Gnetum chinense, a new species of Gnetaceae from southwestern China

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Abstract

Gnetum chinense sp. nov., a new lianoid species of Gnetaceae, is described from southwestern China. The new species is morphologically similar to G. montanum Markgr. in its oblong elliptic leaves and the ovoid to ellipsoid chlamydosperm, but differs from the latter by its shorter male spikes having fewer involucral collars (7–10 vs. 13–18 in G. montanum). We also did a new molecular analysis using one nuclear marker (i.e. nrITS) and four chloroplast markers (i.e. mark gene, rpoC1 intron, psbB-rps12 IGS, and trnF-trnV IGS). The result suggests that this specific clade is sister to a large clade consisting of all other known Chinese lianoid species of Gnetum except G. parvifolium (Warb.) W.C. Cheng.

Keywords

Gnetum, China, morphology, phylogeny, taxonomy

Introduction

Gnetum L., belongs to the monotypic family Gnetaceae of gymnosperms, and contains ca. 40 extant species that are widely distributed in tropical and subtropical forests in Asia, Africa and South America (Yang et al. 2017a). This genus is evergreen, mostly
lianas, rarely trees, and possesses a set of unusual characters for gymnosperms, e.g. dicots-like broad leaves with pinnate venation, female gametophytes lacking archegonia, male and female reproductive units assembled into whorls, male spikes usually having abortive chlamydosperms and appearing to be bisexual, chlamydosperms possessing two outer envelopes, etc. (Pearson 1929; Maheshwari and Vasil 1961; Martens 1971; Gifford and Foster 1989; Friedman and Carmichael 1996). Although the earliest macrofossil of the gnetoid clade is known from the mid-Jurassic in northeastern China, the modern *Gnetum* is believed to diversify in South America and split into the New World clade and the Old World clade around the K-Pg boundary (Hou et al. 2015; Yang et al. 2017b). The Chinese clade diverged from other southeastern Asian species around 38 mya (million years ago, 95% posterior density 27–49 mya), and became more diversified after the earliest Miocene (ca. 21 mya, Hou et al. 2016).

*Gnetum* has a wide range of distribution in southern China (Cheng 1978; Fu et al. 1999). Cheng (1978) recognized seven species in China, while Fu et al. (1999) accepted nine species in the *Flora of China*. Both studies were based on herbarium material only. These traditional taxonomic treatments laid much emphasis on reproductive characters, but variation patterns of important reproductive characters are ambiguous because i) fruiting material is poorly represented in herbaria, and ii) it is difficult to match male and female specimens to a certain species when studying a dioecious taxon like *Gnetum*.

Molecular phylogeny was successfully applied to the delimitation of species of *Gnetum* in combination with morphological characters (Hou et al. 2016; Kim and Won 2016). Kim and Won (2016) applied a barcode method and recognized three species in Cambodia, i.e. *G. macrostachyum* Hook. f., *G. montanum* Markgr., and *G. aff. gracilipes* C.Y. Cheng. Hou et al. (2016) conducted a taxonomic revision based on molecular and morphological data, and recognized the following six lianoid species in China: *G. catasphaericum* H. Shao, *G. formosum* Markgr., *G. luofuense* C.Y. Cheng, *G. montanum* Markgr., *G. parvifolium* (Warb.) W.C. Cheng, and *G. pendulum* C.Y. Cheng.

A new *Gnetum* species was identified when we worked on a *Gnetum* genome project a few years ago. Further morphological and molecular studies on newly collected materials during field investigations in southern China allowed us to describe this species here as new to science.

**Materials and methods**

Plant materials, comprising silica-dried leaves and vouchers, were sampled in Yunnan and Guizhou of southern China. All vouchers were deposited in the Herbarium (PE), State Key Laboratory of Systematic and Evolutionary Botany, Institute of Botany, Chinese Academy of Sciences (Table 1).

Total genomic DNA was extracted from the dried leaf materials using the CTAB method (Doyle and Doyle 1987) and purified using a QIAquick PCR Purification Kit. For phylogenetic studies, it is thought that nrITS and four chloroplast markers including *matK* gene, *rpoC1* intron, *psbB-rps12* IGS, and *trnF-trnV* IGS are highly variable
A new species of \textit{Gnetum} (Kim and Won 2016; Hou et al. 2016). We followed the methods described in Hou et al. (2016), and one nuclear marker (i.e. \textit{nrITS}) and four chloroplast markers (i.e. \textit{matK} gene, \textit{rpoC1} intron, \textit{psbB-rps12} IGS, and \textit{trnF-trnV} IGS) were targeted. \textit{Gnetum} sequences generated in Hou et al. (2016) were downloaded from the GeneBank (Table 2). Sanger sequencing was conducted at Majorbio, Beijing, China. The output files were assembled and edited using Sequencer ver. 4.5 (Gene Codes Corporation, Ann Arbor, Michigan, U.S.A.) and DNA sequences were aligned using Clustal X ver. 2.1 (Larkin et al. 2007) and manually adjusted using BioEdit ver. 7.2.5 (Hall 1999). Sequences of the five markers were concatenated using SequenceMatrix Windows ver. 1.7.8 (Vaidya et al. 2011).

Previous studies suggested that the African species are sister to all Asian species (Won and Renner 2006; Hou et al. 2015), as a result, we chose the African \textit{G. africanum} Welw. as the outgroup. Maximum likelihood (ML) analyses were conducted using the RAxML-HPC2 on XSEDE (8.0.0) executed in the CIPRES portal (http://www.phylo.org/, Stamatakis 2014). The ML bootstrap values (BS) for each node were summarized after 1,000 replicates of bootstrapping iterations. The obtained trees were viewed and edited using FigTree ver. 1.4.0 (http://tree.bio.ed.ac.uk/software/figtree/). Bayesian inference (BI) analyses were performed using MrBayes 3.2.6 (Huelsenbeck and Ronquist 2001) on XSEDE (8.0.0) in CIPRES. The Markov Chain Monte Carlo (MCMC) algorithm was run for 3,000,000 generations with the sampling frequency 1,000. Bayesian posterior probabilities (PP) were calculated for the majority consensus tree of all sampled trees after discarding trees sampled within the burn-in (25%) phase in MrBayes v.3.2.1.

The distribution map was generated using ArcGIS 9.3 (ESRI, Redlands, CA, USA; http://www.esri.com). The photos were taken using digital cameras (Nikon D700 and Olympus TG-3), manually edited and created using Adobe Photoshop CS2 ver. 9.0. Phylogenetic trees were viewed and adjusted using FigTree ver. 1.4.0 (http://tree.bio.ed.ac.uk/software/figtree/).

\begin{table}[h]
\centering
\caption{Sequences of \textit{Gnetum chinense} sp. nov. generated in this study and their vouchers. All vouchers have been deposited in PE.} \\
\begin{tabular}{|c|c|c|c|c|c|c|}
\hline
Collection & Locality & ITS & \textit{matK} & \textit{rpoC1} & \textit{psbB-rps12} & \textit{trnF-trnV} \\
\hline
T. Wan MLP001 & China. Yunnan: Malipo & MT362085 & MT373322 & MT373311 & MT373300 & MT373333 \\
T. Wan MLP002 & China. Yunnan: Malipo & MT373323 & MT373312 & MT373301 & MT373334 & MT373335 \\
T. Wan MLP003 & China. Yunnan: Malipo & MT373324 & MT373313 & MT373302 & MT373335 & MT373336 \\
T. Wan MLP005 & China. Yunnan: Malipo & MT373325 & MT373314 & MT373303 & MT373336 & MT373337 \\
B. Liu & al. 1360 & China. Yunnan: Malipo & MT373326 & MT373315 & MT373304 & MT373337 & MT373338 \\
B. Liu & al. 1441 & China. Yunnan: Malipo & MT373327 & MT373316 & MT373305 & MT373338 & MT373339 \\
B. Liu & al. 1725 & China. Yunnan: Malipo & MT373328 & MT373317 & MT373306 & MT373339 & MT373340 \\
B. Liu & al. 2627 & China. Yunnan: Cangyuan & MT362086 & MT373329 & MT373318 & MT373307 & MT373340 \\
B. Liu & al. 2675 & China. Yunnan: Lancang & MT373329 & MT373319 & MT373308 & MT373341 & MT373342 \\
B. Liu & al. 3045 & China. Yunnan: Jiangcheng & MT373330 & MT373320 & MT373309 & MT373340 & MT373342 \\
C.Y. Deng 12466 & China. Guizhou: Xingyi & MT362084 & MT373321 & MT373310 & MT373299 & MT373332 \\
\hline
\end{tabular}
\end{table}
| Species                          | ITS          | matK         | rpoC1        | psbB-rps12   | trnF-trnV   |
|---------------------------------|--------------|--------------|--------------|--------------|-------------|
| G. catasphaericum_24            | KX234206     | KX234250     | KX234304     | KX234352     | KX234382    |
| G. catasphaericum_31            | KX234205     | KX234249     | KX234303     | KX234351     | KX234381    |
| G. catasphaericum_32            | KX234203     | KX234247     | KX234301     | KX234349     | KX234379    |
| G. catasphaericum_44            | KX234202     | KX234246     | KX234300     | KX234348     |             |
| G. catasphaericum_33            | KX234204     | KX234248     | KX234302     | KX234350     | KX234380    |
| G. cuspidatum_20                | KX234174     | KX234222     | KX234274     | KX234325     |             |
| G. cuspidatum_C52               | KP256660     | KP256698     | KX234273     | KX234324     |             |
| G. diminutum_C101               | KP256664     | KP256702     | KX234275     |             |             |
| G. edule_C4                     | KP256658     | KP256696     | KX234268     | KX234322     |             |
| G. formosum_14                  | KX234200     | KX234230     | KX234298     | KX234346     | KX234377    |
| G. formosum_21                  | KX234201     | KX234231     | KX234299     | KX234347     | KX234378    |
| G. giganteum_37                 | KX234209     | KX234232     | KX234307     | KX234354     | KX234384    |
| G. giganteum_41                 | KX234213     | KX234256     | KX234311     | KX234358     | KX234388    |
| G. giganteum_8                  | KX234211     | KX234254     | KX234309     | KX234356     | KX234386    |
| G. griffithii_1                 | KX234173     | KX234221     | KX234265     | KX234319     |             |
| G. griffithii_102                |             | KX385188     | KX385188     | KX385188     | KX385188    |
| G. griffithii_2                  | KX234170     | KX234219     | KX234263     | KX234317     |             |
| G. griffithii_101               | KX234171     | KX385189     | KX385189     | KX385189     | KX385189    |
| G. griffithii_23                 | KX234172     | KX234220     | KX234264     | KX234318     |             |
| G. griffithii_9                  | KX234179     | KX234244     | KX234296     | KX234344     | KX234375    |
| G. griffithii_16                 | KX234199     | KX234245     | KX234297     | KX234345     | KX234376    |
| G. griffithii_18                 | KX234196     | KX234242     | KX234294     | KX234342     |             |
| G. griffithii_25                 |             | KX234240     | KX234292     | KX234340     | KX234372    |
| G. hainanense_5                 | KX234197     | KX234243     | KX234295     | KX234343     | KX234374    |
| G. hainanense_9                  | KX234192     | KX234237     | KX234289     | KX234337     | KX234369    |
| G. hainanense_10                 | KX234187     | KX385193     | KX385193     | KX385193     | KX385193    |
| G. hainanense_110                | KX234186     | KX385194     | KX385194     | KX385194     | KX385194    |
| G. latifolium_C15               | KP256661     |             | KX34269      |             |             |
| G. leptostachyum_C102            | KP256665     | KP256703     | KX34271      |             |             |
| G. luofuense_19                  | KX234190     | KX234236     | KX34287      | KX234336     | KX234367    |
| G. luofuense_27                  | KX234189     | KX234235     | KX34286      |             |             |
| G. luofuense_28                  | KX234185     | KX234233     | KX34284      | KX234334     | KX234365    |
| G. luofuense_6                   | KX234188     | KX234234     | KX34285      | KX234335     | KX234366    |
| G. luofuense_C61                 | KX234191     | KP256710     | KX34288      |             | KX234368    |
| G. montanum_105                  |             | KX385195     | KX385195     | KX385195     | KX385195    |
| G. montanum_106                  |             | KX385196     | KX385196     | KX385196     | KX385196    |
| G. montanum_17                   | KX234208     | KX234251     | KX34306      | KX234353     | KX234383    |
| G. montanum_29                   | KX234207     |             | KX34305      |             |             |
| G. neglectum_C19                 | KP256667     | KP256705     | KX34270      |             |             |
| G. parvifolium_35                 | KX234178     | KX234224     | KX34277      | KX234327     |             |
| G. parvifolium_108                | KX234176     | KX385190     | KX385190     | KX385190     | KX385190    |
| G. parvifolium_109                | KX234177     | KX385192     | KX385192     | KX385192     | KX385192    |
| G. parvifolium_22                 | KX234181     | KX234227     | KX34280      | KX34330      |             |
| G. parvifolium_26                 | KX234180     | KX234226     | KX34279      | KX234329     |             |
A new species of Gnetum

| Species             | ITS      | matK     | rpoC1    | psbA-rps12 | trnF-trnV |
|---------------------|----------|----------|----------|------------|-----------|
| G. parvifolium_34   | KX234179 | KX234225 | KX234278 | KX234328   | –         |
| G. parvifolium_36   | KX234175 | KX234223 | KX234276 | KX234326   | –         |
| G. parvifolium_40   | KX234182 | KX234228 | KX234281 | KX234331   | –         |
| G. parvifolium_43   | KX234183 | KX234229 | KX234282 | KX234332   | –         |
| G. parvifolium_C50  | KP256675  | KX385191 | KX385191 | KX385191   | KX385191  |
| G. pendulum_103     | –        | KX385197 | KX385197 | KX385197   | KX385197  |
| G. pendulum_104     | –        | KX385198 | KX385198 | KX385198   | KX385198  |
| G. pendulum_38      | KX234212 | KX234255 | KX234310 | KX234357   | KX234387  |
| G. pendulum_4       | KX234217 | KX234260 | KX234315 | KX234362   | KX234391  |
| G. pendulum_45      | KX234216 | KX234259 | KX234314 | KX234361   | KX234390  |
| G. pendulum_46      | KX234215 | KX234258 | KX234313 | KX234360   | KX234389  |
| G. pendulum_47      | KX234218 | KX234261 | KX234316 | KX234363   | –         |
| G. raya_C11         | KP256657  | –        | KX234267 | KX234321   | –         |
| G. tenuifolium_C18  | KP256662  | KP256700 | KX234272 | KX234323   | –         |
| G. africanum_C41    | KP256642  | KP256681 | KX234262 | –          | –         |

**Results**

**Phylogeny**

The ML tree (Fig. 1), in general, was better resolved than the BI tree (Fig. 2). All Chinese lianoid taxa of Gnetum included in this study formed a strongly supported monophyletic group (BS: 100%; PP: 1.00). Ten samples of G. parvifolium constituted a sister group (BS: 100%; PP: 1.00) to a clade consisting of the rest of the lianoid congeners from China included in this study. The analyses revealed the 11 newly collected specimens as a monophyletic group (BS: 100%; PP: 1.00) sister to a clade composed of G. formosum, G. catasphaericum, G. luofuense, G. montanum and G. pendulum. Delimitations between G. montanum and G. pendulum were not resolved. The two samples of G. formosum formed a weakly supported group (BS: 81%; PP < 0.70), which was followed by a split between the strongly supported G. catasphaericum (BS: 98%; PP: 0.99) and a large clade containing a subclade of Gnetum montanum, G. pendulum, G. giganteum H. Shao, and G. gracilipes (BS: 98%; PP: 1.00), and another one of G. hainanense C.Y. Cheng ex L.K. Fu et al. and G. luofuense (BS: 98%; PP: 0.81). Delimitations between the two species were not resolved.

**Taxonomy**

*Gnetum chinense* Y. Yang, Bing Liu & S.Z. Zhang, sp. nov.
urn:lsid:ipni.org:names:77209708-1
Figs 3, 4

**Type.** China. Yunnan: Cang-yuan County, on the way from Ban-hong to Ban-lao Prefecture, forest margin, male cones, March 31st, 2015, B. Liu, Y. Yang & T.W. Xiao 2627 (PE, holotype).
Figure 1. Maximum likelihood tree based on nuclear ribosomal ITS and chloroplast matK, rpoC1, psbB-rps12, and trnF-trnV, showing the robust species clade of *Gnetum chinense* sp. nov. Bootstrap values are displayed when they are greater than 50%.
A new species of *Gnetum* 111

Figure 2. Bayesian inference tree based on nuclear ribosomal ITS and chloroplast *matK*, *rpoC1*, *psbB*-rpo12, and *trnF-trnV*, showing the robust species clade of *Gnetum chinense* sp. nov. Posterior probabilities are shown when they are greater than 0.70.
**Diagnosis.** This species is similar to *G. montanum* in its oblong elliptic leaves and subsessile chlamydosperm, but differs from the latter by its shorter male cones (1–1.5 cm long in the new species vs. 2–3 cm in *G. montanum*) having fewer involucral collars (7–10 in the new species vs. 13–18 in *G. montanum*), nearly sessile or extremely shortly stiped chlamydosperms (vs. markedly stiped, stipes 3–5 mm long in *G. montanum*).

**Description.** Lianas; twigs terete, dichasially branched having swollen nodes. Leaves opposite (Fig. 4a), oblong to elliptic, 11–16 cm long, 4–8 cm wide, base rotund to acute, apex acute to acuminate, pinnately veined, midvein impressed adaxially and elevated abaxially, lateral veins 6–8 (Fig. 4a), more or less elevated on both sides, petioles 1–1.2 cm long, grooved adaxially. Male reproductive shoots terminal, dichasial, branched once or twice (Fig. 4a). Male cones pedunculate, peduncles 2–10 mm long; cylindric, ca. 10–15 mm long, 4 mm in diam., involucral collars 8–10 (Fig. 4b). Chlamydosperms ellipsoid to subglobose, ca. 2.2 cm long, 1.4 cm in diam., apex obtuse, base contracted into an extremely short stalk or subsessile, green when young, and orange when mature (Fig. 4c).

**Distribution.** In Yunnan and Guizhou provinces of China (Fig. 3).

**Habitats.** In evergreen tropical and subtropical forests.

**Etymology.** The specific epithet ‘chinense’ is derived from China.

**Phenology.** Blooming male cones and mature chlamydosperms were found in late May and early November, respectively.
A new species of *Gnetum*

**Conservation.** *Gnetum chinense* is common in evergreen forests in Yunnan and Guizhou. We consider this species to be of Least Concern (LC) under the IUCN Red List Categories and Criteria ver. 3.1 second edition (IUCN 2012).

**Specimens examined.** China. Yunnan: Lan-cang Lahuzu Autonomous County, from Shang-yun to Xi-meng, Apr. 2, 2015, B. Liu, Y. Yang & T.W. Xiao 2675

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**Figure 4.** *Gnetum chinense* sp. nov. **A** branch bearing male cones **B** male cones **C** female cone portion displaying chlamydosperm morphology.
Table 3. A morphological comparison between *Gnetum chinense* and other Chinese lianoid species.

| Species                        | Leaf blade shape                  | Leaf blade length (cm) | Leaf blade width (cm) | Petiole length (mm) | Male spike length (cm) | Male spike involucral collars | Chlamydosperm shape | Chlamydosperm length (cm) | Chlamydosperm stipe length (mm) |
|-------------------------------|-----------------------------------|------------------------|-----------------------|---------------------|------------------------|-------------------------------|---------------------|-----------------------------|-------------------------------|
| *Gnetum catasphaericum*        | Ovate to oblong ovate             | 7–12                   | 4–6.5                 | 6–10                | ca. 2                  | 10–16                         | Oblong, subglobose          | 1.8–2.2                     | 2–6                           |
| H. Shao                       |                                    |                        |                       |                     |                        |                               |                     |                             |                               |
| *G. chinense* sp. nov.         | Oblong to elliptic                | 11–16                  | 4–8                   | 10–12               | 1–1.5                  | 8–10                          | Ellipsoid to subglobose      | ca. 2.2                     | Subsessile                     |
| *G. formosum* Markgr.          | Elliptic to narrowly oblong        | 11–14                  | 4–7                   | 9–10                | ?                      | ?                             | Narrowly oblong, fusiform   | 2–2.5                       | Sessile                       |
| *G. luofuense* C.Y. Cheng      | Elliptic to oblong ovate           | 4.5–16                 | 3–8.5                 | 8–13                | 2–3                    | 12–15                         | Broadly ellipsoid to cylindrical | 1.8–2.5                   | 2–5                           |
| *G. montanum* Markgr.          | Elliptic to oblong                 | 10–28                  | 4.5–13                | 9–26                | 2–3.5                  | 16–25                         | Cylindric ovoid, cylindric ellipsoid | 1.6–2                      | 3–5                           |
| *G. parvifolium* (Warb.)       | Elliptic to narrowly oblong        | 4–11                   | 2–4                   | 5–7                 | 0.8–1.5                | 9–11                          | Ellipsoid                     | 1.3–1.8                     | Sessile                       |
| W.C. Cheng                    | Narrowly elliptic to oblong ovate | 10–18                  | 4–8.5                 | 8–15                | 1–1.5                  | 12–15                         | Elongate ellipsoid            | 3–4                         | 10–30                         |

Data were collected from Hou et al. (2016) and this study.
A new species of Gnetum

(PE); Jiang-cheng County, Qu-shui Prefecture, Apr. 18, 2015, B. Liu 3045 (PE); Ma-li-po County, March 15–17, 2015, T. Wan MLP001, MLP002, MLP003, MLP 005 (PE); Ma-li-po County, Xia-jin-chang Prefecture, Li-jia-wan, May 27, 2011, B. Liu 1360 (PE); Ma-li-po County, Xia-jin-chang Prefecture, Li-jia-wan, Sept. 24, 2011, B. Liu 1441 (PE); Ma-li-po County, Xia-jin-chang Prefecture, Li-jia-wan, Nov. 2, 2012, B. Liu 1725 (PE). Guizhou: unknown collection date, C.Y. Deng CYD12466 (PE).

Discussion

Phylogenies based on molecular data have clearly resolved major lineages of Gnetum, including a South American clade, an African clade, and several Asian clades (Won and Renner 2003, 2005a, 2005b, 2006; Kim and Won 2016; Hou et al. 2015, 2016). Taxonomy of the Asian Gnetum is rather complicated because plants of the genus are usually dioecious woody climbers, and there are few taxonomic characters, so it is difficult to identify species without diagnostic reproductive characters (Kim and Won 2016).

Phylogenetic methods were successfully applied to discover and delimit species of Asian Gnetum (Kim and Won 2016; Hou et al. 2016). Our phylogenetic study found a new specific clade that was not recognized in previous studies; this clade is well resolved (Figs 1, 2; BS: 100%; PP: 1.00).

We did a morphological comparison between our new species and those known lianoid species from China (Table 3), and found that the specimens of this new specific clade are similar to G. montanum in the shape of leaves and chlamydosperms, and to G. parvifolium in the length of the male spikes and number of involucral collars, but differ from G. montanum by their shorter male cones having fewer involucral whorls, and from G. parvifolium by their larger leaves 11–16 cm long and bigger chlamydosperms ca. 2.2 cm long (vs. smaller leaves ca. 4–11 cm long, smaller chlamydosperms 1.3–1.8 cm long).

A few morphological details of the new species are taxonomically important but not known to us, e.g. shape and the number of sterile ovules in male spike. As a result, further field investigations are encouraged.

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