silica gel dried leaf tissues were used for total DNA extraction using a modified CTAB (hexadecyltrimethyl-ammonium bromide) method (Doyle & Doyle, 1987; Takano & Okada, 2002). The entire ITS1-5.8S-ITS2 region was amplified via polymerase chain reaction (PCR) using ITS5P (5'-GGAAGGAGAAGTCGTAACAAGG-3') and ITS8P (5'-CACGCTTCTCCAGACTACA-3') of Möller & Cronk (1997). The thermal cycling parameters were: initial denaturation at 94°C for 30 seconds, primers annealing at 48°C for 2 minutes, and extension at 72°C for 45 seconds. A final extension at 72°C for 7 minutes was done at the end of the amplification. PCR products were then purified by using High Pure PCR Product Purification kit (Roche Diagnostic

Table 2: List of taxa used in this study with information related to taxonomy, collections, vouchers and GenBank accession number. Data are presented in the following sequence: Taxon name, Voucher (if available), Collection site/ Source of DNA (if available) and GenBank accession numbers for ITS1, 5.8S and ITS2. "¯ " indicates voucher and collection site/ source of DNA is not available.

Outgroup:

Burbidgea nitida Hook.f., J. Mood 96p81, —, AF414494; *B. pauciflora* Valeton, S. Sakai 241 KYO, AB097253; *B. pubescens* Ridl., J. Mood 990, —, AF414495; *B. schizocheila* Hort., Rangsiruji & M. Newman s.n. E, AY769821; *B. stenantha* Ridl., —, —, AJ388308; *Riedelia* sp., —, —, AF478785; *Pleuranthodium floccosum* (Valeton) R. M. Sm., —, —, AY742333; *P. trichocalyx* (Valeton) R. M. Sm., —, —, AY742332; *Siamanthus siliquosus* K. Larsen & J. Mood, Living collection RBGE 20001319 E, Thailand, AY769820; *Siliquamomum tonkinense* Baill., Kress #00-6802 US, —, AF478791; *Renealmia alpinia* (Rottb.) Maas, Kress #99-6407 US, Tropical America, AF478778; *R. batternbergiana* Bak., Kress #94-5277 US, Tropical Africa, AF478779.

Ingroup:

Aframomum luteoalbum K. Schum., A. D. Poulsen 708 AAU, Africa, AF414493; *Afra. verrucosum* J.M. Lock, A. D. Poulsen 771 AAU, Africa, AF414492; *Alpinia aenea* Argent, G. Argent *et al*. 0016 E, Indonesia, AY769833; *Alp. aquatica* (Retz.) Roscoe, —, —, AY742335; *Alp. arctiflora* (F. Muell.) Benth., —, —, AY742336; *Alp. argentea* (B. L. Burtt & R. M. Sm.) R. M. Sm., —, Sumatra, AY742337; *Alp. bilamellata* Makino, —, —, AY742339; *Alp. blepharocalyx* K. Schum., Kress #98-6136 US, AF478709; *Alp. caerulea* Benth., —, —, AY742342; *Alp. calcarata* Roscoe, Kress #94-3675 US, China, AF478710; *Alp. carolinensis* Koidz., Kress #99-6404 US, Micronesia, AF478711; *Alp. conchigera* Griff., Kress #00-6706 US, China, AF478712; *Alp. cylindrocephala* K. Schum., —, —, AY742345; *Alp. elegans* K. Schum., Kress #99-6412 US, Philippines, AF478713; *Alp. eremochlamys* K. Schum., —, —, AY742346; *Alp. fax* B. L. Burtt & R. M. Sm., —, —, AY742348; *Alp. formosana* K. Schum., —, —, AY742350; *Alp. foxworthyi* Ridl., Kress #98-6293 US, Philippines, AF478713; *Alp. galanga* (L.) Willd., Kress #94-5263 US, Ex hort. Hawaii, AF478715; *Alp. guangdongensis* S. J. Chen & Z. Y. Chen, —, —, AY742352; *Alp. haenkei* C. Presl, —, —, AY742354; *Alp. hainanensis* K. Schum., —, —, AY742355; *Alp. hansenii* R. M. Sm., A. Julius 155 BORH, Sabah, DQ507828; *Alp. havilandii* K. Schum., Cultivated, Mountain Garden, K. Park, Sabah, DQ507829, *Alp. hookeriana* Valeton, —, —, AY742356; *Alp. intermedia* Gagnep., Kress #97-5780 US, Japan, AF478716; *Alp. japonica* (Thunb.) Miq., ¯, ¯, AY742358; *Alp. jianganfen* T. L. Wu, —, —, AY188289; *Alp. ligulata* K. Schum., ¯, Borneo, AY742361; *Alp. maclurei* Merr., —, —, AY742362; *Alp. monopleura* K. Schum., —, —, AY742363; *Alp. nanchuanensis* Z. Y. Zhu, —, —, AY188290; *Alp. napoensis* H. Dong & G. J. Xu, —, —, AF254466; *Alp. nigra* (Gaertn.) B. L. Burtt, —, —, AF254459; *Alp. nieuwenhuizii* Valeton, —, Sabah, DQ507830; *Alp. novaepommeraniae* K. Schum., —, —, AY742368; *Alp. nutans* K. Schum., —, —, AY742369; *Alp. officinarum* Hance, Kress #00-6614 US, China, AF478718; *Alp. oxyphylla* Miq., —, —, AY742372; *Alp. pinetorum* Loes. AY742373; *Alp. pinnanensis* T. L. Wu & Senjen Chen, —, —, AF254470; *Alp. polyantha* Fang, —, —, AY745692; *Alp. pricei* Hayata, —, —, AY742374; *Alp. pumila* Hook "f.", Kress #97-6119 US, China, AF478719; *Alp. rosea* Elmer, —, Philippines, AY742377; *Alp. rubricaulis* K. Schum., —, Sumatra, AY742378; *Alp. rugosa* J. -P. Liao ined., —, —, AY742379; *Alp. sibuyanensis* Elmer, —, Philippines, AY742381;

Table 2 continues:

Alp. species #2, —, —, AY742383; *Alp*. species #1, —, —, AY742382; *Alp. stachyoides* Hance, —, —, AY742384; *Alp. strobiliformis* T. L. Wu & Senjen Chen var. *glabra* T. L. Wu, —, —, AF254471; *Alp. suishaensis* Hayata, —, —, AY742385; *Alp. tonkinensis* Gagnep., —, —, AY742386; *Alp. vittata* Bull., Kress #99-6415 US, Polynesia, AF478720; *Alp. warburgii* K. Schum., ¯ , Sumatra, AY742388; *Alp. zerumbet* (Pers.) B. L. Burtt & R. M. Sm., ¯, ¯, AY742389; *A*. aff. *calycodes* Baker, W. Baker 1051 K, Indonesia, AY769834; *Amomum angustipetalum* S. Sakai & Nagam., S. Sakai 389 KYO, Sarawak, AB097245; *Amo. calyptratum* S. Sakai & Nagam., S. Sakai 363 KYO, Sarawak, AB097239; *Amo. dimorphum* M. F. Newman, S. Sakai 372 KYO, Sarawak, AB097244; *Amo. durum* S. Sakai & Nagam., S. Sakai 362 KYO, Sarawak, AB097241; *Amo. oliganthum* K. Schum., S. Sakai 370 KYO, Sarawak, AB097243; *Amo. roseisquamosum* Nagam. & S. Sakai, S. Sakai 188 KYO, Sarawak, AB097246, *Amo. somniculosum* S. Sakai & Nagam., S. Sakai 373 KYO, Sarawak, AB097247; *Amo. villosum* Lour., Kress #01-6978 US, —, AF478724; *Etlingera triorgyalis* (Baker) R. M. Sm., L.B. Pedersen & B. Johans. 1065 C, —, AF414475; *Etl. yunnanensis* (T. L. Wu & Senjen Chen) R. M. Sm., W.J. Kress 95-5511 US, —, AF414468; *Hornstedtia gracilis* R. M. Sm., J. Mood 996, AF414482; *H. hainanensis* T. L. Wu & Senjen Chen, Kress #97-5769 US, —, AF478766; *Leptosolena haenkei* C. Presl, —, —, AY742331; *Plagiostachys albiflora* Ridl. 1, A. Julius & P. Jimbau 2 BORH, Sabah, DQ507835; *P. albiflora* Ridl. 2, A. Julius & A. Takano AT34 BORH, HYO, Sabah, DQ507834; *P. brevicalcarata* Julius & A. Takano, A. Julius & A. Takano AT35 SAN, HYO, Sabah, DQ507839; *P. crocydocalyx* (K. Schum.) B. L. Burtt & R. M. Sm., A. Julius & A. Takano AT1 SAN, HYO, Sabah, DQ507837; *P. glandulosa* S. Sakai & Nagam., S. Sakai 374 KYO, Sarawak, AB097251; *P. lasiophylla* Gobilik & A. L. Lamb, Cultivated. Sandakan, Sepilok, RDC, Evo. Trail, Sabah, DQ507843; *P. longicaudata* Julius & A. Takano, A. Julius & A. Takano AT76 SAN, HYO, Sabah, DQ507832; *P. megacarpa* Julius & A. Takano, A. Julius *et al.* AGS2 SAN, HYO, Sabah, DQ507844; *P. mucida* Holttum, Khaw Siok Hooi 741 E, KEP, Malay Peninsula, AY769841; *P. oblanceolata* Gobilik & A. L. Lamb, A. Julius *et al.* ATW34 BORH, HYO, Sabah, DQ507848; *P. breviramosa* complex, A. Julius & A. Takano AT63 SAN, HYO, Sabah, DQ507842; *P. parva* J. Cowley, AMGB 1 BORH, SNP, Sabah, DQ507840; *P. roseiflora* Julius & A. Takano, A. Julius & A. Takano AT64 SAN, HYO, Sabah, DQ507846; *P. strobilifera* (Baker) Ridl. 1, A. Julius & A. Takano AT61 BORH, Sabah, DQ507833; *P. strobilifera* (Baker) Ridl. 2, ¯, Sabah, DQ507849; *P. strobilifera* (Baker) Ridl. #3, S. Sakai 361 KYO, Sarawak, AB097252; *P. parva* complex, A. Julius & A. Takano AT65 SAN, HYO, Sabah, DQ507841; *P. viridisepala* Julius & A. Takano, A. Julius 198 SAN, HYO, Sabah, DQ507838; *P.* species 3, Cultivated. Sandakan, Sepilok, RDC, Evo. Trail, Sabah, DQ507836; *P. odorata* C. K. Lim, Kress #99-6330 US, Thailand, AF478772; *P.* species #1, Kress #00-6745US, Sabah, AF478773; *P*. aff. *albiflora*, Khaw Siok Hooi 745 KEP & E, Peninsular Malaysia, AY769840 *P.* aff. *megacarpa*, A. Julius *et al.*, ATS2 BORH, Sabah, DQ507845; *P.* aff. *breviramosa* complex, A. Julius & A. Takano AT2 BORH, Sabah, DQ507831.

GmbH, Germany). Automated sequencing was conducted using ABI Prism® BigDyeTM Terminator Ready Cycle Sequencing kits on an Applied Biosystems HITACHI 3100 Genetic Analyzer Automated Sequencer, using both the PCR primers and another two internal primers, ITS2K (5'-GGCACAACTTGCGTTCAAAG-3') (Rangsiruji *et al*., 2000) and ITS3P (5'-GCATCGATGAAGAACGTA-3') (Möller & Cronk, 1997).

Phylogenetic Analysis

Following the published sequence of the 5.8S rDNA gene and the ITS region in *P. glandulosa* (AB097251), sequence boundaries of 5.8S rDNA gene and both ITS1 and ITS2 regions of the 22 taxa yielded in this study were determined. The aligned matrix was then submitted to the GenBank with accession number from DQ507828 to DQ507849. Clustal X version 1.8 (Thompson *et al*., 1997) was used for multiple alignment of complete sequences with default settings. Nucleotide composition and G+C content were analyzed using MEGA Ver.3.0 (Kumar *et al*., 2004). Phylogenetic analysis was performed using PAUP* Version 4.0b8 (Swofford, 2001). Characters were left unordered and equally weighted. Maximum parsimony (MP) analysis of the ITS sequence data was conducted using heuristic search methods (10 Random Addition Replicates) with Tree Bisection Reconnection (TBR) branch swapping to find the most parsimonious trees, COLLAPSE option in effect, MULTREES and steepest descent options were not in effect. In order to evaluate the relative support of the clades, bootstrap analysis was executed using 1000 replicates heuristic with TBR.

RESULTS

Phylogenetic Analysis

Alignment of the sequences yielded a data matrix of 640 total characters, of which 260 were phylogenetically informative. The complete ITS sequences of *Plagiostachys* varied in length from 542 to 574 bp data matrix (without coded gap). The length of ITS1 ranged from 159 to 164 bp with a GC content of 55.6%, the 5.8S ranged from 183 to 186 bp and GC of 50.1%, while that of ITS2 was greater and ranged from 198 to 226 bp with GC content of 58.6%.

The strict consensus of 100 equally parsimonious trees with accompanying bootstrap values were given in Figure 1 (length = 1094; CI = 0.482; RC = 0.389). The homoplasy index (HI) of the data was 0.518. As shown in Figure 2, *Plagiostachys* made a strongly supported clade (BS = 96%) with some *Alpinia* species of Smith's (1990) section *Alpinia* subsections *Cenolophon* and *Paniculatae*. The *Plagiostachys* can be further subdivided into three major subclades (A, B and C) with moderate to strong support (BS > 70%). Subclade A composed of species found only in Borneo and moderately supported with 76% bootstrap value. Three small groups of species can be found within this first subclade: (i) *P. lasiophylla*, *P. megacarpa* Julius & A. Takano, *P*. aff. *megacarpa*, *P. oblanceolata, P. roseiflora* Julius & A. Takano, *Plagiostachys* sp. 3 (BS = 61%), (ii) three replicates of *P. strobilifera* (BS = 64%), and (iii) *P. parva, P. parva* complex and *Plagiostachys* sp. 2 (BS = 91%). Thirteen taxa of *Plagiostachys* formed the moderate-supported subclade B with 72% bootstrap value. Species consists within this second subclade can be found in Borneo, Malay Peninsula and Thailand. Three small groups of species are found in *Plagiostachys* subclade B: i) *P. breviramosa* complex and *P*. aff. *breviramosa* (BS = 78%), ii) *P*. aff. *albiflora* and *Plagiostachys odorata* C. K. Lim (BS=76%), and iii) *P. albiflora* Ridl., *P*. *brevicalcarata* Julius & A. Takano, *P*. *glandulosa* S. Sakai & Nagam., *P. longicaudata* Julius & A. Takano, *P. mucida* Holttum and *Plagiostachys* sp. 4 (BS = 75%). The third well-supported subclade C composed of two species, *P. crocydocalyx* (K. Schum.) B. L. Burtt & R. M. Sm. and *P. viridisepala* Julius & A. Takano, which are only found in Borneo (BS = 99%).

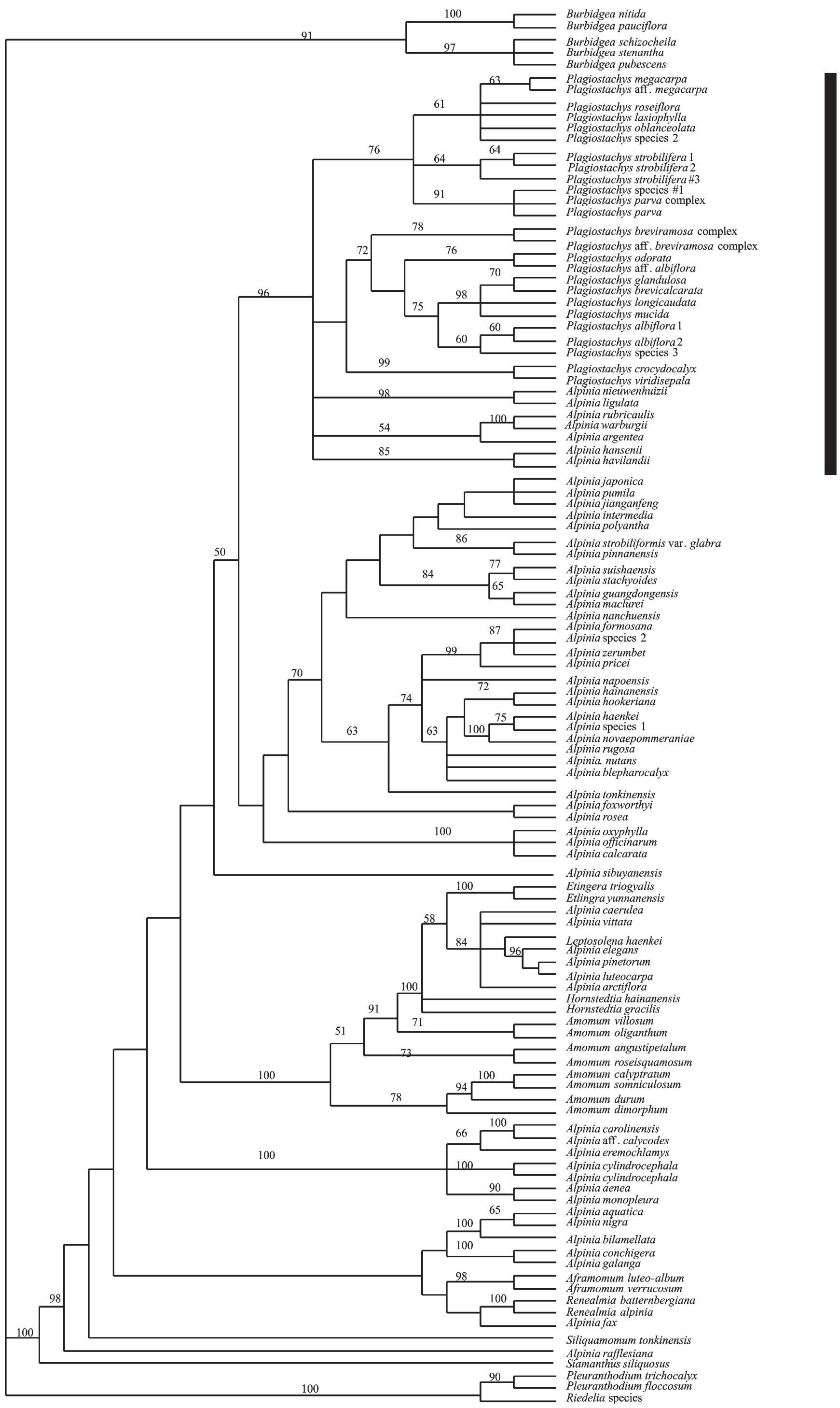

Figure 1: Strict consensus tree of 100 equally parsimonious trees with emphasis on the *Plagiostachys* resulting from the ITS sequence data (length = 1094; CI = 0.483 excluding informative characters; HI=0.517) showing bootstrap values from the parsimony analysis (above the line if $\geq$ 50 %)
(Abbreviation: ITS = Internal Transcribed Spacer; CI = Consistency Index; HI = Heuristic Index)

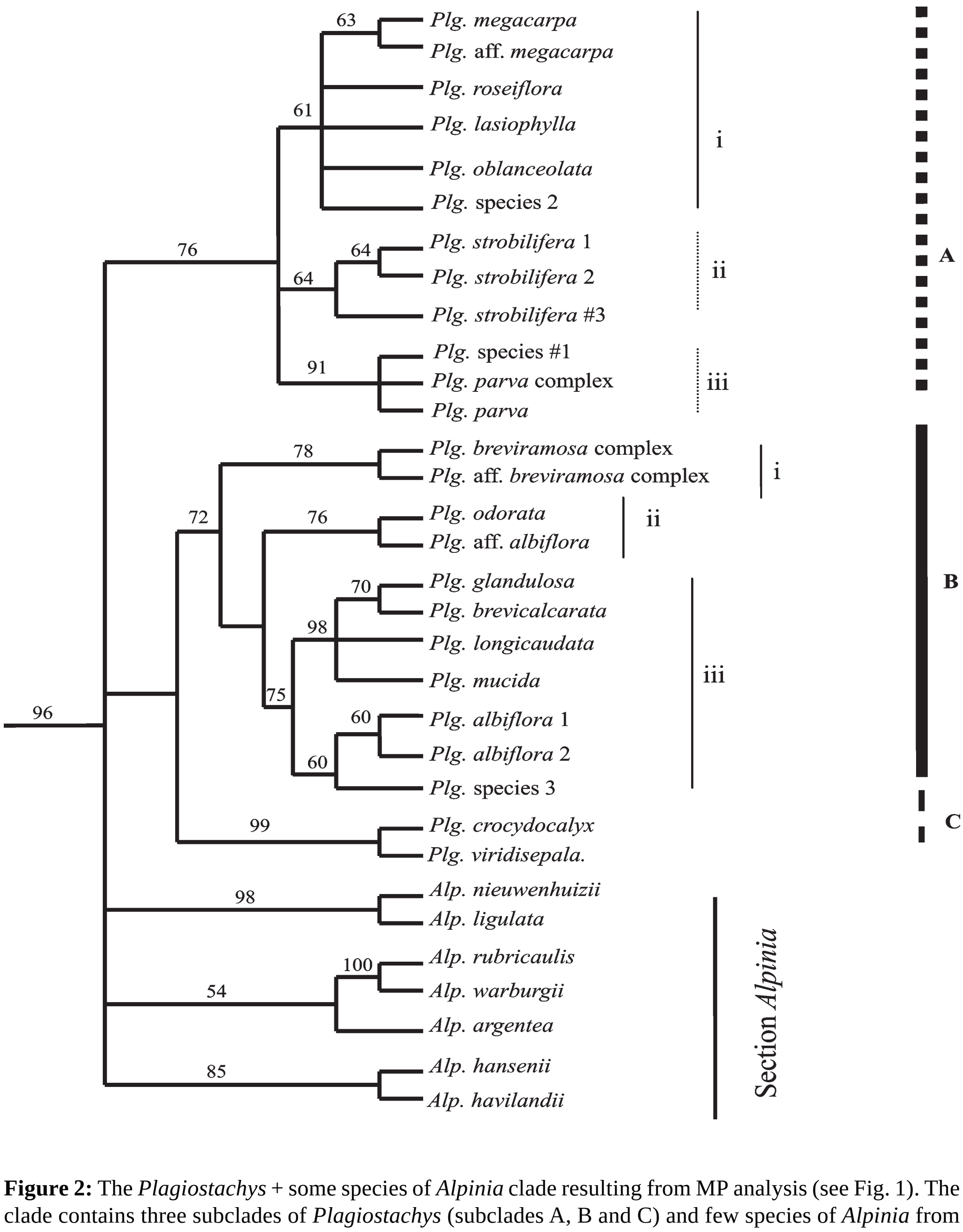

Figure 2: The *Plagiostachys* + some species of *Alpinia* clade resulting from MP analysis (see Fig. 1). The clade contains three subclades of *Plagiostachys* (subclades A, B and C) and few species of *Alpinia* from section *Alpinia*. Bootstrap values for MP is shown above the line (if ≥ 50 %)
(Abbreviation: *Alp. = Alpinia; Plg. = Plagiostachys*)

MORPHOLOGICAL STUDY

The status of vegetative and floral characters examined was summarized in Table 3. In each character, it is stable within species. Only one species, *P. albiflora* matched with Smith's criteria in all morphological status, however, the rest of the species showed mixed character status of groups 1 and 2 as in *P. breviramosa* and *P. parva*.

Figure 3 shows the distribution of morphological character status which was used by Smith (1985) for informal groupings of *Plagiostachys*. As suggested from this study, *Plagiostachys* is subdivided into three major subclades (A, B and C) with moderate to strong support (BS > 70%), and each character used by Smith was scattered into these three subclades: not mucilaginous inflorescence was found in the species of subclade A and part of subclade B, and mucilaginous status was found in part of subclade B and subclade C. Both character status of ligule (bilobed vs. truncate), of bracteole (distinctly tubular and early decaying vs. tubular at base or open and persistent), of style (adnate to vs. free from the corolla wall), and of capsule (oblong-ovoid or pyriform vs. globose) are also found in subclades A and B. Instead, the hairiness of capsules seems to be useful character: all species bearing glabrous capsules were gathered in the subclade B, and species with pubescent capsules were in the subclades A and C. Therefore, the combination of those inflorescence and hairiness of capsules could be used to distinguish the three subclades: the species of subclade A has not mucilaginous inflorescence status and pubescent capsules, those of subclade B show not – or mucilaginous inflorescence but glabrous capsules, and those of subclade C has mucilaginous inflorescence but pubescent capsules.

DISCUSSION

Phylogenetic Position of *Plagiostachys* and its Relationship with Related Genera

The present ITS analysis focused on phylogenetic position of *Plagiostachys* showed that the species of the genus are divided into three major subclades A, B and C with 76%, 72% and 99% bootstrap supports respectively. These subclades consist of strongly supported clade with *Alpinia nieuwenhuizii* Valeton and *A. ligulata* K. Schum. (BS = 98%) + *A. rubricaulis* K. Schum., *A. warburgii* K. Schum. and *A. argentea* B. L. Burtt & R. M. Sm (BS = 54%) + *A. hansenii* R. M. Sm. and *A. havilandii* K. Schum. (BS = 85%). Our result is basically congruent with Kress *et al*. (2002, 2005 & 2007), since most of the members of the clade are that of *A. glabra* clade of Kress *et al*. (2005), together with *A. hansenii* and *A. havilandii*, the newly analyzed taxa. The reason why these two *Alpinia* were added was because both species have lateral inflorescence as in *Plagiostachys*. It is interesting both *Alpinia* also become sisters taxa to *Plagiostachys*, since it might indicate that the event having lateral inflorescence had happened only in the *Plagiostachys* + some species of *Alpinia* clade. However, other *Alpinia* species in this clade have a terminal inflorescence, and as shown in Figure 2, subclades within the *Plagiostachys* + some species of *Alpinia* clade are unresolved, so the phylogenetic relationships among these subclades of *Plagiostachys*'s and *Alpinia*'s remain unknown. Therefore, we could not discuss the evolution of lateral inflorescence further. At least we could mention that the species of *Plagiostachys* and *Alpinia* do not mix up with each other in these subclades. By adding more data such as extending the taxon sampling on both *Plagiostachys* and *Alpinia*, and employing more than one gene markers as others did (e.g. ITS and *trn*L-F by Ngamriabsakul *et al*., 2004; ITS and rps16 by Pedersen, 2004; ITS and *matK* by Xia *et al*.,

Table 3: Comparison of some keys morphological characters for Bornean *Plagiostachys*

Taxa	Characteristics						
	Inflorescence	***Bracteole***	***Calyx***	***Capsule***	***Style***	***Ligule***	***Floral colour***
P. albiflora	Mucilaginous	Distinctly tubular, early decaying, only the very basal part remaining	Fleshy	Pyriform, glabrous	Adnates to the corolla wall for 3 mm above the epigynous glands	Bilobed	Translucent pink, white-yellow labellum with pink venation
P. bracteolata	Non-mucilaginous	Open to the base	Papery	Globose, pubescent	Adnates to the corolla wall for 2 mm above the epigynous glands	Bilobed	Whitish or pale yellow, white-yellow labellum with pink venation
P. brevicalcarata	Mucilaginous	Distinctly tubular, early decaying, only the very basal part remaining	Fleshy	Globose to subglobose, angled, glabrous	Adnates to the corolla wall for 2 mm above the epigynous glands	Bilobed	Translucent-pink with yellow labellum
P. breviramosa	Non-mucilaginous	Distinctly tubular, early decaying	Papery	Pyriform, firm, sometimes ridged, glabrous	Adnates to the corolla wall for 3 mm above the epigynous glands	Bilobed	Translucent with yellow labellum
P. breviramosa complex	Non-mucilaginous	Distinctly tubular, early decaying	Papery	Pyriform, globose, sometimes ridged, glabrous	Adnate to the corolla wall for 1 mm above the epigynous glands	Bilobed	Translucent or translucent-pink with yellow labellum
P. crocydocalyx	Mucilaginous	Distinctly tubular, early decaying, only the very basal part remaining	Fleshy	Globose, pubescent	Free from the corolla wall	Bilobed	White or pale cream with some pink, labellum white-yellow
P. glandulosa	Mucilaginous	Distinctly tubular, early decaying, only the very basal part remaining	Fleshy	Globose, glabrous	Adnate to the corolla wall for 3.5 mm above the epigynous glands	Bilobed	Pink with yellow labellum
P. lasiophylla	Non-mucilaginous	Tubular at base and free above	Papery	Oblong-ovoid, pubescent	Adnate to the corolla wall for 1 mm above the epigynous glands	Bilobed	Pinkish-red

Table 3 Continues:

Species	Characteristics						
	Inflorescence	***Bracteole***	***Calyx***	***Capsule***	***Style***	***Ligule***	***Floral colour***
P. longicaudata	Mucilaginous	Distinctly tubular, early decaying, only the very basal part remaining	Papery	Subglobose to pyriform, glabrous	Adnate to the corolla wall for 1 – 3 mm above the epigynous glands	Bilobed	Translucent-deep purple with yellow labellum
P. megacarpa	Non-mucilaginous	Distinctly tubular, early decaying	Papery	Globose, pubescent	Free from the corolla wall	Bilobed	Pinkish, labellum rose-pink, yellow at tip with pink venation
P. oblanceolata	Non-mucilaginous	Tubular at base and free above	Papery	Globose, ridged, pubescent	Adnate to the corolla wall for 10 mm above the epigynous glands	Bilobed	Pinkish, pinkish labellum with pink venation
P. parva	Non-mucilaginous	Tubular at base, free above	Papery	Oblong-pyriform, pubescent	Adnate to the corolla wall above the epigynous	Bilobed	Pinkish-red, labellum pink-yellow with pink venation
P. parva complex	Non-mucilaginous	Tubular and free above	Papery	Oblong-pyriform, globose, ridged, pubescent	Adnate to the corolla wall for 3 mm above the epigynous glands	Bilobed	Pinkish, labellum pink-yellow with pink venation
P. roseiflora	Non-mucilaginous	Distintcly tubular, early decaying only the very basal part remaining	Papery	Globose, pubescent	Free from the corolla wall	Bilobed	Pinkish, yellowish-pink with pink venation
P. strobilifera	Non-mucilaginous	Tubular at base, free above	Papery	Globose, pubescent	Free from the corolla wall	Truncate o r	Pinkish-red, labellum yellow with some pink
P. strobilifera complex	Non-mucilaginous	Tubular at base and free above	Papery	Globose, glabrescent	Free from the corolla wall	Bilobed	Pink, yellowish-orange pink labellum with pink venation
P. viridisepala	Mucilaginous	Distintcly tubular, early decaying only the very basal part remaining	Fleshy	Subglobose, pubescent	Free from the corolla wall	Bilobed	Yellow with pale yellow labellum

2004), we could obtain more resolved tree and answer how we should treat *Plagiostachys* in the near future. At the moment, the possibility of monophyly of *Plagiostachys* is still remain, therefore we suggest to leave *Plagiostachys* as an independent genus.

Evaluation of Smith's Informal Grouping within Bornean *Plagiostachys*

Our examination of morphological characters used by Smith's informal grouping (Tables 1 & 3) revealed that only one species could completely satisfy her criteria. Additionally, the molecular phylogenetic analysis showed that *Plagiostachys* is divided into three subclades with moderate to strong support and not correspond to previous informal grouping (Fig. 3). Therefore, we conclude that Smith's grouping should no longer be used for Bornean *Plagiostachys* as Cowley (1999) and Sakai & Nagamasu (2003) suggested.

Evaluation of the Morphological Characters Used for Classification in *Plagiostachys*

In molecular phylogenetic analysis, we found three subclades in *Plagiostachys*: subclades A, B and C (Fig. 2), and among morphological characters examined, the status of ligule, bracteole, floral colour and calyx were scattered into these subclades (Fig. 3). This indicates these characters are useful for species recognition but less significant for infrageneric grouping. On the contrary, combination of those inflorescence (mucilaginous vs. not-mucilaginous) and hairiness of capsules (pubescent vs. glabrous) could be used to recognize the three subclades. Smith (1985) used the shape of the capsule to distinguish between groups 1 and 2, but did not mention the hairiness of capsules. However, as inflorescence character, also it could be a useful character for classification.

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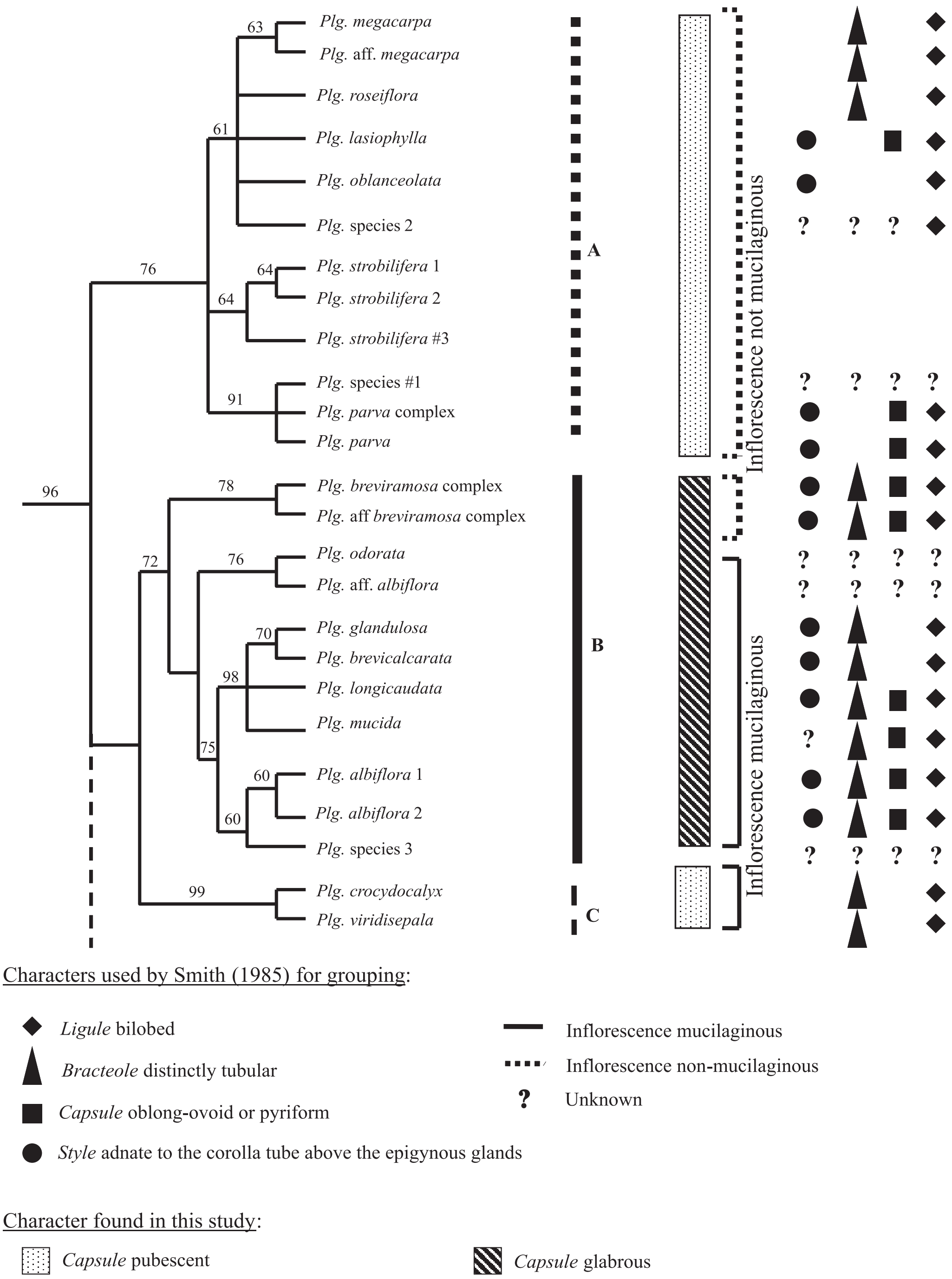

Figure 3: The *Plagiostachys* clade extracted from strict consensus tree showing the distribution of morphological characters previously used by Smith (1985) and character indicated in this study for the grouping of Bornean *Plagiostachys* (Abbreviation: *Alp.* = *Alpinia; Plg.* = *Plagiostachys*)

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