A new *Haniffia* species (Zingiberaceae) and a new generic record from Sarawak, Malaysian Borneo

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### Abstract

**Background:** *Haniffia* Holttum is a genus of three described species of terrestrial gingers hitherto restricted to Peninsular Thailand and various localities in Peninsular Malaysia.

**Results:** With generic placement confirmed using nrITS, *trn*K and *mat*K plastid sequence data, *Haniffia santubongensis* S.Y. Wong & P.C. Boyce is described as a taxonomically novel species representing a new generic record for Borneo, to where it is endemic to Mount Santubong, Kuching Division, NW Sarawak, Malaysian Borneo. An identification key to all species is given and *H. santubongensis* is illustrated from living plants.

**Conclusion:** *Haniffia santubongensis* is the fourth species of *Haniffia* so far described, and the first occurring on sandstone.

**Keywords:** *Haniffia santubongensis*; Mount Santubong; Phylogeny; Taxonomy

### Background

*Haniffia* Holttum is a genus of three described species of terrestrial gingers hitherto restricted to Peninsular Thailand and various localities in Peninsular Malaysia. The three described species are all seemingly locally endemic. The type species, *H. cyanescens* (Ridl.) Holttum, is restricted to Bukit Tanga (Negeri Sembilan, Peninsular Malaysia), with a variety, *H. cyanescens* var. *penangiana* C.K. Lim, occurring on Pulau Pinang and Kedah. The most recently recognized species, *H. flavescent* Y.Y. Sam & Julius (Sam et al. 2009) is known only from Endau Rompin National Park (Johor, Peninsular Malaysia). The sole extra-Malaysian species, *H. albiflora* K. Larsen & Mood, is confirmed only from Nam Tok Chatwarin, Naratiwat, Thailand. A summary of the taxonomic history of *Haniffia* Holttum is presented by Larsen and Mood (2000).

### DNA extraction, amplification and sequencing

Genomic DNA was extracted using a modified CTAB protocol. ITS, *trn*K intron and *mat*K gene were amplified using the same set of primers as in Leong-Škorničková et al. (2011). PCR products were purified using GenJet PCR purification kit (Thermo Scientific, Vilnius, Lithuania) and sent for sequencing in forward and reverse directions at First BASE Laboratories Sdn. Bhd., Selangor, Malaysia. Sequences were edited, assembled and aligned using MUSCLE (Edgar 2004) as implemented in Geneious Pro v5.6.4 (Biomatters Ltd., Auckland, New Zealand; www.geneious.com; Drummond et al. 2012). Two newly generated sequences were deposited into GenBank under accession numbers KJ452785 (*trn*K/*mat*K) and KJ452784 (ITS), and combined with sequences included in Leong-Škorničková et al. (2011). When the placement of the new sequences was confirmed to fall within the *Kaempferia* Clade, then the final data matrix was reduced to include all the species in the *Kaempferia* Clade with *Cautleya gracilis* (Sm.) Dandy and *Roscoea cautleoides* Gagnep. selected as outgroups. Table 1 shows the list of species included for the final data matrix. The data matrix was deposited into TreeBASE (reviewer access URL: http://purl.org/phylo/treebase/phylows/study/TB2:S15361?x-access-code=f78126f9da891d3c6999dd52dafa8f77&format=html).

### Methods

**Plant material**

Fresh leaf material of *Haniffia santubongensis* was collected from the type locality, Mount Santubong. The type specimen with the spirit material was deposited to SAR.
Table 1 List of species included in this study with vouchers (herbarium location) and GenBank accession numbers for DNA sequences used in the phylogenetic analyses

| Taxon                               | Voucher               | trnK (including matK) | ITS          |
|-------------------------------------|-----------------------|-----------------------|--------------|
| Haniffia albiflora K. Larsen & Mood | Kress #99-6370 (US)   | AF47885               | AF478756     |
| Haniffia cyanescens (Ridl.) Holttum | Julius et al. FR56069 | JF82553               | JF825533     |
| Haniffia flavescens Y.Y. Sam & Julius| Julius et al. FR57598 | JF82553               | JF825534     |
| Haniffia santubongensis S.Y. Wong & P.C. Boyce| P.C. Boyce & S.Y. Wong ZI22 | KJ452785 | KJ452784 |
| Newmania orthostachys N.S. Lý & Skorničk. | Lý 470 (VNM, E, P, SING) | JF825540 | JF825537 |
| Newmania serpens N.S. Lý & Škorničk. | Lý 332 (VNM, E, P, SING) | JF82541 | JF825536 |

Phylogenetic analyses
Phylogenetic analyses were performed with PAUP*4.0b10 (Swofford 2002) for maximum parsimony (MP) reconstruction with all characters equally weighted. The most parsimonious trees were obtained with heuristic searches of 1,000 replicates with random stepwise sequences addition, tree bisection-reconnection (TBR) branch swapping, collapse of zero-length branches, with the multiple-tree option in effect, and saving up to 10,000 trees from each random sequence addition.

The most suitable nucleotide substitution model for each of the gene regions was selected in jModeltest ver. 0.1.1 (Posada 2008) using Akaike information criterion (AIC). General time reversible (GTR + I + G) was the nucleotide substitution model selected. Maximum likelihood (ML) analyses were carried out using RAxML 7.2.6 (Stamatakis et al. 2008). Maximum likelihood bootstrap values were obtained by running 10,000 replicates. Bayesian phylogenetic analyses were performed with MrBayes ver. 3.1.2 (Huelsenbeck and Ronquist 2001). Markov chain Monte Carlo (MCMC) was repeated twice to assure parameter convergence. The MCMC algorithm was run for 2,000,000 generations with one cold and three heated chains, starting from random trees and sampling one out of every 100 generations. Convergence was assessed by using the standard deviation of split frequencies as convergence index with values < 0.005 interpreted as indicating good convergence. The first 10% of trees were discarded as burn-in. Remaining trees were used to construct 50% majority-rule consensus trees.

Results and discussion
Morphology and biogeography
In overall appearance H. santubongensis is most similar to H. cyanescens, sharing with that species a wide bluish labellum and semi-glossy fruits. However, H. santubongensis is clearly distinct from H. cyanescens by the lateral staminodes with an oblique bifid tip, the labellum distally notched (not deeply divided), and differences in labellum colouration and patterning (most notably the presence of a median yellow callus in H. santubongensis).

Haniffia santubongensis represents a new generic record for Borneo where it is locally endemic to Gunung Santubong. Combined with the three locally endemic species in Peninsular Malaysia and P. Thailand, it provides further compelling evidence that these Haniffia species, along with numerous other examples in families as diverse as the aroids, the palms, Rubiaceae Juss., and the genus Hanguana Blume, represent relictual fragments of the Riau Pocket phytochore (Ashton 2005; Corner 1960).

Molecular analyses
The combined ITS-plastid dataset contained the new species and 19 species in the Kaempferia Clade recognized in Leong-Škorničková et al. (2011) together with two outgroup species. The dataset comprises 2,115 characters: 1,682 characters were constant, 251 variable, but parsimony uninformative, and 182 (8.6%) were parsimony informative. The data analyses produced 32 shortest trees of length 633 steps with a Consistency Index = 0.7615 and Retention Index = 0.7729. The tree topologies obtained from three analyses are similar and consistent with the results from Leong-Škorničková et al. (2011). Figure 1 shows the partial tree obtained from maximum likelihood analysis. The new species is shown to be a sister taxon to H. albiflora and H. flavescens. Haniffia cyanescens is supported basally to the three Haniffia species. The new species of Haniffia differs by nine nucleotide substitutions (two in trnK intron and seven in ITS), an addition of 17 bps (trnK intron), an eight bp substitution (ITS), and seven bp deletion (ITS).

Taxonomic treatment
Key to Haniffia species

1a. Corolla lobes and staminodes pale yellow; labellum pale yellow with golden yellow median band, apex emarginate. . . . . . . . . . . H. flavescens
1b. Corolla lobes and staminodes white; labellum white, white with purple veins or dark blue-violet; apex bilobed. . . . . . . . . . 2
2a. Leaf blade 10–147×72–3.5 cm; ligule with long white hairs; flowers 2–5 in each inflorescence. S Thailand. . . . . . . . . H. albiflora
2b. Leaf blade 17–21 × 3–4.9 cm; ligule glabrous or sparsely pubescent; flowers 5–7 in each inflorescence. Malaysia. . . . . 3
3a. Lateral staminodes bifid at apex; labellum distally notched, with a central yellow median band. NW Borneo. . . . . . H. santubongensis
3b. Lateral staminodes not bifid; labellum deeply split, without a central yellow median band. Peninsular Malaysia. . . . . . 4
4a. Labellum dark blue-violet with white veins; lateral staminodes obovate to oblanceolate. . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . system
Seeds ellipsoid-ovoid, 6–7 × 3–4 mm, glossy brown, turning greyish-black, aril thick, white when fresh.

Ecology
Partially shaded, deep sandy peat podzols of ridge kerangas in Dryobalanops-dominated hill forest; ca 200–250 m asl.

Distribution
Haniffia santubongensis is known only from the type locality where it occurs as two separate, dense, populations.

Etymology
The species epithet is derived from the name of the type locality.

Conclusion
Haniffia santubongensis is the fourth species of Haniffia and represents a new generic record for Borneo.

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
SYW and IHO carried out the molecular genetic studies, participated in the sequence alignment and data analyses. SYW and PCB dealt with the species description. SYW drafted the manuscript. All authors read and approved the final manuscript.

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References

Ashton PS (2005) Lambir’s forest: The world’s most diverse known tree assemblage? In: Roubik DW, Sakai S, Hamid Karim AA (eds) Pollination Ecology and The Rain Forest: Sarawak Studies. Springer, New York, pp 191–216
Corner EJH (1960) The Malayan flora. In: Purchon RD (ed) Proceedings of the Centenary and Bicentenary Congress of Biology. University of Singapore, Singapore, pp 21–24
Drummond AJ, Ashton B, Buxton S, Cheung M, Cooper A, Duran C, Heled J, Kearse M, Markowitz S, Moir R, Stones-Havas S, Sturrock S, Swidan F, Thierer T, Wilson A (2012) Geneious v5.6. Available from http://www.geneious.com
Edgar RC (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research 32:1792–1797
Huelsenbeck JP, Ronquist F (2001) MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17:754–755
Larsen K, Mood J (2000) Revision of the genus Haniffia (Zingiberaceae). Nord J Bot 20:285–289
Leong-Škorníková J, Lý N-S, Poulsen AD, Tosh J, Forrest A (2011) Newmanizia: a new ginger genus from central Vietnam. Taxon 60:1386–1396
Posada D (2008) jModelTest: phylogenetic model averaging. Mol Biol Evol 25:1258–1266
Sam YY, Julius A, Chew MY (2009) Haniffia flavescens (Zingiberaceae): a new species from Peninsular Malaysia. Bot Stud 50:359–364
Stamatakis A, Hoover P, Rougemont J (2008) A rapid bootstrap algorithm for the RAXML web-servers. Syst Biol 57:770–771
Swofford DL (2002) PAUP*: Phylogenetic Analysis Using Parsimony (* and other methods) Version 4.0 Beta 10. Sinauer Associates, Sunderland, doi:10.1186/s40529-014-0051-9

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