Distribution of *Holttumochloa* (Poaceae: Bambusoideae) in China with description of a new species revealed by morphological and molecular evidence

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** A B S T R A C T **

*Holttumochloa* K.M. Wong is a genus endemic to Malay Peninsula with three described species ([Wong, 1993]): *Holttumochloa korbuensis* K.M. Wong, *Holttumochloa pubescens* K.M. Wong and *Holttumochloa magica* (Ridley) K.M. Wong, which is the type species. *Holttumochloa* differs from other taxa of paleotropical woody bamboos (PWB), especially in its unique branching pattern. Branch complements of *Holttumochloa* are derived from multiple primary buds at the culm nodes, whereas they are derived from a solitary bud in other PWB taxa. Phylogenetically, *Holttumochloa* was revealed to be closely related to *Kinabaluchloa* K.M. Wong, which consists of two species each endemic to the Malay Peninsula and Borneo, respectively ([Goh et al., 2010, 2013]). However, the two genera have different branching patterns and pseudospikelete features ([Wong, 1993]). Pseudospikelets of *Kinabaluchloa* are each subtended by a large bract which is almost as long as the lemmas, whereas pseudospikelets of *Holttumochloa* are each subtended by a small bract that is distinctly smaller than the lemmas.

We collected specimens of a slender bamboo without inflorescences from Diaoluo Mountain during a field expedition to the Island of Hainan, South China in 2014. It occurs in montane forests above elev. 600 m. Its vegetative characters are different from all recognized bamboo species in China. After a general literature survey, we found that it resembles *Kinabaluchloa nebulosa* K.M. Wong in the following vegetative characters: culm tips entangled with tree crowns; internodes white-waxy; nodes prominent with a thick girdle at sheath base; buds none at the lowermost nodes; culm sheaths pale purplish green covered with black hairs, ligules with pale brown bristles, blades linear, reflexed. But it is different from *K. nebulosa* in having shorter and thinner culms and bearing no golden-brown hair ring at the sheath base. During a second expedition in 2015, we found a flowering clump of the same bamboo. After further investigation and comparison with the literature and type specimens of *Holttumochloa* and *Kinabaluchloa*, we found that it belongs to *Holttumochloa* rather...
than *Kinabaluchloa* in the diagnostic characters of the flowering specimens: pseudospikelets each subtended by a bract distinctly smaller than the lemmas; ovary with the hairy summit, obovoid-ovoid when young and becoming cylindrical gradually. Corroborated by the molecular phylogeny, we concluded that it should be a new species of *Holttumochloa*, despite its vegetative similarities with *Kinabaluchloa*. Herein, this new species is described and illustrated.

Materials and methods

We compared our samples with images of type specimens of *H. magica*, *K. nebulosa* and *Kinabaluchloa wrayi* on the website of the Royal Botanic Gardens, Kew (http://apps.kew.org/herbcat/navigator.do), and consulted protologues of relevant taxa as well. Vegetative characters were measured in the field and in the herbarium, and the pseudospikelets were analyzed under a stereo microscope (ZEISS SteREO Discovery.V12) facilitated by the Germplasm Bank of Wild Species in Kunming Institute of Botany, Chinese Academy of Sciences.

In order to investigate the phylogenetic position of *Holttumochloa hainanensis*, some species of *Holttumochloa*, *Kinabaluchloa* and other closely related PWBs, particularly of the Bambusinae, were selected to reconstruct the molecular phylogeny. Partial sequence of the nuclear granule-bound starch synthase I (*GBSSI*) gene was used to study the molecular phylogeny. In total, DNA sequences of 27 species of PWBs were obtained from previously published work (Goh et al., 2013). The sequence of *H. hainanensis* was generated following Yang et al. (2008) and has been deposited in GenBank (KY679198). Sequences were aligned by MAFFT v7.184 (Katoh and Standley, 2013) and adjusted manually where necessary. Maximum likelihood (ML) analysis was performed with RAxML-HPC2 v8.2.4 (Stamatakis, 2014). Maximum parsimony analysis was carried out in PAUP* version 4.0b10 (Sinauer, Sunderland, Massachusetts). Parameter settings for both analyses followed Zhou et al. (in press).

**Results**

Inferring from the molecular phylogeny of *GBSSI* (Fig. 1), *Kinabaluchloa* and *Holttumochloa* form a well-supported clade (90/96) except for *K. wrayi*, whose phylogenetic position remains unresolved. This clade can be distinguished from its sister clade, the *Bambusa*—*Dendrocalamus*—*Gigantochloa* (BDG) complex, with high statistical support. The *GBSSI* phylogeny recovered a monophyletic *Holttumochloa*, within which *H. hainanensis* was resolved to be sister to *H. magica* with strong support (97/99).

**Discussion**

The close relationship between *Kinabaluchloa* and *Holttumochloa* has been revealed in previous phylogenetic studies (Goh et al., 2010, 2013; Zhou et al., in press). The two genera formed a monophyletic group in Goh et al. (2013). However, both *Kinabaluchloa*...
and Holttumochloa were not monophyletic genera in the plastid phylogeny, and H. hainanensis was sister to a clade consisting of H. magicca and K. nebulosa (Zhou et al., in press). In contrast, both Kinabaluchloa and Holttumochloa were resolved to be monophyletic in the nuclear phylogeny (Goh et al., 2013). Since both genera are endemic to a narrow geographical region and have similar vegetative characters, except for different primary buds, hybridizations are likely to have occurred in the evolutionary history of the two genera. In addition, GBSSI phylogeny further confirmed our recognition of the new species. Based on previous studies and our results, some morphological characters of bamboos, including diagnostic features previously used, are of little phylogenetic significance. In the case of lineages occurring in diverse habitats, characters might be variable due to different climates, soil and other environmental factors. Therefore, some functional traits are probably of less phylogenetic significance, such as habit, leaf size, and culm wall thickness. In contrast, characters less likely to be affected by external factors, such as primary bud number and characters of inflorescences, pseudospikelets and florets, are more likely to be phylogenetically informative. Since Kinabaluchloa and Holttumochloa are closely related phylogenetically and are endemic to adjacent geographical areas with similar habitats, they are similar in many vegetative characters but different in characters of pseudospikelets, florets and primary branch buds. Structures of pseudospikelets and florets are important criteria for classification and sometimes overweight vegetative similarities in the taxonomy of paleotropical woody bamboos.

The discovery of this new species greatly expands the distribution area of Holttumochloa northward, and it is of great biogeographical significance since Holttumochloa has previously only been recorded from the Malay Peninsula. The flora of Hainan has shown higher similarity to that of the Malay Peninsula than southern Guangdong, which is geographically closer to Hainan (Zhu, 2016). Also, the Southeast Asian mainland was directly connected to western Malaysia until the early Pliocene, and there was no geographical barrier between them during the Tertiary (Hall and Holloway, 1998). This might explain the close affinity of flora between the two areas. Coincidentally, Trigonobalanus verticillata Forman has also been found to be disjunctively distributed in Hainan, Malaysia and Indonesia, and their affinities were further confirmed by cytological studies (Ng and Lin, 2008; Chen and Sun, 2010). Both cases illuminate the affinity between tropical floras of Hainan and Malesia at the species or sister-species level. However, phylogeographical studies at the comparative population level are needed to further understand this intriguing disjunction.

**Taxonomic treatment**

海南多枝竹 (多枝竹属) (新拟) hai nan duo zhi zhu.

*Holttumochloa hainanensis* M.Y.Zhou & D.Z.Li, sp. nov (Figs. 2 and 3).

It is morphologically similar to *H. korbuensis*, but differs from the latter species by having thicker and white-waxy culms, longer leaf blades, larger pseudospikelets with 2 fertile florets and longer anthers.

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**Fig. 2.** *Holttumochloa hainanensis*. A. Habit. B. Culm node with branchlets. C. Culm sheath. D. Shoot. E. Node and culm. F. and G. Pseudospikelets. H. Rachilla internode. I. Bract. J. Lemma. K. Palea.
Type:—CHINA. Hainan: Lingshui County, Benhao Township, Diaoluo Mountain, Lower montane forest, elev. 915 m, 18.7222° N, 109.8736° E, 27 September 2015, Zhou Meng-Yuan 062 (zmy062) (Holotype: KUN).

Sympodial bamboo. Rhizomes pachymorph. Clumps in forest understory with culm tips entangled with tree crowns or drooping over to the ground, erect at base. Culms 7–8 m long, 0.5–1.3 cm in diameter, wall thin; internodes 21–56 cm long, lower ones shorter than upper ones, dark purplish when young, white-waxy; nodes prominent with a thick girdle at the sheath base, glabrous; buds unseen, none at the lowermost 6–7 nodes. Branch complements clusters of slender subequal branchlets encircled half of nodes, dark purplish green. Culm sheaths deciduous, covered with densely black or dark brown deciduous bristles all over, green or apex purplish, apex concave; auricles absent or inconspicuous with marginal oral

Fig. 3. Holttumochloa hainanensis. A. Upper part of culm sheath, abaxial view. B. Culm node with branchlets. C. Culm with culm sheath. D. Part of flowering branch. E. Pseudo-spikelets. F. Pistil. G. Bract. H. Transitional glume. I. Lemma. J. Palea. K. Stigmas. Illustrated by Ms. Ling Wang (KUN).
setae; ligule with pale brown setae to 1 cm long; blades linear, reflexed, green. Leaves 4–5 per branchlet; leaf blade linear-lanceolate, 10–13 cm long × 0.5–1 cm wide, glabrous; pseudopetiole <1 mm; auricles absent or inconspicuous, fimbriate; ligule 0.2–0.3 mm. Inflorescences iteractic, borne on terminal nodes of leafy branches or nodes of leafless branches; pseudospikelets several clustered at each node of branches. Pseudospikelets fusiform, 10–15 mm long, 1–2 basal bracts, 2 gernemenous bracts, one transitional glume, 2 fertile florets, and one terminal vestigial floret; rachilla internodes between florets flattened, finely hairy, ca. 2.3 mm long. Florets lemma ca. 6 mm, acute, margins with fine short hairs; palea ca. 6 mm, 2-keeled, margins enfolded, apex of the keels pale ciliate; lodicule 3; stamens 6, anthers ca. 4 mm with marginationed tips; filaments filiform and free; stigmas 3, plumose; ovary obvoid with a thickened and short-hairy apex.

**Etymology:** The specific epithet refers to its distribution on the Hainan Island.

**Additional specimen examined:** CHINA. Hainan: Lingshui County, Benhao Town, Diaoluo Mountain, 10 June 2014, Zhou Meng-Yuan 033 (zmy033) (KUN).

**Keys to species of *Holttumochloa***

1. Leaf blades densely pale-hairy on lower side ...........H. pubescens
2. Pseudopetiole 2–3 mm; leaf sheath auricle bristly; pseudospikelets 2–3 cm long; perfect florets 4–5; rachilla internodes 3–5 mm ..............................................H. magica
3. Pseudopetiole shorter than 1.5 mm; leaf sheath auricles absent or inconspicuous; pseudospikelets shorter than 1.5 cm, perfect florets 2–3; rachilla internodes 2–3 mm .............................................H. hainanensis
4. Pseudopetiole 1–1.5 mm; culm 0.5–1.3 cm diameter, covered by white wax; leaf blade 10–13 cm long; pseudospikelets 1–1.5 cm; perfect florets 2; anthers ca. 4 mm long .....................................H. korbuensis

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