

## Phylogenetic study of the *Hottarum* Group (Araceae: Schismatoglottideae) utilising the nuclear ITS region

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**ABSTRACT.** Recent phylogenetic analyses of the tribe Schismatoglottideae (Araceae) elucidated a well-supported but internally unresolved crown group comprising *Schismatoglottis sarikeensis* (Bogner & M.Hotta) A.Hay & Bogner, previously placed in the genus *Hottarum* Bogner & Nicolson, the genus *Phymatarum* M.Hotta, and a number of species either novel or hitherto placed in *Schismatoglottis* Zoll. & Moritzi. The clade is particularly interesting in that it is centred in northern central Sarawak (Malaysian Borneo), north of the Lupar Divide and appears to represent an autochthonous radiation point for evolutionary activity isolated from the major tribal radiations in south-western Sarawak. Former *Hottarum* species (with the exclusion of *H. truncatum*) transferred to *Piptospatha* and *Schismatoglottis* are misplaced. All except *Bakoa lucens* (Bogner) P.C.Boyce & S.Y.Wong belong to this supra-Lupar Divide grouping. This study was undertaken to test the validity and phylogeny of the genus *Hottarum* utilising the nuclear ITS region.

**Keywords.** *Hottarum*, ITS region, Lupar Divide, phylogeny, *Phymatarum*, *Schismatoglottis*

### Introduction

The genus *Hottarum* Bogner & Nicolson previously comprised of four rheophytic species, all endemic to Borneo (Mayo et al. 1997). These include the type species, *Hottarum truncatum* (Bogner 1978), from Sg. (river) Kakus, Tatau, Bintulu, Sarawak, and a further three species: *H. lucens* (Bogner 1983), *H. sarikeense* (Bogner & Hotta 1983) and *H. kinabaluense* (Bogner 1984). *Hottarum brevipedunculatum* (Okada & Mori 2000) was subsequently described.

In dismantling *Hottarum*, Bogner & Hay (2000) placed the constituent taxa of *Hottarum* (i.e., including the type) into *Piptospatha*, and *H. sarikeense* was placed into *Schismatoglottis*, based purely on morphological characters. *Piptospatha sensu* Bogner & Hay (2000) differs from *Schismatoglottis* by its unconstricted spathe and in having seeds with an extended micropylar appendage. *Piptospatha sensu* Wong & Boyce (2010b) is further defined from *Schismatoglottis* by the peduncle erect at fruit dispersal, with the persistent lower fruiting spathe forming a funnel-form splash cup,

and pistils connate into a syncarpium (or rarely free but coherent).

Phylogenetic analyses of the tribe Schismatoglottideae (Wong et al. 2010), with a well-supported molecular profile and compelling morphological peculiarities, supported the removal of *H. lucens* to a new genus, *Bakoa* (= *Bakoa lucens*; Wong & Boyce 2010a) and *H. kinabaluense* to the generically novel *Ooia* (= *Ooia kinabaluensis*; Wong & Boyce 2010b). Recently, another former *Hottarum* species, *H. brevipedunculatum* was shown to represent a second species of *Bakoa* (*B. brevipedunculata*; Wong 2011). These transfers leave a core of species, including the nomenclatural type (*Hottarum truncatum*), *Hottarum sarikeense*, *Schismatoglottis josefii* A.Hay, and three undescribed species in a *Phymatarum* + *Hottarum* clade.

## Materials and methods

### *Sampling*

Twenty taxa were selected. Nine taxa formed the ingroup, including the type of *Hottarum*, *H. truncatum*; *H. sarikeense* (two accessions), *Schismatoglottis josefii*, and its putative sister taxa, *Phymatarum borneense* (two accessions), and three unplaced taxa (*Schismatoglottis* sp. A [AR-114], *S.* sp. B [AR-135] and *S.* 'petradoxia' [AR-920]). Eleven outgroup taxa were selected based on the results from Wong et al. (2010), and comprise *Schismatoglottis* (2 species), *Aridarum* (7 species) and *Bucephalandra* (2 accessions). Appendix A lists all the taxa and its respective localities, together with voucher information and GenBank accession numbers. Vouchers are deposited with the Herbarium of the Sarawak Forestry Department (SAR).

### *DNA extraction, PCR and sequencing*

Total DNA was extracted using a modified version of the 2X CTAB protocol (Doyle & Doyle 1987) with the addition of PVP (PolyVinylPyrrolidone) as described by Gauthier et al. (2008). ITS1 (Internal Transcribed Spacer 1) and ITS 2 were amplified using the primer pairs 1F/1R and 3F/4R, respectively (White et al. 1990). Polymerase chain reactions (PCRs) were conducted in a total reaction volume of 20 µl comprising 1X buffer, 0.1mM dNTP mix, 0.2mM of each primer, 2.0mM MgCl<sub>2</sub>, 2 units Taq DNA polymerase and 2 µl of DNA extract). 1 µl of DMSO was added to each reaction for improved amplification.

PCR conditions included an initial 2-min denaturation at 95°C, 40 cycles of 1-min at 95°C (denaturation), 1-min at 50°C-60°C (annealing), and 2-min at 72°C (extension), followed by a final 10-min extension at 72°C. PCR products were visualised on 1.5% or 2.0% agarose gels. Desired products were purified and sent for sequencing.

### *Sequence alignment and phylogenetic analyses*

All sequences obtained were manually checked, edited, assembled and aligned using the BioEdit version 7.0.5 (Hall 1999). Gaps were treated as insertions or deletions of nucleotides (indels).

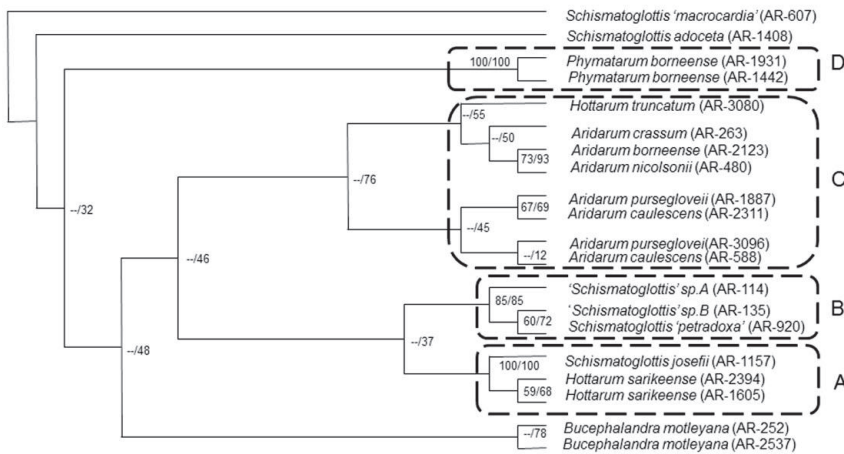
Maximum parsimony (MP) analyses were performed using PAUP\* v.4.0b10 (Swofford 2002) according to the parameters described by Wong et al. (2010), except that 100,000 trees were saved at the second round of tree bisection-reconnection (TBR) branch swapping. Tree topologies were interpreted with bootstrap values generated from RAxML (Randomized Axelerated Maximum Likelihood; Stamatakis et al. 2008) for 100 replicates and repeated 10 times to generate 1000 replicates. Bootstrap support values were taken as weak (50–74%), moderate (75–84%) or strong (85–100%) as applied by Richardson et al. (2000).

## Results and discussion

### *Analysis of the ITS region*

Total aligned nucleotides for the ITS region for 20 taxa comprise 879 bp. The sequence length varies from 770 bp (*Aridarum crassum*, AR-1605) to 803 bp (*Phymatarum borneense*, AR-1931). A large deletion of indels (46 bp) was found in the type, *Hottarum truncatum* (AR-3080) at the position 159 to 204. Indels were found less beyond the position 500 bp for all 20 taxa. The ITS region was rich with the GC nucleotide. All characters are of the type ‘unord’ and have equal weight. 771 characters from the entire sequences are constant and 58 variables characters (6.6%) are parsimony-uninformative. The remaining 50 characters (5.7%) were parsimony-informative, resulting in 29 most parsimonious trees with a tree length of 136 steps, consistency index (CI) of 0.85 and a retention index (RI) of 0.82. These trees also generated a rescaled consistency index (RC) of 0.69 and homoplasy index (HI) of 0.15. CI and HI with the exclusion of uninformative characters were 0.72 and 0.28, respectively.

The tree topology of the maximum parsimony (MP) 50% majority rule (not shown) differed in the *Bucephalandra* + *Hottarum truncatum* (AR-3080) and the *Aridarum* clades formed, as compared with the maximum likelihood (ML) tree (Fig. 1) from RAxML. The bootstrap values (BS) for the MP tree were generated and stopped at 411 replicates due to computing limitations. Both MP and ML trees strongly support the previous phylogenetic study by Wong et al. (2010). *Schismatoglottis josefii* together with *H. sarikeense* formed a monophyletic clade (Clade A in Fig. 1) with parsimony bootstrap value,  $BS_{PA}$  and likelihood bootstrap value,  $BS_{ML}$  of 100%. Clade A is weakly associated with Clade B (37%  $BS_{ML}$ ). Clade B itself is well supported (85%) and comprises the three unplaced taxa (*Schismatoglottis* sp. A, *Schismatoglottis* sp. B and *Schismatoglottis* ‘*petradoxa*’). The *Schismatoglottis* sp. B groups with *Schismatoglottis* ‘*petradoxa*’ with weak  $BS_{PA}$  and  $BS_{ML}$  of 60% and 72%, respectively. *Phymatarum borneense* (Clade D) is recovered as a monophyletic genus ( $BS_{PA}/BS_{ML}=100\%$ ). This genus is sister to the rest of the taxa including clusters containing *Hottarum truncatum* (type species of *Hottarum*) + *Aridarum* (Clade C). *Hottarum truncatum* (AR-3080) received low likelihood bootstrap support for its association with the *Aridarum* species ( $BS_{ML}=55\%$ ) in Clade C. As this is considered insignificant, the *Hottarum truncatum* + *Aridarum* clusters are taken as remaining unresolved.



**Fig. 1.** The maximum likelihood tree obtained from RAxML with ITS sequences. Numbers next to branches are values for  $BS_{PA}/BS_{ML}$ .  $BS_{PA}$  = bootstrap value for maximum parsimony.  $BS_{ML}$  = bootstrap value for maximum likelihood.

## Conclusion

*Hottarum truncatum* (= *Piptospatha truncata*, the type species for *Hottarum*), is shown to be separated from *H. sarikeense* in this study. The rest of the species formerly placed in *Hottarum* have been shown to be misplaced as well. The former *Hottarum* species: *H. lucens* (= *Piptospatha lucens*) and *H. brevipedunculatum* (= *Piptospatha brevipedunculata*) were transferred to a new genus, *Bakoa* P.C.Boyce & S.Y.Wong (Boyce & Wong 2008, Wong 2011) and *Hottarum kinabaluense* (= *P. kinabaluensis*) was transferred to a novel genus, *Ooia* (Wong & Boyce 2010b).

Although *H. truncatum* is sister to *Aridarum*, the weak likelihood bootstrap values do not support the placement of *Hottarum truncatum* within *Aridarum*. A group comprising *Schismatoglottis josefii* and *H. sarikeense*, and another group of 3 novel species from central Sarawak, are well supported clades (with 100% and 85% support, respectively). However, their generic assignment awaits the results of analyses with additional gene regions.

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**Appendix A.** Taxa included in the study: GenBank accession numbers for sequences used, voucher specimen numbers and collection localities. '*Schismatoglottis*' = unplaced and undescribed taxa; '*macrocardia*' and '*petradoxa*' = nomenclatural taxa yet to be described; taxa are arranged alphabetically following the taxonomic position of Mayo et al. (1997), Bogner & Hay (2000), Hay & Yuzammi (2000) and Wong et al. (2010).

| Species                        | GenBank accession no. | Voucher number | Collection locality / coordinates  |
|--------------------------------|-----------------------|----------------|--|
| <i>Aridarum borneense</i>      | JN544438              | AR-2123        | Sungai Bungen, Kubah National Park, Matang, Kuching, Sarawak, 01°36'30.9"N 110°11'35.0"E                   |
| <i>Aridarum caulescens</i>     | JN544440              | AR-2311        | Melinau Gorge, Mulu National Park, Nanga Medamit, Limbang, Sarawak, GPS position not available.            |
| <i>Aridarum caulescens</i>     | JN544428              | AR-588         | Bukit Satiam, Bintulu, Sarawak, 02°58'47.6"N 112°56'37.5"E   |
| <i>Aridarum crassum</i>        | JN544426              | AR-263         | Gunung Gaharu, Pantu, Sri Aman, Sarawak, 01°01'19.5"N 110°52'52.8"E  |
| <i>Aridarum nicolsonii</i>     | JN544427              | AR-480         | Trail above Camp Permai, Santubong, Kuching, Sarawak, 01°45'49.0"N 110°19'07.4"E                           |
| <i>Aridarum purseglovei</i>    | JN544435              | AR-1887        | Bukit Satiam, Bintulu, Sarawak, 02°59'13.3"N 112°55'57.5"E   |
| <i>Aridarum purseglovei</i>    | JN544444              | AR-3096        | Sg. Likau, GT Plantations, Tatau, Bintulu, Sarawak, 02°43'53.6"N 113°25'19.1"E                             |
| <i>Bucephalandra motleyana</i> | JN544425              | AR-252         | Gunung Gaharu, Pantu, Sri Aman, Sarawak, 01°02'39.5"N 110°53'18.3"E  |
| <i>Bucephalandra motleyana</i> | JN544442              | AR-2537        | Bukit Kelam, Sintang, Kalimantan Barat, Indonesia, 00°05'30.1"N 111°39'03.3"E                              |
| <i>Hottarum sarikeense</i>     | JN177489              | AR-2394        | Sg. Pedali, Lubok Antu, Sri Aman, Sarawak, 01°11'58.9"N 112°03'27.0"E                                      |
| <i>Hottarum sarikeense</i>     | JN544434              | AR-1605        | Sg. Lepong, Sarikei, Sarawak, 01°57'12.9"N 111°30'34.9"E   |
| <i>Hottarum truncatum</i>      | JN544443              | AR-3080        | Sg. Pandan Kecil, Trail behind Camp C, GT Plantations, Tatau, Bintulu, Sarawak, 02°42'40.1"N 113°20'37.9"E |



|                                       |          |             |   |
|---------------------------------------|----------|-------------|---|
| <i>Phymatarum borneense</i>           | JN544433 | AR-1442     | Belaga Road, Sebauh, Bintulu, Sarawak,<br>03°03'34.3"N 113°42'16.4"E  |
| <i>Phymatarum borneense</i>           | JN544436 | AR-1931     | Trail to Deer Cave, Mulu National Park,<br>Mulu, Miri, Sarawak, 04°02'23.8"N<br>114°48'54.6"E                   |
| <i>Schismatoglottis adoceta</i>       | JN544432 | AR-<br>1408 | Road junction, km 10 Bakun- Bintulu-<br>Miri, Kapit, Sarawak, 02°50'51.7"N<br>114°01'57.6"E                     |
| <i>Schismatoglottis josefii</i>       | JN544431 | AR-1157     | Sg. Pedali, Nanga Sumpa, Batang<br>Ai, Lubok Antu, Sri Aman, Sarawak,<br>01°11'58.9"N 112°03'27.0"E             |
| <i>Schismatoglottis 'macrocardia'</i> | JN544429 | AR-607      | Bukit Satiam, Bintulu, Sarawak,<br>02°59'26.1"N 112°55'54.4"E   |
| <i>Schismatoglottis 'petradoxa'</i>   | JN544430 | AR-920      | Km 65 road to Camp Gahada, Rejang<br>Wood Concession, Nanga Gaat, Kapit,<br>Sarawak, 01°41'59.7"N 113°31'13.7"E |
| ' <i>Schismatoglottis</i> ' sp. A     | JN544423 | AR-114      | Sg. Piat, Nanga Gaat, Kapit, Sarawak,<br>01°38'09.1"N 113°24'09.9"E   |
| ' <i>Schismatoglottis</i> ' sp. B     | JN544424 | AR-135      | Stream below Camp Gahada, Nanga<br>Gaat, Kapit, Sarawak, 01°41'49.4"N<br>113°26'16.3"E                          |

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