

Occultocarpon, a new monotypic genus of Gnomoniaceae on *Alnus nepalensis* from China

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Received: 8 April 2011 / Accepted: 22 May 2011 / Published online: 4 June 2011
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Abstract Microfungi in the Gnomoniaceae (Diaporthales, Ascomycetes) comprise species commonly reported as pathogens and endophytes on trees and herbaceous hosts primarily from temperate forests of North America, Europe, and Japan. The diversity of Gnomoniaceae in China is poorly known, although several plant families that occur there specifically the Betulaceae are considered important hosts. An exploratory trip to Yunnan, China, resulted in the discovery of several members of the Gnomoniaceae. In this paper a new monotypic genus, *Occultocarpon* and its species, *O. ailaoshanense*, are described and illustrated. A phylogeny based on three genes (LSU, *rpb2*, *tefl-α*) reveals that *O. ailaoshanense* belongs to the Gnomoniaceae and forms a

branch distinct from the currently known genera. *Occultocarpon ailaoshanense* is characterized by perithecia with thin, central to eccentric necks in groups embedded in a stroma and oblong elliptical-elongated, one-septate ascospores. *Occultocarpon ailaoshanense* occurs on the bark of branches of *Alnus nepalensis* (Betulaceae) in Yunnan, China.

Keywords *Ascomycota* · Betulaceae · Diaporthales · Gnomoniaceae · Systematics · Yunnan

Introduction

The Gnomoniaceae (Diaporthales, Ascomycetes) comprise species of microfungi commonly reported as pathogens and endophytes on trees and herbaceous hosts primarily from temperate forests of North America, Europe, and Japan. Collections made in these regions and recent systematic work suggest that several genera of Gnomoniaceae have followed the geographic distribution of and diversified on certain host families such as Betulaceae, Fagaceae, and Salicaceae (Mejía et al. 2011a, b; Walker et al. 2010). China is considered to be the center of diversity for the Betulaceae, one of the major host families of Gnomoniaceae (Chen et al. 1999; Sogonov et al. 2008). The Gnomoniaceae of China are poorly known, although species of *Apiognomonina*, *Gnomonia*, *Linospora*, and *Pleuroceras* have been reported (Eriksson and Yue 1988; Tai 1937, 1979; Teng 1996).

An exploratory trip to collect species of Gnomoniaceae was conducted in Yunnan, China, in July, 2008, by the first author. This province was selected because it is considered a biodiversity “hot spot” (Myers et al. 2000; Xu and Wilkes 2004). Eighty-nine species of Betulaceae including 56 endemic species occur in China and more than 40 of those

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are known in Yunnan (Li and Skvortsov 1999). Other important host families of Gnomoniaceae such as Fagaceae, Juglandaceae, and Salicaceae occur in Yunnan (Fang et al. 1999; Huang et al. 1999; Li and Skvortsov 1999; Lu et al. 1999). A new monotypic genus of Gnomoniaceae from *Alnus nepalensis* D. Don, based on collections made during this trip, is herein described and illustrated.

Materials and methods

Specimens of branches of *Alnus nepalensis* with perithecia were placed in paper bags, air dried, and transported to the laboratory for processing. Observations and measurements of structures and culturing of specimens were done as in Mejía et al. (2008). Isolates sequenced for this study comprise two cultures of *Occultocarpon ailaoshanense*, CBS 129146 = LCM524.01 ex BPI 879253 and CBS 129147 = LCM522.01 ex BPI 879254, and a culture of *Ophiognomonia melanos-tyla* (CBS 129144 = LCM389.01 ex BPI 879257). More information about these cultures appears in Table 1 along with Genbank numbers of new sequences. Other specimens and DNA sequences included in the phylogenetic analyses are from Sogonov et al. (2008).

DNA extraction, amplification, and sequencing

Extractions of DNA were done as in Mejía et al. (2008) employing a Fast Prep FP 120 with lysing Matrix A (MP Biomedicals, Solon, OH, USA) for mechanical lysis and the Puregene Cell Kit (Qiagen Inc., Valencia, CA, U.S.A.). Three gene regions were amplified and sequenced. A region in the RNA polymerase second largest subunit (*rpb2*) was amplified with primers fRPB2-5F and fRPB2-7cR (Liu et al. 1999) and sequenced as in Mejía et al. (2008). A region of the translation elongation factor 1- α gene (*tef1- α*) was amplified and sequenced as in Sogonov et al. (2008) using PCR primers (EF1-728F, EF1-1567r) and the internal sequencing primer EF1-1199R (Carbone and Kohn 1999; see Mejía et al. 2011b; Rehner 2001). Approximately 1,200 base pairs of the 5' region of the nuclear ribosomal large subunit (LSU) were amplified and sequenced as in Castlebury et al. (2002).

Phylogenetic analyses

Editing of sequences and analyses of conflict among genes were prepared as described in Sogonov et al. (2008). The three genes were aligned individually and concatenated into a single alignment for phylogenetic analyses. The alignment was deposited in TreeBase with study ID 11308. Maximum parsimony (MP) analysis was conducted as described by Sogonov et al. (2008) using PAUP* v 4b10

Table 1 Cultures with DNA sequences generated in this study

Taxon	Specimen	Culture	Country	Host	Collector	LSU	ITS	<i>rpb2</i>	<i>tef1-α</i>
<i>Occultocarpon ailaoshanense</i>	BPI879253	CBS 129146 (=LCM524.01)	China: Yunnan	<i>Alnus nepalensis</i>	L. C. Mejía	JF779853	JF779849	JF779856	JF779863
<i>Occultocarpon ailaoshanense</i>	BPI879254	CBS 129147 (=LCM522.01)	China: Yunnan	<i>Alnus nepalensis</i>	L. C. Mejía	JF779852	JF779848	JF779857	JF779862
<i>Ophiognomonia melanos-tyla</i>	BPI879257	CBS 129144 (=LCM389.01)	Germany: Hesse	<i>Tilia cordata</i>	L. C. Mejía & T. Hoffman	JF779854	JF779850	JF779858	JF779860

(Swofford 2002). Support for branches was estimated with 1,000 parsimony bootstrap replications (Felsenstein 1985), with MULTREES and TBR on and 10 random sequence additions per bootstrap replicate.

Bayesian analysis using the program MrBayes v3.1.2 (Huelsenbeck and Ronquist 2001), was also performed. The best model for each gene was estimated using the program MrModeltest v.2 (Nylander 2004). The Bayesian analysis was completed as detailed in Sogonov et al. (2008) with 2,000,000 generations and burn-in = 50,000. Three species of *Cryphonectria* were selected as outgroup taxa based on the relatively close relationship of the Cryphonectriaceae with the Gnomoniaceae (see Castlebury et al. 2002). A 50% majority rule consensus phylogram was computed using 7,800 trees saved after the burn-in period (50,000 generations).

Results

Phylogenetic analyses

No conflicts among individual gene trees were observed and sequences from the three genes were concatenated into a single alignment containing LSU (1,231 bp), RPB2 (1,061 bp), and *tef1-α* (443 bp). Maximum parsimony (MP) analysis resulted in 132 equally parsimonious trees (CI=.277, RI=0.663). A 50% majority rule consensus tree from the MP analysis was obtained and used for comparison with results from the Bayesian analysis. The model GTR + I + G (nst = 6 rates = invgamma statefreqpr = dirichlet (1,1,1,1)) proved to be the best fitting model for each of the three genes and was applied for the Bayesian analysis. The same phylogeny was obtained by maximum parsimony and Bayesian analyses, each with clades representing 11 genera of Gnomoniaceae including the new genus *Occultocarpon* supported by both methods. The consensus phylogram obtained from the Bayesian analysis is presented in Fig. 1 with Bayesian posterior probabilities (PP) and MP bootstrap support indicated at the nodes. *Occultocarpon ailaoshanense*, the only species known in this genus, forms a distinct branch in the Gnomoniaceae and is a part of a larger unsupported clade that includes *Plagiostoma*, *Apiognomonia*, and *Amphioporthes hranicensis*. Additionally, this clade is contained within a clade containing seven genera, four of which occur primarily on Betulaceae (*Cryptosporella*, *Ditopella*, *Gnomonia*, and *Occultocarpon*) (Fig. 1).

Taxonomy

***Occultocarpon* L. C. Mejía & Zhu L. Yang, gen. nov.**
MycoBank number: 519819

Etymology: L. Occultus—hidden, Gr. Karpos—fruit, referring to the hidden nature of the perithecia that occur below the host surface.

Perithecia nigra, immersa, cinereis vel brunneis stromatibus super perithecia, subglobosa, tenui, centrali vel eccentrico collo. Asci cylindracei, elongati, natantes libere in perithecio. Ascospores hyalinae, breves, elongatae oblongo-ellipticae, extremitatibus rotundatis, 1-septatae.

Perithecia black, in groups scattered in host branches, immersed in and pushing up host periderm, with grey to brown, scanty stroma above perithecia, with thin central to eccentric necks protruding from periderm and extending beyond surface, cream yellow mycelium at base of perithecia, perithecia collapsing from bottom when dry. Asci cylindrical-elongated, apical ring visible as two slightly reniform bodies, with eight ascospores arranged obliquely parallel or biseriately. Ascospores hyaline, short, oblong elliptical-elongated, one-septate, multiguttulate.

Anamorph Unknown.

Type species: *Occultocarpon ailaoshanense* L. C. Mejía & Zhu L. Yang

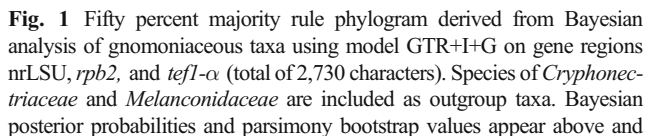
***Occultocarpon ailaoshanense* L. C. Mejía & Zhu L. Yang, sp. nov.** Fig. 2

MycoBank number: 519820

Etymology: From Ailaoshan, referring to the location where this species was first collected.

Perithecia nigra, immersa, cinereis vel brunneis stromatibus super perithecia, subglobosa, diametro × altitudo = 471–480(–489) × (363–)364–369(–375) μm, tenui, centrali vel eccentrico collo, (284–)386–496(–504) μm longo. Asci cylindracei, elongati, natantes libere in perithecio, (57–)64–72(–78) × (13–)15–17(–21) μm. Ascospores hyalinae, breves, elongatae oblongo-ellipticae, extremitatibus rotundatis, 1-septatae, (16–)18–22(–29) × (3–)4(–5) μm, l:w (3.8–)4.6–5.8(–7.6).

Perithecia black, in groups of up to five, immersed in and pushing up host periderm, with grey to brown stroma above perithecia, subglobose, diam × height = 471–480(–489) × 363–369(–375) μm (mean = 477 × 367, SD 10, 6.4, n = 3), with thin central to eccentric necks protruding from periderm and extending beyond surface, length (284–)386–496(–504) μm (mean = 425, SD 122, n = 3), basal diameter (44–)45–53(–61) μm (mean = 50, SD 9.8, n = 3), distal diameter (33–)36–43(–47) μm (mean = 40, SD 7.4, n = 3), and hyaline ostiolar opening. Cream yellow mycelium at base of perithecia, perithecia collapsing from bottom when dry. Asci cylindrical, elongated, floating free in perithecia, (57–)64–72(–78) × (13–)15–17(–21) μm (mean = 67 × 16, SD 6.4, 1.9, n = 22), apical ring 2–4 μm diam., visible as two slightly reniform bodies, with eight ascospores arranged obliquely parallel or biseriate. Asco-



below branches. The type species of each genus is in bold. *Occultocarpon* forms a distinct branch within the *Gnomoniaceae*. Among the genera associated primarily with *Betulaceae*, only three species, *Cryptosporella hypodermia*, *C. tiliae*, and *C. wehmeyeriana* are not associated with *Betulaceae*. *Species names updated based on Mejía et al. 2011a, b.

Holotypus: P.R. CHINA. Yunnan, Jingdong County, Ailaoshan Mountain, on the road, at 2,381 m above sea level, 24° 31' 00.9" N, 101° 00'47.1" E, on dead, still attached branches of *Alnus nepalensis*, 14 Jul 2008, coll. Luis C. Mejía LCM524 (HOLOTYPE BPI879253, derived cultures CBS 129146 = LCM524.01 and LCM524.02).

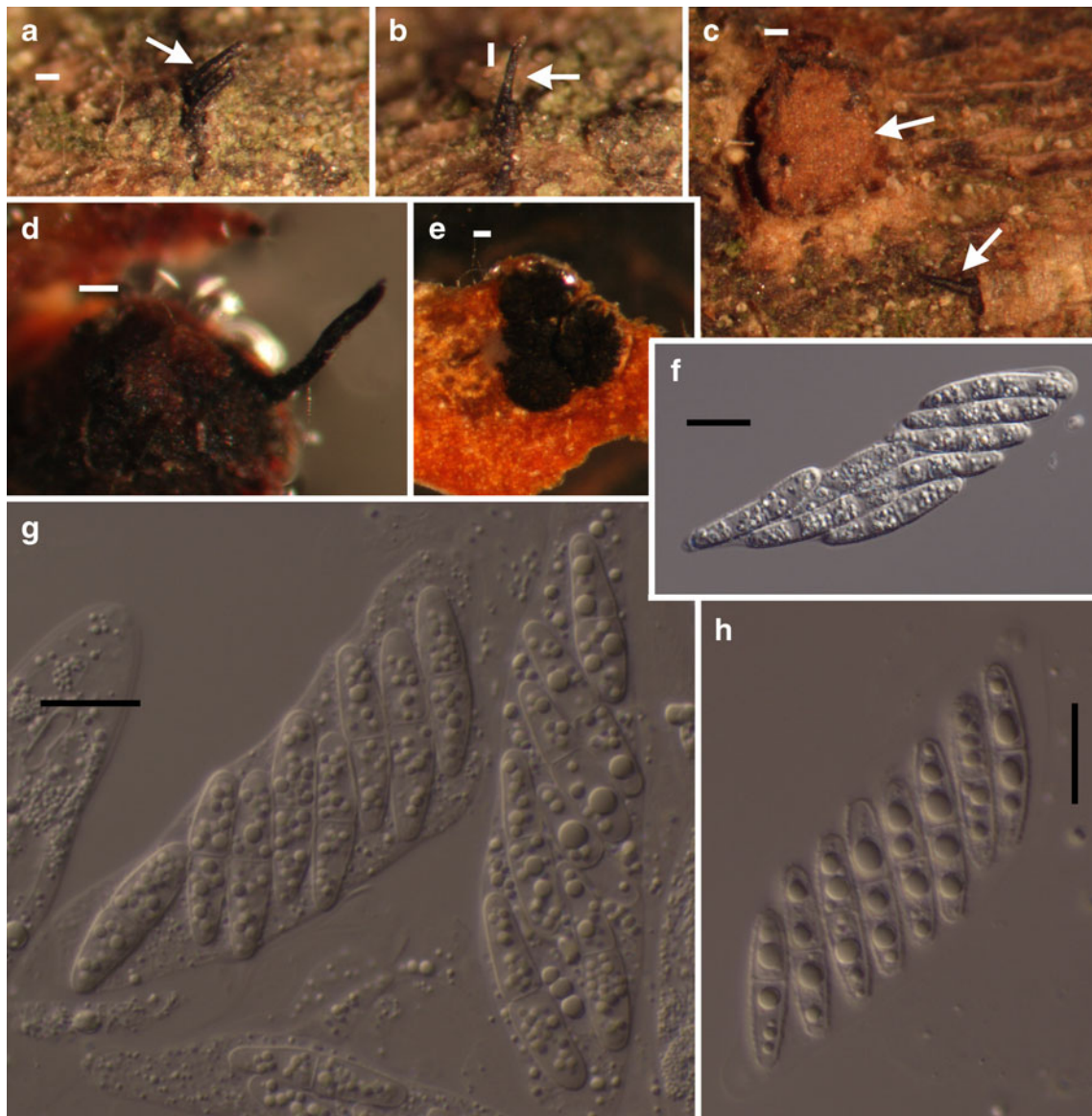


Fig. 2 *Occultocarpon ailaoshanense*. **a-b** Thin perithecial necks protruding the host bark periderm (indicated by the arrow). **c** Exposed stroma after removal of host periderm (upper arrow) and perithecial neck protruding the host periderm (lower arrow). **d** Perithecium seen

from the side. **e** Group of perithecia as seen from the bottom after peeling off the host periderm. **f-h** Asci with ascospores. **a-c, g-h** BPI 879253 (holotype). **d-e** BPI 979255. **f** BPI 879254. Scale bars **a-e** = 100 μm; **f-h** = 10 μm

Other specimens examined: P.R. CHINA. Yunnan, Jingdong County, Ailaoshan Mountain, on the road, at 2,381 m above sea level, 24° 31' 00.9" N, 101° 00' 47.1" E, ca. 100 m from the holotype location, on *Alnus nepalensis*, 14 Jul 2008, LCM522 (BPI879254, derived cultures CBS CBS 129147 = LCM522.01 and LCM522.02); LCM561 (BPI879255, derived cultures LCM561.02, CBS 129145 = LCM561.04).

Discussion

Ten monophyletic teleomorphic genera were supported in the Gnomoniaceae based on Sogonov et al. (2008).

The newly discovered *Occultocarpon ailaoshanense* is now placed as an additional teleomorphic genus in this family by a three-gene phylogeny (Fig. 1). *Occultocarpon ailaoshanense* contains features that are common to other species of the Gnomoniaceae such as black perithecia that collapse from the bottom when dry, arranged in groups, upright perithecial necks that protrude through the host periderm, a refractive apical ring in the asci, and elliptical, guttulate ascospores. Additionally *O. ailaoshanense* occurs immersed in bark, as is common for Gnomoniaceae, and its host is *Alnus nepalensis*, a species of the Betulaceae, host to many species of Gnomoniaceae.

A combination of morphological features also indicates that *O. ailaoshanense* represents a distinct genus and species. These include grouped perithecia embedded in a stroma within the bark of the host each with a thin, central to eccentric upright neck that have a distal diameter less than 50 µm. Other genera and species of Gnomoniaceae such as *Amphiportha hranicensis* and species of *Cryptosporella* and *Plagiostoma* have grouped perithecia in bark (Mejía et al. 2011a, b); however, the necks of these taxa are generally greater than 50 µm diameter. *Plagiostoma exstocollum* is a species with thin necks that grows on *Corylus* (Betulaceae); however, the necks of this species are marginal (Mejía et al. 2011b). The ascospore morphology of previously described, bark-inhabiting species of Gnomoniaceae is different than that of *O. ailaoshanense* (see Barr 1978; Mejía et al. 2008; Monod 1983; Sogonov et al. 2008). When inside the ascus, the ascospores of *O. ailaoshanense* appear cylindrical and resemble those of *Ditopella ditopa*, type species of *Ditopella*. When outside the asci, the ascospores of *O. ailaoshanense* are oblong elliptical-elongated with the upper cells slightly wider than the lower ones. Additionally, asci of *O. ailaoshanense* contain eight ascospores per ascus in contrast to those of *Ditopella ditopa* that contain 32 ascospores per ascus. Perithecia of *Ditopella* are solitary and scattered in host tissue, not in groups as in *O. ailaoshanense*. The ascospores of species of *Cryptosporella* are generally non-septate (Mejía et al. 2011a) while those of *Occultocarpon* are one-septate. Interestingly *Cryptosporella*, *Ditopella*, and *Occultocarpon* are all bark-inhabiting genera associated with the Betulaceae.

In addition to *O. ailaoshanense*, *Plagiostoma yunnanense*, another species of Gnomoniaceae from Yunnan, was collected during this same trip and described (Mejía et al. 2011b). The discovery of *O. ailaoshanense* and *P. yunnanense* indicates that the diversity of gnomoniaceous fungi in China is greater than has been reported. Although it was expected that additional species of Gnomoniaceae in association with Betulaceae would be found in China during this trip, factors such as the amount of time spent collecting and the seasonality of perithecial production in species of Gnomoniaceae limited these efforts. In Europe and North America, most species of Gnomoniaceae start producing perithecia early in the spring but this varies with species both in timing and duration. Near Lijiang in Yunnan, we collected leaves of *Corylus* (Betulaceae) with empty perithecia having the morphology of *Gnomonia*. Collecting earlier in the year may yield more specimens of Gnomoniaceae with ascospores. DNA sequences deposited in Genbank suggest that additional species of Gnomoniaceae exist in China. Phylogenetic analyses including ITS sequences of endophytic fungi isolated from a host in the Betulaceae from China place these fungi in the Gnomoniaceae (see Mejía et al. 2011a). Unfortunately

voucher specimens are not available for those DNA sequences.

By focusing our collecting efforts on species of Betulaceae, Fagaceae, and Salicaceae several new species of Gnomoniaceae have been discovered in temperate forests of Europe and North America, as well as in neotropical montane forests (Mejía et al. 2011a, b). These findings together with the discovery of *O. ailaoshanense* on *A. nepalensis* and the recently described *P. yunnanense* suggest that additional undiscovered species of Gnomoniaceae exist in China. Yunnan, a biodiversity hotspot with many plant species from host families associated with species of Gnomoniaceae, seems an attractive region for the discovery of new taxa in this family.

Acknowledgement This work was funded by the National Science Foundation Partnerships for Enhancing Expertise in Taxonomy (NSF 03–28364). Additional funding for field work by LCM was received through Rutgers University, New Brunswick, New Jersey, from the Spencer Davis Research Award from the Department of Plant Biology and Pathology, and from the Myron Backus Award from the Mycological Society of America. ZLY was supported by the Hundred Talents Program of the Chinese Academy of Sciences. Christian Feuillet kindly provided translations of the diagnoses into Latin.

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