

# *Amylostereum orientale* sp. nov. (Basidiomycota, Russulales) and first report of *A. areolatum* in China based on morphological and molecular characters

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*Amylostereum orientale* S. H. He & Hai J. Li on *Cunninghamia lanceolata* is described from China as a new species, and *A. areolatum* is reported for the first time from China. *Amylostereum orientale* is very similar to *A. laevigatum* in morphology, but the former has distinctly ellipsoid basidiospores and the host is a plant native in China. Representatives of the new taxon form a distinct clade in the phylogenetic tree inferred from the analysis of ITS rDNA sequences. A complete description with illustrations of the new species is provided.

*Amylostereum* Boid with *A. chailletii* (Fr.) Boid as its type is a fascinating genus, as some species of the genus are known as symbionts of mycophagous horn-tails (Slippers et al. 2003). Species of this genus are characterized by numerous thick-walled and apically encrusted cystidia in hymenium and context, nodose-septate generative hyphae and distinctly amyloid basidiospores (Eriksson and Ryvarden 1973). For the time being, only four species have been recognized worldwide, and all of them occur on gymnosperm trees (Tabata et al. 2000). Traditionally, *Amylostereum* has been placed in the Stereaceae due to its morphological similarity to *Stereum* Hill ex Pers. Phylogenetic analysis based on DNA sequences, however, showed that *Amylostereum* is close to *Echinodontium* Ellis & Everh. and should be placed in the monotypic family Amylostereaceae (Tabata et al. 2000).

Two species of *Amylostereum* (*A. chailletii* and *A. laevigatum* (Fr.) Boidin) have been recorded in China (Dai et al. 2004, Dai 2011). In 2010, an intensive investigation of wood-inhabiting fungi in Yellow Mountain National Park, Anhui Province was carried out, and later several new species and Chinese new records were published (Cui and Jia 2011, Cui et al. 2011, He and Dai 2012). Recently, an unknown *Amylostereum* species on *Cunninghamia lanceolata* collected from this area was carefully studied, and both morphological and molecular results indicate that it is different from all hitherto known species of the genus. Therefore, we describe *Amylostereum orientale* sp. nov. in the present paper. In addition, several specimens collected from Yunnan Province were identified

as *A. areolatum* (Chaillet ex Fr.) Boidin, and this cosmopolitan species is herewith reported for the first time from China. These two new records for China are here described and illustrated.

## Material and methods

Voucher specimens were deposited in the herbarium BJFC, and the microscopic procedure followed Dai (2010). In presenting the size range of spores and cystidia, 5% of the measurements were excluded from each end of the range, and the measurements were given in parentheses. In the text the following abbreviations are used: L = mean spore length, W = mean spore width, Q = variation in the L/W ratios between studied specimens, n = the number of spores measured from given number of specimens. CB is the abbreviation of Cotton Blue in lactic acid. CB– = acyanophilous. Special color terms follows Petersen (1996).

The F-130 Phire® Plant Direct PCR Kit was employed for DNA extraction and PCR amplification from herbarium specimens according to the manufacturer's instructions. Approximately 600 base pairs of the ITS region were amplified with primers ITS5 (GGA AGT AAA AGT CGT AAC AAG G) and ITS4 (TCC TCC GCT TAT TGA TAT GC; White et al. 1990), using the following procedure: initial denaturation at 98°C for 5 min, followed by 39 cycles of 98°C for 5 s, 58°C for 5 s and 72°C for 5 s, and a final extension of 72°C for 10 min. DNA sequencing was performed at Beijing Genomics Inst., and all the newly

generated sequences were submitted to GenBank (<www.ncbi.nlm.nih.gov>).

Sampled species and their voucher specimen information and GenBank accession numbers are listed in Table 1. The phylogeny was inferred from the analysis of 25 ITS sequences from five *Amylostereum* and three *Echinodontium* species. *Stereum annosum* Berk. & Broome was selected as an outgroup (Tabata et al. 2000). The sequences were aligned using ClustalX 1.83 (Chenna et al. 2003). Alignments were optimized manually in BioEdit (Hall 1999). Maximum parsimony analyses were performed using PAUP\* (Swofford 2002). Gaps in the alignments were treated as missing data. Trees were generated using 100 replicates of random stepwise addition and tree-bisection reconnection (TBR) branch-swapping algorithm, with all characters given equal weight. Branch supports for all parsimony analysis were estimated by performing 1000 bootstrap replicates (Felsenstein 1985) with a heuristic search of 10 random-addition replicates for each bootstrap replicate. The tree length (TL), consistency indices (CI), retention indices (RI), rescaled consistency indices (RC) and homoplasy index (HI) were calculated for each generated tree. Trees were figured in TreeView (Page 1996).

***Amylostereum orientale* S. H. He & Hai J. Li  
sp. nov. (Fig. 1, 2)**

Mycobank no. MB 801336

*Carpophorum annuum*, *resupinatum*, *adnatum*, 50–200 µm *crassum*. *Hymenophorum laeve*, *submurinum* vel *griseosepiaceum*. *Hymenium* et *subhymenium* *adsunt*. *Systema*

*hypharum monomiticum*, *hyphae generatoriae fibulatae*. *Cystidia abunda*, *encrustata*, (17–)20–50 × 4–7(–8) µm. *Sporae ellipsoideae*, *amyloideae*, CB–, 5–7(–7.5) 3.5–4.2(–4.5) µm.

**Type:** China, Anhui Prov., Huangshan County, Yellow Mountain National Park, on fallen trunk of *Cunninghamia lanceolata*, 22 Oct 2010 S. H. He 479 (holotype: BJFC).

**Etymology**

The epithet ‘*orientale*’ (Latin) refers to the known distribution of the species in eastern Asia.

**Description**

Basidiocarps annual, resupinate, closely adnate, coriaceous, first as small colonies, later confluent up to 20 cm or more in longest dimension, 50–200 µm thick. Hymenophore smooth, azonate, pale mouse gray to grayish brown, usually not cracked or sometimes finely cracked when dry; margin thinning out, indistinct, paler or concolorous with hymenophore surface. Hymenium and subhymenium present. Subhymenium composed of several rows of overlapping cystidia and interspersed agglutinated generative hyphae. Hyphal system monomitic; generative hyphae with clamp connections, thin- to thick-walled, agglutinated, vertically arranged, 2–5 µm in diameter. Cystidia numerous, subulate, some slightly sigmoid, yellowish brown, thick-walled, with acute tips, encrusted with fine crystals in the apical part, (17–)20–50 × 4–7(–8) µm, projecting up to 10 µm above the hymenium, with encrusted part 10–25 µm long; juvenile cystidia subulate, hyaline, thin-walled, smooth.

Table 1. A list of species, voucher specimen/isolate information and accession numbers of ITS sequences used in the phylogenetic analysis. \* = sequences obtained from the current study.

Species	Specimen/isolate	Host	Locality	ITS accession no.
<i>Amylostereum areolatum</i>	Yuan 2059	<i>Abies</i> sp.	China	JX049993*
	Yuan 2090	<i>Abies</i> sp.	China	JX049992*
	B1350	<i>Pinus densiflora</i>	Japan	AF218389
	N36	<i>Picea abies</i>	Latvia	FJ903375
	AH1-01	<i>Sirex noctilio</i>	USA	GQ422464
<i>A. chailletii</i>	RLG8273	—	USA	HM461077
	B32	<i>Picea abies</i>	Latvia	FJ903304
	B3/2/1/2/A2	<i>Abies alba</i>	Slovenia	AM981207
	NMG 5	—	China	HM776410
<i>A. ferreum</i>	CBS637.84	<i>Podocarpus lambertii</i>	Brazil	HM461082
	CBS634.84	<i>Podocarpus lambertii</i>	Brazil	AF218390
<i>A. laevigatum</i>	Kotiranta 20108	<i>Juniperus</i> sp.	Finland	JX049990*
	Kotiranta 20661	<i>Thuja</i> sp.	Finland	JX049991*
	CBS626.84	<i>Juniperus nana</i>	France	HM461080
	CBS627.84	<i>Juniperus nana</i>	France	HM461081
	CBS624.84	<i>Juniperus nana</i>	France	AF218396
	NH12863	—	Sweden	AF506407
<i>A. orientale</i>	olrim409	<i>Picea abies</i>	Sweden	AY781246
	He 468	<i>Cunninghamia lanceolata</i>	China	JX049989*
	He 479	<i>Cunninghamia lanceolata</i>	China	JX049987*
	He 480	<i>Cunninghamia lanceolata</i>	China	JX049988*
<i>Echinodontium japonicum</i>	B 1375	<i>Quercus gilva</i>	Japan	AF218399
<i>E. tinctorium</i>	B 1122	<i>Tsuga</i> sp.	USA	AF218397
	DAOM 16666	—	—	AY854088
<i>E. tsugicola</i>	B 1377	<i>Tsuga diversifolia</i>	Japan	AF218398
Outgroup				
<i>Stereum annosum</i>	FPL8562	—	USA	AF218401



Figure 1. A basidiocarp of *Amylostereum orientale* sp. nov. on *Cunninghamia lanceolata* (the holotype).

Basidia clavate, with four sterigmata and a basal clamp connection,  $20\text{--}26 \times 4\text{--}6 \mu\text{m}$ ; basidioles in shape similar to basidia, but slightly smaller. Basidiospores ellipsoid, hyaline, thin-walled, smooth, amyloid, CB–,  $5.0\text{--}7.0(-7.5) \times 3.5\text{--}4.2(-4.5) \mu\text{m}$ ,  $L = 6.19 \mu\text{m}$ ,  $W = 3.95 \mu\text{m}$ ,  $Q = 1.57$  ( $n = 90/1$ ).

#### Additional specimens examined

*Amylostereum orientale* (paratypes): China, Anhui Prov., Huangshan County, Yellow Mountain National Park, on fallen trunk of *Cunninghamia lanceolata*, 22 Oct 2010 S. H. He 468 and 480 (paratypes: BJFC).

*Amylostereum areolatum*: China, Yunnan Prov., Shangri-La County, on fallen trunk of *Pinus*, 29 Aug 2006 H. S. Yuan 1919; on stump of *Picea*, 29 Aug 2006 H. S. Yuan 1933; on stump of *Abies*, 1 Sep 2006 H. S. Yuan 2059; on fallen trunk of *Abies*, 1 Sep 2006 H. S. Yuan 2090.

#### Phylogenetic analysis

Seven ITS sequences were newly generated in this study (GenBank accession no. JX049987–JX049993, Table 1). The alignment of 26 ITS sequences of nine species resulted in 560 base pairs with 75 parsimony informative characters. Five equally parsimonious trees were yielded from the analysis with  $TL = 255$ ,  $CI = 0.867$ ,  $RI = 0.878$ ,  $RC = 0.761$  and  $HI = 0.133$  (Fig. 3). The strict consensus tree showed that species of *Amylostereum* and *Echinodontium* were well separated from the outgroup, and sequences of *A. orientale* appeared as a distinct lineage with strong support (bootstrap value = 99%). This indicated that *A. orientale* was different from all other species sampled in the phylogenetic analysis. The sequences of *Amylostereum areolatum*, including those newly obtained from Chinese specimens,

formed a strongly supported clade (bootstrap value = 98%) in the phylogenetic tree.

#### Discussion

In morphology, *Amylostereum orientale* is very similar to *A. laevigatum*, both of which have resupinate and thin basidiocarps without hyphal layers, and a monomitic hyphal system. *Amylostereum laevigatum* is a common species in Europe, and has been reported on *Cupressus*, *Juniperus*, *Taxus* and cultivated *Thuja* (Eriksson and Ryvarden 1973, Bernicchia and Gorjón 2010). However, data on spore sizes and shapes of this species are not consistent among different descriptions (cylindrical or narrowly ellipsoid,  $7\text{--}12 \times 3\text{--}4 \mu\text{m}$  in Eriksson and Ryvarden 1973; cylindrical to fusiform,  $7\text{--}8 \times 3\text{--}4 \mu\text{m}$  in Bernicchia and Gorjón 2010; ellipsoid to oblong-ellipsoid,  $5.9 \times 3.8$  or  $7.2 \times 4.0$  on average in Boidin and Lanquetin 1984). *Amylostereum orientale* differs from *A. laevigatum* in having distinctly ellipsoid basidiospores ( $5\text{--}7 \times 3.5\text{--}4.2 \mu\text{m}$ ,  $Q = 1.57$ ) and a host of *Cunninghamia lanceolata* which is native to China. Moreover, in the phylogenetic tree inferred from the analysis of ITS rDNA sequences, *A. orientale* and *A. laevigatum* formed distinct clades (Fig. 3).

*Amylostereum laevigatum* has also been reported from Japan, where it was isolated from a woodwasp (*Urocerus* sp.) and basidiomes of the fungus were found on *Cryptomeria* and *Chamaecyparis* (Tabata and Abe 1997, 1999, Tabata et al. 2000). However, in the phylogenetic trees of Tabata et al. (2000), sequences of *A. laevigatum* from Japan formed a clade distinct from those in France. Compared with the specimens from France, *A. laevigatum* from Japan were reported to have smaller basidiospores and the hosts were different, viz. *Cryptomeria* and *Chamaecyparis* which

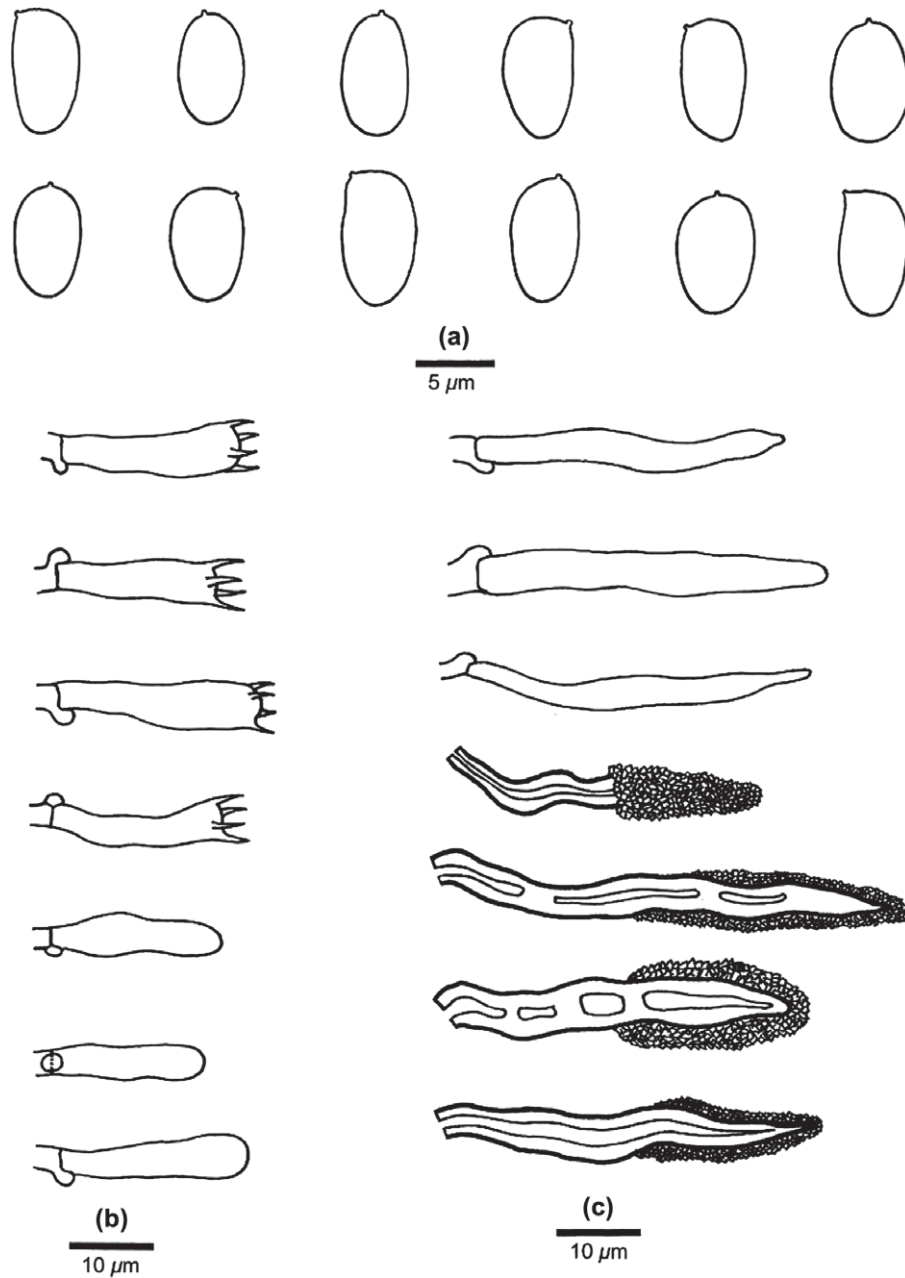


Figure 2. Microscopic structures of *Amylostereum orientale* sp. nov. (a) basidiospores, (b) basidia and basidioles, (c) cystidia. Drawn from the holotype.

are native to Asia (Tabata et al. 2000). Although the specimens of Japanese '*A. laevigatum*' are not available for us, one ITS sequence from Japan (GenBank accession no. AF218395) nested within the *A. orientale* clade rather than the European *A. laevigatum* clade (including sequences from Finland, France and Sweden) in our analysis (data not shown). Therefore, we suppose that the Japanese '*A. laevigatum*' may be conspecific with *A. orientale*, though studies of more specimens of these two similar species are needed.

*Amylostereum areolatum*, a cosmopolitan species reported from many countries is a symbiont of several siricid woodwasps including *Sirex noctilio* (Wilson et al. 2009). Recently, several specimens collected from Yunnan Province, southwestern China were identified as *A. areolatum* (Fig. 4) based on morphological and molecular evidences, and it is the first time to report this species in China. However, the woodwasps associated with the species in China are unknown.



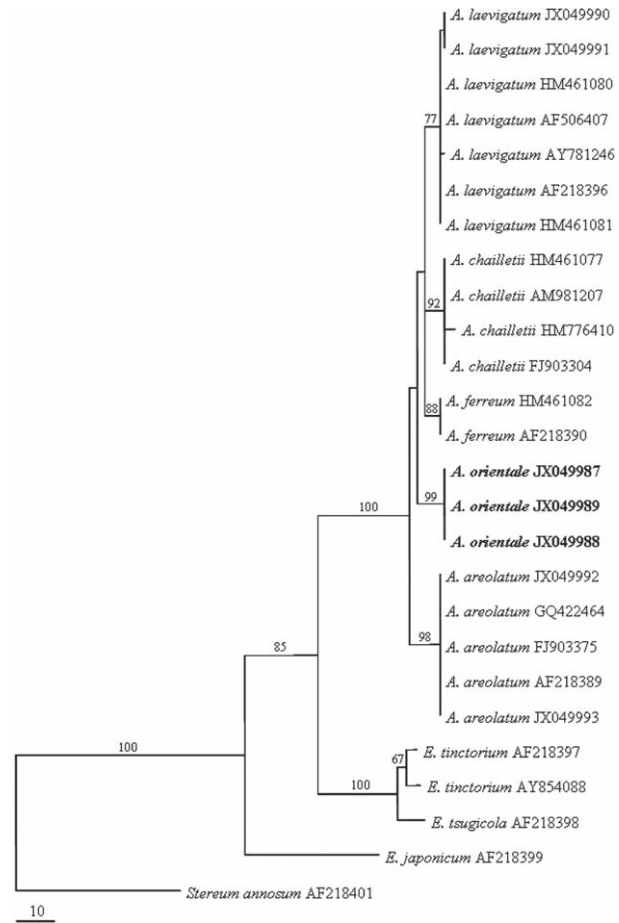


Figure 3. Strict consensus tree obtained from Maximum Parsimony analysis of ITS sequences of *Amylostereum* and *Echinodontium*. *Stereum annosum* was used as outgroup. Parsimony bootstrap values (> 50%) are shown. Bold entries denote the new species.



Figure 4. Basidiocarps of *Amylostereum areolatum* on *Picea* (Yuan 1933).

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