ABSTRACT

This study seeks to better understand the phylogenetic relationships of SE Asian woody bamboos belonging to the Paleotropical alliance of the Bambuseae tribe because, apart from a clearly circumscribed Melocanninae, other subtribes (Bambusinae, Racemobambosinae) are not yet clearly demarcated and a number of genera are of uncertain affiliation. The definition of key Bambusinae genera, such as *Bambusa* (itself with a contentious scheme of subgenera), *Dendrocalamus* and *Gigantochloa* is problematic because of morphological overlaps and it is unclear how these mainly erect-suberect bamboos are related to a suite of SE Asian climbing genera, including *Dinochloa*, *Holttumochloa*, *Kinabaluchloa*, *Maclurochloa*, *Mullerochloa*, *Neololeba*, *Racemobambos*, *Soejatmia*, *Sphaerobambos* and *Temburongia*, many of which have been contentiously placed in *Bambusa* sensu lato in the past. This study utilized DNA sequence data for a nuclear gene region (Granule Bound Starch Synthase, *GBSS*I) and 3 chloroplast regions (*rps16-trnQ, trnC-rpoB* and *trnD-T* intergenic spacers) for 35 species (representing 5 genera) of erect-suberect Bambusinae, 13 species (10 genera) of climbing bamboos, and 3 Melocanninae species. The phylogenetic analyses recovered six major lineages within the ingroup: (1) the BDG complex (*Bambusa*, *Dendrocalamus*, *Gigantochloa*, *Maclurochloa*, *Phuphanochloa*, *Thyrsostachys* and *Soejatmia*), (2) the *Holttumochloa-Kinabaluchloa* clade, which is sister to the BDG complex, (3) the *Dinochloa-Mullerochloa-Neololeba-Sphaerobambos* (DMNS) clade, (4) the *Racemobambos* clade, (5) *Temburongia simplex*, and (6) the Melocanninae clade. Significant incongruence between cpDNA and *GBSS*I topologies indicates widespread reticulate evolution within the BDG complex in the past, which could account for its taxonomic complexities. This is corroborated by demonstration of an existing natural hybridization between *Dendrocalamus pendulus* and *Gigantochloa scortechinii*, where *GBSS*I haplotypes of the hybrid were highly similar to the species-specific *GBSS*I sequences of the parental species. The extensive introgressive hybridization in the BDG complex was not detected among the other climbing bamboo lineages, suggesting that the BDG complex and the climbing bamboo lineages are of different subtribes. Overall, this study suggests that the Bambusinae should be delimited by only the BDG complex and the climbing bamboo lineages are distinct from the Bambusinae. Equivocal relationships among the DMNS clade, the *Racemobambos* clade and *T. simplex* do not permit clear subtribal recognition for these three clades. The Bambusinae is centred in mainland SE Asia, with decreasing representation towards, and a solitary representative in, Australia. In contrast, the present-day geographical distribution of the DMNS clade suggests that this clade could be of Australasian origin, and the subsequent diversification within this clade could have occurred with dispersal towards SE Asia. Other details of the phylogenetic results variously indicate vicariance across the S China Sea (*Kinabaluchloa* spp.) and the New Guinea–Sulawesi–Borneo–S Philippines pathway as a dispersal corridor between Australia and SE Asia (DMNS clade). These results, together with findings from other published studies, contribute important indications that past introgression, reticulate evolution and ploidy increases could have been a significant feature in the development of the several woody bamboo lineages.

ABSTRAK

Kajian ini bertujuan untuk memahami dengan lebih mendalam hubungan filogenetik bagi buluh *Paleotropical* di Asia Tenggara yang tergolong dalam *tribe* Bambuseae. Ini adalah kerana selain daripada Melocanninae, *subtribe* yang lain (Bambusinae dan Racemobambosinae) masih belum ditentukan sempadannya dan hubungan antara genus-genus juga masih tidak dapat dipastikan. Definisi genus-genus utama bagi Bambusinae, iaitu seperti *Bambusa* (yang mana ia sendiri mempunyai skim subgenus yang meragukan), *Dendrocalamus* dan *Gigantochloa* adalah bermasalah kerana ciri-ciri morfologi mereka yang bertindih. Selain daripada itu, masih tidak dapat dipastikan bagaimana buluh-buluh yang kebanyakannya bersifat menegak ini mempunyai hubungan dengan sekumpulan genus-genus buluh yang bersifat memanjat, termasuklah *Dinochloa*, *Holttumochloa*, *Kinabaluchloa*, *Maclurochloa*, *Mullerochloa*, *Neololeba*, *Racemobambos*, *Soejatmia*, *Sphaerobambos* and *Temburongia*, yang mana kebanyakannya (secara masih dalam kontroversi) pernah diklasifikasikan sebagai *Bambusa sensu lato* sebelum ini. Kajian ini menggunakan data jujukan DNA bagi satu bahagian gen nuklear (*Granule Bound Starch Synthase*, *GBSS*I) dan 3 bahagian kloroplas (*rps16-trnQ*, *trnC-rpoB* and *trnD-T intergenic spacers*) untuk 35 spesies (yang mewakili 5 genus) buluh bersifat menegak dari Bambusinae, 13 spesies (mewakili 10 genus) dari buluh bersifat memanjat, dan 3 spesies dari Melocanninae. Analisa filogenetik menemukan enam keturunan utama di dalam kumpulan yang dianalisis: (1) kompleks BDG (*Bambusa*, *Dendrocalamus*, *Gigantochloa*, *Maclurochloa*, *Phuphanochloa*, *Thyrsostachys* dan *Soejatmia*), (2) *Holttumochloa-Kinabaluchloa*, (3) *Dinochloa-Mullerochloa-Neololeba-Sphaerobambos* (DMNS), (4) *Racemobambos*, (5) *Temburongia simplex*, dan (6) Melocanninae. Percanggahan yang signifikan di antara pokok filogenetik cpDNA dan *GBSS*I menunjukkan bahawa kompleks BDG pernah mengalami evolusi retikulasi yang meluas, yang mana proses ini mungkin adalah penyebab kepada kerumitan taksonomi kompleks tersebut. Keputusan ini juga disokong dengan kewujudan kacukan semula jadi di antara *Dendrocalamus pendulus* dan *Gigantochloa scortechinii*, di mana jujukan-jujukan *GBSS*I hibrid adalah sangat serupa dengan jujukan *GBSS*I yang spesifik kepada spesies induknya. Kacukan *introgressive* yang meluas dalam kompleks BDG tidak dapat dikesan dalam keturunan buluh memanjat yang lain, maka ini menyokong cadangan bahawa kompleks BDG dan keturunan buluh memanjat adalah dari *subtribe* yang berlainan. Secara keseluruhannya, kajian ini menunjukkan bahawa Bambusinae sepatutnya dibatasi dengan kompleks BDG sahaja dan buluh-buluh keturunan memanjat adalah berbeza daripada Bambusinae. Hubungan filogenetik antara keturunan DMNS, *Racemobambos* dan *T. simplex* yang meragukan menyebabkan *subtribe* mereka belum dapat dikenalpasti. Taburan buluh-buluh Bambusinae adalah berpusat in tanah besar Asia Tenggara dan perwakilannya semakin berkurangan ke arah Australia (hanya satu spesies sahaja terdapat di Australia). Sebaliknya, taburan geografi semasa bagi keturunan DMNS membayangkan bahawa kumpulan buluh ini mungkin berasal dari Australasia dan disebarkan ke arah Asia Tenggara. Butir-butir lain dalam keputusan filogenetik kajian ini mencadangkan proses *vicariance* yang merentasi Laut Cina Selatan (ditunjukkan oleh *Kinabaluchloa* spp.) dan laluan New Guinea–Sulawesi–Borneo–S Philippines sebagai koridor penyebaran di antara Australia dan Asia Tenggara (ditunjukkan oleh keturunan DMNS). Keputusan-keputusan kajian ini, bersama dengan keputusan kajian-kajian lain yang telah diterbitkan, menyumbang bukti-bukti penting bahawa *introgression* yang lalu, evolusi retikulasi dan peningkatan ploidi adalah ciri-ciri yang signifikan dalam perkembangan evolusi bagi beberapa keturunan buluh.

ACKNOWLEDGMENTS

I would like to express my deepest gratitude to my supervisor, Dr. Wong Khoon Meng, who has given me this tremendous opportunity to study the bamboos and guided me through this research. I am also very much indebted to Dr. Chandran Somasundram, my co-supervisor, who supervised me on the molecular aspects of this project.

This work would have been impossible without kind help of many institutions and individuals who helped make available the diverse array of samples used: Forest Research Centre, Sabah; Rimba Ilmu Botanical Garden, University of Malaya; South China Botanical Garden; South West Forestry College, Kunming; the Kunming World Expo; David Bowman, Nicholas Hogarth, Prof. Yuji Isagi, Dr. Shingo Kaneko, Lin Rushun, Y.W. Low, Janna Nilsson, Dr. Joan T. Pereira, Dr. M. Sugumaran, Dr. Yang Han Qi, Ye Jing, Dr. Yong Kien Thai, Tran Van Tien and Zheng Chaohan.

My special thanks go to Dr. Koichi Kamiya (Ehime University, Japan), who generously taught me the methods to analyze my data and helped me in shaping my ideas about hybridization in bamboos. I also thank Dr. Lynn G. Clark (Iowa State University), Dr. Amanda E. Fisher (Rancho Santa Ana Botanic Garden), Dr. Donald C. Franklin (Charles Darwin University), Dr. Scot A. Kelchner (Idaho University), Dr. K.C.Koshy (Tropical Botanical Garden and Research Institute, Kerala), Dr. Sarawood Sungkaew (Kasetsart University) and Prof. Xia Nian He (South China Botanical Garden) for their valuable comments and constructive suggestions on this work.

I thank members from the Postharvest Biotechnology Laboratory for being a wonderful team during my research. Kit Yew Sen established the molecular cloning work station, Daniel Lee Kian Poh and Loo Zhang Xin assisted me in some molecular work, Arina Arshad and Punitha Vasanthan kept me accompanied when I worked after office hour, and N. Doraisamy, Hue Seow Mun, Wijenthiran Kunasekaran, Rebecca Ow Phui San, Zuliana Razali and Nadiah Saruan, have also helped me in various ways. Weekly lunch chats with Dr. Tung Heng Fung, one of the advisors of the Postharvest Biotechnology Lab, have always been insightful.

I was financially supported by a Postgraduate Fellowship of the University of Malaya from year 2009 to 2011. This work received fundings from the University of Malaya research grants, FS275/2008C, PS151/2008B and PS293/2009C, as well as the H.M.Burkill Research Fellowship 2012 awarded by the Singapore Botanic Gardens.

This work is dedicated to my beloved family for their continuous support and trust in me in completing my studies.

List of Abbreviations

µl	=	microliter
µM	=	micromolar
DNA	=	deoxyribonucleic acid
dNTP	=	deoxy-N-triphosphate
e.g.	=	for example
et al.	=	et alia (and others)
i.e.	=	that is
km	=	kilometer
$MgCl_2$	=	magnesium chloride
mM	=	mili-molar
Mt.	=	Mount
MYA	=	million years ago
N	=	North
NW	=	Northwest
S	=	South
s.n.	=	sine numero (without number)
SE	=	Southeast
sp.	=	species
SW	=	Southwest
syn.	=	synomym
viz.	=	namely

List of Appendices

List of Figures

List of Plates

List of Tables

List of Taxonomic Authorities

Tribes or subtribes

Arundinarieae Nees ex Ascherson & Graebner
Bambusinae J.S.Presl.
Hickeliinae Dendragrostis Nees von Esenbeck ex Munro
Melocanninae Bentham
Olyreae Kunth ex Spenner
Racemobambosinae Stapleton

Genera

Ampelocalamus S.L.Chen, T.H.Wen & G.Y.Yang
Bambusa Schreber
Bonia Balansa
Brachystachyum Keng
Cephalostachyum Munro
Chusquea Kunth
Cyrtochloa S.Dransf.
Dendrocalamus Nees
Dinochloa Büse
Fimbribambusa Widjaja
× *Gigantocalamus* K.M.Wong
Gigantochloa Kurz
Greslania Balansa
× *Hibanobambusa* Maruy. & H.Okamura
Hickelia A. Camus
Holttumochloa K.M.Wong
Kinabaluchloa K.M.Wong
Leptocanna L.C.Chia & H.L.Fung
Maclurochloa K.M.Wong
Melocalamus Benth.
Melocanna Trinius
Mullerochloa K.M.Wong
Neololeba Widjaja
Neomicrocalamus P.C. Keng
Neosinocalamus Keng f.
Oreobambos K.Schum,
Oxytenanthera Munro
Phuphanochloa Sungkaew & Teerawat.
Phyllostachys Torr.
Pleioblastus Nakai
Pseudostachyum Munro
Sasa Makino & Shibata
Sasaella Makino

Schizostachyum Nees
Sellulocalamus W.T.Lin
Semiarundinaria Makino
Soejatmia K.M.Wong
Sphaerobambos S. Dransf.
Temburongia S.Dransf. & K.M.Wong
Temochloa S. Dransf.
Vietnamosasa Nguyen,

Species

Arundinaria appalachiana Triplett, Weakly & L.G.Clark
A. gigantea Nutt.
A. tecta Muhl.
Bambusa arnhemica F.Muell.
B. balcooa Roxb.
B. bambos (L.) Voss
B. blumeana J.A. & J.H.Schultes
B. boniopsis McClure
B. burmanica Gamble
B. cerosissima McClure
B. chungii McClure
B. distegia (Keng & P.C.Keng) L.C.Chia & H.L.Fung
B. eutuldoides var. viridivittatta (W.T.Lin) L.C.Chia
B. farinacea K.M.Wong
B. flexuosa Munro
B. gibba McClure
B. grandis (Q.H.Dai & X.L.Tao) Ohrnb.
B. griffithiana Munro
B. heterostachya (Munro) Holttum
B. intermedia Hsueh & T.P.Yi
B. laxa K.M.Wong
B. multiplex (Lour.) Raeuschel ex J.A. & J.J.Schultes
B. oldhamii Munro
B. pervariabilis McClure
B. polymorpha Munro
B. remotiflora Kuntze
B. sinospinosa McClure
B. stenoaurita (W.T.Lin) T.H.Wen
B. surrecta (Q.H.Dai) Ohrnb.
B. textilis McClure
B. tulda Roxb.
B. tuldoides Munro
B. valida (Q.H.Dai) W.T.Lin
B. ventricosa McClure
B. vulgaris Schrad. ex Wendl.

Chimonobambusa quadrangularis (Fenzi) Makino
Dendrocalamus asper (Schult.) Backer ex Heyne
D. dumosus (Ridl.) Holttum
D. elegans (Ridl.) Holttum
D. farinosus (Keng & Keng f.) L.C.Chia & H.L.Fung
D. giganteus Munro
D. hirtellus Ridl.
D. jianshuiensis Hsueh & D.Z.Li
D. khoonmengii Sungkaew, Teerawat. & Hodk.
D. latiflorus Munro
D. longispathus (Kurz) Kurz
D. membranaceus Munro
D. ovatus N.H.Xia & L.C.Chia
D. peculiaris Hsueh & D.Z.Li
D. pendulus Ridl.
D. semiscandens Hsueh & D.Z.Li
D. sinuatus (Gamble) Holttum
D. strictus (Roxb.) Nees
D. tomentosus Hsueh & D.Z.Li
D. tsiangii (McClure) L.C.Chia & H.L.Fung
Dinochloa malayana S.Dransf.
D. scabrida S.Dransf.
Dinochloa trichogona S.Dransf.
× *Gigantocalamus malpenensis* K.M.Wong
Gigantochloa achmadii Widjaja
G. albopilosa K.M.Wong
G. albovestita (Holttum) K.M.Wong
G. apus (J.A. & J.H. Schultes) Kurz
G. atroviolacea Widjaja
G. atter (Hassk.) Kurz
G. balui K.M.Wong
G. hasskarliana (Kurz) Backer ex Heyne
G. holttumiana K.M.Wong
G. latifolia Ridl.
G. ligulata Gamble
G. manggong Widjaja
G. nigrocilliata Kurz
G. pruriens Widjaja
G. ridleyi Holttum
G. rostrata K.M.Wong
G. scortechinii Gamble
G. thoii K.M.Wong
G. wrayi Gamble
Guadua cf. chacoensis (Rojas) Londoño & Peterson
Holttumochloa korbuensis K.M.Wong
H. magica (Ridl.) K.M.Wong

H. pubescens K.M.Wong
× *Hibanobambusa tranquillans*
Kinabaluchloa nebulosa (Stapf) K.M.Wong
K. wrayi (Stapf) K.M.Wong
Maclurochloa montana (Ridl.) K.M.Wong
M. baccifera (Roxb.) Kurz
Mullerochloa moreheadiana (F.M. Bailey) K.M.Wong
N. affinis (Rendle) P.C.Keng
Neololeba amahussana
Neololeba atra (Lindl.) Widjaja
Phuphanochloa speciosa Sungkaew & Teerawat
Racemobambos gibbsiae (Stapf.) Holttum
R. prainii (Gamble) Keng f. & T.H.Wen
R. hepburnii S. Dransfield
R. setifera Holttum
Sasa senanensis Rehder
S. veitchii Rehder
Sasaella bitchuensis (Makino) Koidz.
S. ramosa Makino
Schizostachyum aciculare Gamble
S. brachycladum Kurz
S. gracile (Munro) Holttum
S. grande Ridl.
S. iraten Steud.
S. jaculans Holttum
S. latifolium Gamble
S. lengguanii K.M.Wong
S. terminale Holttum
S. zollingeri Steud.
Soejatmia ridleyi (Gamble) K.M.Wong
Sphaerobambos hirsuta S.Dransf.
S. philippinensis (Gamble) S.Dransf.
S. subtilis S.Dransf.
Temburongia simplex S. Dransf. & K.M.Wong
Thyrsostachys siamensis Gamble
Vietnamosasa cilliata (A.Camus) T.Q.Nguyen
Yushania niitakayamensis (Hayata) Keng f.
Y. tessellata (Holttum) S.Dransf.

TABLE OF CONTENTS