**RESEARCH ARTICLE** 



# Additions to tribe Chromosereae (Basidiomycota, Hygrophoraceae) from China, including Sinohygrocybe gen. nov. and a first report of Gloioxanthomyces nitidus

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#### Abstract

Sinohygrocybe gen. nov., typified by S. tomentosipes sp. nov., is described upon morphological and molecular evidence. The new genus is characterised by its sinuate to subdecurrent or short deccurent, usually furcate and interveined and relatively distant lamellae, dry and whitish tomentose stipe, thin-walled ellipsoid to oviod, non-constricted basidiospores and particularly elongated basidia and a ratio of basidiospore to basidium length of >5 to 8; it is close to genera *Chromosera* and *Gloioxanthomyces* of the tribe Chromosereae, but morphologically differs from *Chromosera* in less umbilicate basidiomata, tomentose stipe and usually longer basidia and differs from *Gloioxanthomyces* in more robust basidioma and less glutinous pileus and/or stipe surface. Phylogenetic analyses, with ITS-LSU-RPB2 data, also indicate that *Sinohygrocybe* forms a very distinct and independent clade at the generic level. In addition, a Chinese new record *G. nitidus* is described here.

#### Keywords

East Asia, new record species, new taxa, phylogeny overview

## Introduction

Hygrophoraceae Lotsy (Hymenomycetes, Basidiomycota) is a large family in Agaricales, including 26 genera and over 600 species (Lodge et al. 2014). In a six-gene phylogenetic tree of Agaricales, Hygrophoraceae, Pterulaceae Corner, Typhulaceae Jülich and some small groups formed a Hygrophoroid clade, which is one of the six largest clades in Agaricales (Matheny et al. 2006); and in a genome based mushroom tree of life, Hygrophoraceae and Clavariaceae Chevall. are representative families of the suborder Hygrophorineae Aime, Dentinger & Gaya, which is one of the seven suborders of the Agaricales (Dentinger et al. 2016). Traditionally, the family Hygrophoraceae referred to a group of agaricoid, waxy-gilled and white-spored mushrooms; and a majority of the members are classified in the type genus Hygrophorus Fr. and genus Hygrocybe (Fr.) P. Kumm. Morphological characters of the Hygrophoraceae taxa are relatively simple (usually without annulus or volva and a cystidiate) amongst the agaric fungi and their basidioma colours are often very susceptible to the environmental conditions and developmental stages, making their classification and identification difficult, so it is often challenging to make correct identification and taxonomy of them just according to morphological recognition (Young 2005). Modern molecular techniques have been revolutionising the taxonomy and phylogeny of Hygrophoraceae.

Lodge et al. (2014) had conducted the most comprehensive molecular phylogenetic study on the family until now, therefore their systematic viewpoint on Hygrophoraceae is adopted in this paper. According to their study, the family could be divided into four groups at subfamily level, i.e. subfamily Hygrophoroideae E. Larss., Lodge, Vizzini, Norvell & S.A. Redhead, Hygrocyboideae Padamsee & Lodge, Lichenomphalioideae Lücking & Redhead and Cuphophylloid grade. The subfamily Hygrocyboideae could be divided into three tribes, i.e. tribe Chromosereae, Humidicuteae and Hygrocybeae; and the tribe Chromosereae included two sister genera, *Chromosera* Redhead, Ammirati & Norvell and *Gloioxanthomyces* Lodge, Vizzini, Ercole & Boertm.

*Chromosera*, the type genus of the tribe Chromosereae, was erected to accommodate *Omphalina cyanophylla* (Fr.) Quél. which was originally described from Sweden and combined as *C. cyanophylla* (Fr.) Redhead, Ammirati & Norvell (Redhead et al. 1995, 2012). Now, another four species, formerly placed in *Hygrocybe* or *Hygrophorus*, are also classified into *Chromosera*, i.e. *C. citrinopallida* (A.H. Sm. & Hesler) Vizzini & Ercole originally described from USA, *C. lilacina* (P. Karst.) Vizzini & Ercole originally described from the northern Fennoscandia, *C. viola* (J. Geesink & Bas) Vizzini & Ercole originally described from Belgium and *C. xanthochroa* (P.D. Orton) Vizzini & Ercole originally described from Scotland (Lodge et al. 2014).

*Gloioxanthomyces* is a small genus with only two known species, the type species *G. vitellinus* (Fr.) Lodge, Vizzini, Ercole & Boertm. originally described from Europe and *G. nitidus* (Berk. & M.A. Curtis) Lodge, Vizzini, Ercole & Boertm. from North America (Crous et al. 2004, Lodge et al. 2014). Before the recognition of *Gloioxanthomyces*, those two species were usually placed in the genus *Hygrocybe* as *H. vitellina* (Fr.) P. Karst and *H. nitida* (Berk. & M.A. Curtis) Murrill, respectively. Morphologi-

cally, the main differences between the two species were in their basidiospore sizes: *G. nitidus* had ellipsoid to oblong basidiospores, measuring 7–10 × 5–6 µm with Q = 1.3–1.8; while *G. vitellinus* had subglobose basidiospores, measuring 6.5–8.5 × 5–7 µm with Q=1.1–1.6 (Boertmann 1990). Since their differences were limited, the two taxa seemed to be conspecific (Boertmann 2011). However, according to the phylogenetic analyses with ITS data by Boertmann (2012), the European collections clearly clustered together as the *G. vitellinus* species clade, while the North American materials independently formed another group as the *G. nitidus* species clade, thus they could actually be sharply defined as two separated sister species.

During the studies on the Chinese Hygrophoraceae in recent years, some collections morphologically corresponding to tribe Chromosereae were collected. Comprehensive observation and analyses revealed some interesting findings, which can contribute to the taxonomic knowledge of the tribe. In this paper, we aim to: 1) formally describe a new genus of tribe Chromosereae from East Asia based upon morphological and molecular analyses and present a Chinese new record of *Gloioxanthomyces nitidus*; 2) reconstruct the phylogeny of the family Hygrophoraceae using 3 gene regions, i.e. the internal transcribed spacer region (ITS), the large subunit nuclear ribosomal RNA region (nrLSU) and the nuclear RPB2 6F to 7.1R region (RPB2). Detailed studies were therefore conducted and the results are presented as follows.

## Materials and methods

#### Morphological studies

Specimens were photographed and annotated in the field and then dried in an electric drier. Macroscopic descriptions were gained from the original field notes and photographs. Colour descriptions followed Kornerup and Wanscher (1978). Tissue sections were immersed in 5% potassium hydroxide (KOH) and/or 1% Congo Red solution for microscopical examinations, but in distilled water for colour descriptions of basidia, pileipellis and stipitipellis. From a mature specimen, over 40 basidiospores and 20 basidia were randomly selected and measured under a light microscope in KOH. The notation (a)b–c(d) was used to describe dimensions where the range b–c representing 90% or more of the measured values and a, d were the extreme values. The length/width ratio of spores was presented as Q and the mean ratio was presented as  $Q_m$ . The studied specimens were deposited in the Fungal Herbarium of Guangdong Institute of Microbiology (GDGM), Guangzhou, China.

#### Molecular studies

Genomic DNA was extracted from the herbarium specimens using the Sangon Fungus Genomic DNA Extraction kit (Sangon Biotech Co., Ltd., Shanghai, China) according to the manufacturer's instructions. The ITS, LSU and RPB2 gene regions were amplified by Polymerase Chain Reaction, using universal primers ITS1F/ITS5 and ITS4 (White et al. 1990; Gardes and Bruns 1993), LR0R and LR5 (http://biology.duke. edu/fungi/mycolab/primers.htm) and RPB2-6F and RPB2-7.1R (Matheny 2005), respectively. Amplified products were sequenced by Beijing Genomic Institute (BGI) using the same primers. The abi format sequences were assembled by SeqMan version 7.1.0 (DNAStar, Inc.) and then the assembled sequences were submitted to GenBank.

In this study, two datasets were constructed. The first one is an ITS-LSU-RPB2 matrix of the family Hygrophorceaeae for making a comprehensive phylogenetic tree and analysing the positions of the new taxa; most known species of Hygrophoraceae with available sequences from reliable sources were included in the dataset, each of them having at least an LSU sequence and *Typhula phacorrhiza* (Reichard) Fr. was selected as the outgroup referred from Yang et al. (2013) and Lodge et al. (2014). The second dataset is an ITS matrix of the tribe Chromosereae and *Hygrocybe conica* (Schaeff.) P. Kumm. and *H. conica* var. *conicoides* (P.D. Orton) Boertm. were chosen as outgroups. Each gene was independently aligned on the online MAFFT service (Katoh et al. 2017), then combined by the Geneious software (Biomatters Ltd.) for the first dataset. Maximum likelihood phylogenetic trees were generated by the RAxML software (Stamatakis 2014) on the CIPRES service (Miller et al. 2010) with 1000 bootstrap replications using the default options.

## Results

#### Molecular phylogenetic results

The combined 3-gene dataset composed of 120 samples (Table 1), including 5 newly sequenced samples and 115 published ones. In the final matrix, the ITS, LSU and RPB2 regions comprised positions 1 to 1751, 1752 to 2873, 2874 to 3759, respectively. In the 3-gene Maximum Likelihood tree (Fig. 1), the four Chinese collections (GDGM43351 and GDGM43347 from Sichuan province, GDGM50075 and GDGM50149 from Hunan province) formed a strong monophyletic clade with 100% bootstrap support, which was near the *Chromosera-Gloioxanthomyces* clade composed of members of *Chromosera* and *Gloioxanthomyces* with 76% bootstrap support.

The ITS dataset included 30 samples of all known taxa of tribe Chromosereae and 2 *Hygrocybe* sequences chosen as the outgroups, the matrix length is 679 bp. In the ITS Maximum Likelihood tree (Fig. 2), collections of the species *G. nitidus* and *G. vitellinus* were clustered together with 93% and 100% support values, respectively and the North American and the East Asian *G. nitidus* were clustered as sister groups with 93% support value; all the members of *Chromosera* (except *C. viola*), *Gloioxanthomyces* and *Sinohygrocybe* were clustered together with 95%, 93% and 100% support values, respectively; and the *Chromosera-Gloioxanthomyces* clade was presented as the sister clade of the *Sinohygrocybe* clade with strong support value (100%).

Table	I. Sequences infor	mation of samples	used for the ITS-LS	U-RPB2 combine	ed tree. Newly	generated
sequen	ces were bold.					

Species name	Isolate/voucher ID	ITS	LSU	RPB2
Acantholichen albomarginatus	MDF543	KT429797	KT429809	_
Acantholichen campestris	DIC595b	KT429798	KT429810	KT429818
Acantholichen galapagoensis	MDF057	KT429784	KT429799	KT429811
Acantholichen galapagoensis	MDF058	KT429785	KT429800	KT429812
Acantholichen galapagoensis	MDF089	KT429786	KT429801	_
Acantholichen galapagoensis	MDF090	KT429787	KT429802	KT429813
Acantholichen galapagoensis	MDF093	KT429790	KT429803	KT429814
Acantholichen galapagoensis	MDF094	KT429791	KT429804	KT429815
Acantholichen galapagoensis	MDF100	KT429792	KT429805	KT429816
Acantholichen pannarioides	MDF352	KT429795	KT429807	KT429817
Acantholichen pannarioides	Bungartz 5593	EU825953	EU825953	_
Acantholichen sorediatus	DIC335	KT429794	KT429806	_
Acantholichen variabilis	MDF679	KT429796	KT429808	_
Ampulloclitocybe clavipes	DJL06TN40	_	KF381542	KF407938
Ampulloclitocybe clavipes	AFTOL-ID 542	AY789080	AY639881	AY780937
Arrhenia auriscalpium	Lutzoni Lamoure 910824-3	U66428	U66428	_
Arrhenia lobata	Lutzoni Lamoure 910824-1	U66429	U66429	_
Cantharellula umbonata	RDY-1366 (SFSU)	KF381519	AF261443	_
Cantharocybe brunneovelutina	DJL-BZ-1883 (holotype)	KX452404	HM588721	_
Cantharocybe gruberi	AFTOL-ID 1017	DQ200927	DQ234540	DQ385879
Cantharocybe gruberi	AH24539	JN006422	JN006420	_
Cantharocybe virosa	TENN 63483(holotype)	KX452405	JX101471	_
Chromosera citrinopallida	DUKE8895	U66435	U66435	_
Chromosera citrinopallida	D. Boertmann 2006/2	KF291072	KF291073	_
Chrysomphalina chrysophylla	AFTOL-ID 1523	_	DQ457656	DQ192180
Chrysomphalina chrysophylla	S.A. Redhead 7700	_	U66430	U66430
Chrysomphalina grossula	OSC 113667	_	EU652372	EU644703
Chrysomphalina grossula	OSC 113683	_	EU652373	EU644704
Cora minor	Luecking 15243	EU825968	EU825968	_
Cuphophyllus acutoides var. pallidus	CFMR TN-257	-	KF291097	_
Cuphophyllus adonis	MES-152	_	KF291036	KF291037
Cuphophyllus aff. pratensis	PBM-752	_	DQ457650	KF442252
Cuphophyllus aurantius	CFMR PR-6601	_	KF291100	KF291102
Cuphophyllus bicolor	DJL-PR-2	_	KF291056	_
Cuphophyllus flavipes	Hattori-JP-6	-	KF291045	KF291047
Cuphophyllus fornicatus	D. Boertmann 2009/94	_	KF291124	_
Cuphophyllus pratensis	DJL-Scot-8	_	KF291058	_
Cuphophyllus sp.	AM01	_	HM026542	

Species name	Isolate/voucher ID	ITS	LSU	RPB2
Dictyonema glabratum	AFTOL-ID 1995	DQ917656	DQ917661	_
Dictyonema glabratum	Luecking 15581	EU825958	EU825958	_
Dictyonema glabratum	Luecking 16563	EU825956	EU825956	_
Dictyonema glabratum	R06	EU825959	EU825959	_
Dictyonema glabratum	R11	EU825960	EU825960	_
Dictyonema glabratum	R18	EU825961	EU825961	_
Dictyonema glabratum	R20	EU825963	EU825963	_
Gliophorus aff. psittacinus	CFMR JP-4	KF291079	KF291080	-
Gliophorus graminicolor	TJB-10048	KF381520	KF381545	KF407936
Gliophorus psittacinus	D. Boertmann 2002/10	KF291075	KF291076	KF291078
Gloioxanthomyces nitidus	GDGM41710	MG712283-4	MG712282	MG711911
Haasiella splendidissima	Herbarium Roux n. 3666	JN944398	JN944399	-
Haasiella splendidissima	Herbarium Roux n. 4044	JN944400	JN944401	-
Haasiella splendidissima	JVG1071013-1	JN944395	JN944396	-
Haasiella venustissima	A. Gminder 971488	KF291092	KF291093	-
Haasiella venustissima	E.C. 08191	JN944393	JN944394	-
Humidicutis sp. 2	CFMR PR4047	-	KF291151	KF291149
Humidicutis sp. 2	DJL-2103 CFMR PR-6524	KF291150	KF291151	
Humidicutis sp. 3	D.J. Lodge DJL-BZ-3	KF291110	KF291111	_
Hygroaster albellus	AFTOL ID 1997	KF381521	EF551314	KF381510
Hygroaster nodulisporus	AFTOL-ID 2020	_	EF561625	KF381511
Hygrocybe acutoconica	CFMR JP-2	KF291161	KF291162	
Hydrocybe aff citringuirens	DIL05TN10	KF291090	KF291091	
Hygrocybe aff. conica	PBM 918	AV854074	DO071739	AV803747
Hygrocybe aff prieta	DII -B7-65	KF291168	KF291169	111003/1/
Hygrocybe caespitosa	DMWV-03-737	KF291104	KF291105	KF291107
Hygrocybe cantharellus	AFTOL-ID 1714	DO490628	DO457675	1(12)110/
Hygrocybe ceracea	D Boertmann 2002/7	KF291108	KF291109	
Hygrocybe cf. acutoconica	DII 04NC2	KF291117	KF291118	KF291120
Hygrocybe chloochlora	DIL-BZ-32	EU435147	EU435147	
Hygrocybe chlorophana	Boertmann 2002/9	EU435148	EU435148	KF381513
Hyprocybe coccinea	AFTOL-ID 1715	DO490629	DO457676	DO472723
Hyprocybe coccinea	Boertmann02/8	EU435146	EU435146	KF291114
Hyerocybe constrictospora	D. Boertmann 2007/38	KF291115	KF291116	
Hygrocybe glutinipes var. rubra	DJL05NC9	EU435149	EU435149	_
Hygrocybe helobia	AK-124	KF291182	KF291183	_
Hygrocybe hypohaemacta	DJL-BZ-105	EU435150	EU435150	KF291165
Hygrocybe konradii var. konradii	Boertmann 2004/6	KF306329	KF306330	_
Hygrocybe lepida	Boertmann 2002/2	KF306333	KF306334	_
Hygrocybe melleofusca	DJL-PR-EV	KF291154	KF291155	_
Hygrocybe miniata	AK-110	KF291179	KF291180	

Species name	Isolate/voucher ID	ITS	LSU	RPB2
Hygrocybe miniata f. longipes	AFTOL-ID 1891	DQ490630	DQ457677	DQ472724
Hygrocybe noninquinans	DJL-PR-1	KF291127	KF291129	KF291128
Hygrocybe occidentalis var. occidentalis	Cancerel PR 02	EU435151	EU435151	-
Hygrocybe punicea	DJL-SCOT-B2	KF291133	KF291134	-
Hygrocybe purpureofolia	DJL04NC1	KF291192	KF291193	
Hygrocybe reidii	DJL-ENG-15-2006	KF291158	KF291159	
Hygrocybe rosea	DJL-PR-4	KF291197	KF291198	_
Hygrophorus agathosmus	EL2-00	-	AY586660	_
Hygrophorus cossus	SJ94064	AY548963	AY548963	
Hygrophorus hyacinthinus	SJ950830	-	HM143012	_
Hygrophorus olivaceoalbus	SJ91060	-	AY586662	-
Hygrophorus russula	JP-3	KF291216	KF291217	KF291219
Hygrophorus sordidus	AFTOL-1338	DQ490632	AF042562	_
Lichenomphalia umbellifera	J. Geml-2	U66445	U66445	KF381515
Neohygrocybe ingrata	GWG H. ingrata 23-10-06 (ABS)	KF291225	KF291226	_
Neohygrocybe ingrata	TN-62 voucher DJL05TN62	KF381525	KF381558	KF381516
Neohygrocybe ingrata	CFMR NY-43	-	KF291223	KF291224
Neohygrocybe ovina	K(M) 187568	KF291228	KF291229	-
Neohygrocybe ovina	GWG H. ovina Rhosisaf (ABS)	KF291233	KF291234	KF291236
Neohygrocybe subovina	WRWV04-752 (DEWV 5366)	_	KF291142	KF291138
Neohygrocybe subovina	CFMR NC-61	KF291136	KF291137	-
Neohygrocybe subovina	DJL04TN16 (GRSM 77065)	KF291140	KF291141	_
Omphalina epichysium	Redhead3140	U66442	U66442	_
Omphalina grossula	Gulden 417/75	-	U66444	U66444
Omphalina hudsoniana	LUTZ-920728.4a	U66446	U66446	_
Omphalina obscurata	Lam L73-101	U66448	U66448	_
Omphalina philonotis	LUTZ930804-5	U66449	U66449	_
Omphalina sphagnicola	LUTZ930810	U66453	U66453	-
Omphalina velutina	LUTZ-930812.1	U66454	U66454	-
Omphalina velutipes Lamoure	L77	U66455	U66455	-
<i>Omphalina wynniae</i> A. H. Smith	82899	_	U66457	U66457
Porpolomopsis aff. calyptriformis	DJL05TN80	KF291246	KF291247	KF291249
Porpolomopsis calyptriformis	EB-ENG-3	KF291242	KF291243	KF291245
Porpolomopsis lewelliniae	TJB-10034	KF291238	KF291239	KF291241
Pseudoarmillariella bacillaris	HKAS76377	KC222315	KC222316	-
Pseudoarmillariella ectypoides	AFTOL-ID 1557	DQ192175	DQ154111	DQ474127
Sinohygrocybe tomentosipes	GDGM43351	MG685872	MG696901	MG696905
Sinohygrocybe tomentosipes	GDGM43347	_	MG696900	MG696904
Sinohygrocybe tomentosipes	GDGM50075	MG685873	MG696902	MG696906
Sinohygrocybe tomentosipes	GDGM50149	MG685874	MG696903	MG675232
Typhula phacorrhiza	TP21	AF134710	AF393079	AY218525



**Figure 1.** Phylogenetic overview of the family Hygrophoraceae inferred from ITS-LSU-RPB2 data using Maximum Likelihood (ML) method. *Typhula phacorrhiza* was selected as outgroup. Bootstrap values (≥50%) were presented around the branches. The newly generated sequences are shown in bold.



Figure 1. Continued.

#### Taxonomy

#### *Sinohygrocybe* C.Q. Wang, Ming Zhang & T.H. Li, gen. nov. MycoBank: MB824821

**Diagnosis.** Differs from *Chromosera* and *Gloioxanthomyces* by its less omphalioid, more robust basidiomata, dry to subviscid pileus, dry and white tomentose stipe, more elongated basidia, higher length ratio (up to 8 times) of basidia to basidospores.

**Etymology.** *Sino-* refers China, the holotype's location of the genus; *-hygrocybe* indicates that it is a *Hygrocybe*-like genus.

Type species. Sinohygrocybe tomentosipes C.Q. Wang, Ming Zhang & T.H. Li

**Description.** Basidiomata medium-sized, subcaespiotose. Pileus convex to applanate, slightly depressed in the centre, yellow, orangish-yellow to orange, dry to subviscid, slightly when wet, never strongly gelatinised or glutinous. Lamellae adnate to decurrent, concolorous with pileus, with usually furcate and interveined lamellulae. Stipe yellow to whitish or almost concolorous with pileus, yellow or covered by white to yellowish-white tomentum. Basidiospores ellipsoid to oblong, ovoid, Qm = 1.6-1.7,



**Figure 2.** Phylogenetic overview of the tribe Chromosereae inferred from ITS data using ML method. Two *Hygrocybe conica* sequences were rooted as outgroups. Bootstrap values ( $\geq$ 50%) are shown around the branches. GenBank accession numbers of downloaded sequences were added after the species name and the collection locations were added at the ends. NA, EA and EU referred to North America, East Asia and Europe, respectively. The newly generated sequences are shown in bold.

not constricted, thin-walled, inamyloid, hyaline, smooth; basidia usually 4-sterigmate,  $41-80 \mu m$  long, ratio of basidia to basidiospore length over 5 (up to 8), with basal clamp connection. Pileipellis and stipitipellis a cutis. Lamellar trama subregular. Clamp connections present throughout.

# *Sinohygrocybe tomentosipes* C.Q. Wang, Ming Zhang & T.H. Li, sp. nov. MycoBank: MB824824

Figs 3, 4

**Diagnosis.** Differs from the other members of the tribe Chromosereae by its larger and more robust basidiomata, concolorous yellow pileus, lamellae and the subsurface of stipe, usually furcate and interveined lamellae and lamellulae, white fibrillose stipe surface, long basidia (up to  $80 \mu m$ ), ratio of basidia to basidiospore length over 5 and even up to 8.



**Figure 3.** Basidiomata of *Sinohygrocybe tomentosipes* (**a–b** GDGM43351 **c–d** GDGM43352 **e** GDGM43347 **f** GDGM50075 **g–h** GDGM50149). Scale bars: 2 cm.

Etymology. The species epithet *tomentosipes* refers to the tomentose stipe.

**Type.** China. Sichuan Province, Panzhihua City, Yanbian County, Gesala Ecotourism Area, at 27°16'N, 101°26'E, alt. 3100 m, 24 Aug 2013, Ming Zhang (GDGM43351, holotype).



Figure 4. Line drawings of *Sinohygrocybe tomentosipes*. **a** Basidiomata **b** Basidiospores **c** Basidia **d** Elements of pileipellis cells **e** Elements of gill trama.

**Description.** Basidiomata small to medium-sized. Pileus 2.5–6 cm diam., convex to applanate, usually slightly depressed in the centre, smooth, dry but subviscid when wet, light yellow to vivid yellow (3A5–8) or to deep yellow (4A5–8), or light orange to dark orange (5A5–8), becoming paler when dry; margin even, straight or upturned and occasionally split when mature. Lamellae up to 7 mm wide, adnate to sinuate or decurrent, distant, 17–22 lamellae per pileus, with 1–3 lamellulae between two complete lamellae, usually furcate, often interveined or anastomosing at lamella base, thick, concolorous with the pileus; lamellar base and lamellulae irregular and occasionally the whole hymenophore irregular; lamellar edge even and concolorous. Context concolorous with lamellae and pileus, unchanged when cut. Stipe 4–6.5 × 0.6–1.2 cm, central or occasionally eccentric, subcylindrical, moderately to densely covered with white tiny adpressed fibres. Odour indistinct.

Basidiospores 8–10(–10.5) × (4.5–)5–7(–7.5)  $\mu$ m, Q = (1.3–)1.5–1.8, Q<sub>m</sub> = 1.6–1.7, ellipsoid to ellipsoid-oblong, ovoid, not constricted, thin-walled, hyaline, smooth.

Basidia 41–80 × 4–10  $\mu$ m, strongly elongated, narrow clavate, 4-spored, thin-walled; sterigmata up to 10  $\mu$ m long; ratio of basidia to basidiospore length over 5 and up to 8. Hymenophoral trama subregular, yellow, made up of thin-walled hyphae 3–15  $\mu$ m wide and usually less than 100  $\mu$ m long and some conducting elements. Pileipellis a cutis, made up of repent hyphae 3–9  $\mu$ m wide with the terminal elements 30–80  $\mu$ m long. Stipitipellis a cutis, with thin-walled hyphae (5–7  $\mu$ m wide). Clamp-connections present in all tissues.

Habitat and known distribution. Gregarious, caespitose, or scattered in broadleaf forest in subtropical temperate transition zone, so far known only from Sichuan and Hunan Provinces in China.

Additional specimens examined. CHINA, Sichuan Province, Panzhihua City, Yanbian County, Gesala Eco-Tourism Area, at 27°16'N, 101°26'E, alt. 3100 m, 24 Aug 2013, Ming Zhang (GDGM43347), Chao-Qun Wang (GDGM43352); Hunan Province, Zhuzhou City, Yanling County, Taoyuandong National Nature Reserve, at 26°19'N, 114°00'E, alt. 1534 m, 23 Nov 2013, Chao-Qun Wang (GDGM50075 and GDGM50149).

# *Gloioxanthomyces nitidus* (Berk. & M.A. Curtis) Lodge, Vizzini, Ercole & Boertm., Fungal Diversity 64: 50 (2014)

Figs 5, 6

= Hygrophorus nitidus Berk. & M.A. Curtis, Ann. Mag. nat. Hist., Ser. 2 12: 424 (1853).

**Description.** Pileus 1.5–3.5 cm wide, convex to nearly plane with a slightly depressed disc, strongly glutinous, yellow, light orange yellow to apricot yellow, even whitish-yellow when mature, clearly striate at margin; pileus margin usually slightly undulating, slightly incurved when young, expanded to flat or partially uplifted when mature. Context thin, yellow to nearly concolorous with pileus, hygrophanous and translucent. Lamellae arcuate-decurrent, narrow at both ends, bright yellow or slightly orange yellow, waxy and fragile, subdistant, usually having 1–3 unequal lamellulae between two lamellae; lamellar edge even, usually gelatinised and sometimes translucent. Stipe  $2.5-6 \times 0.2-0.5$  cm, cylindrical, hollow, yellow to slightly greenish-yellow, smooth, sticky or glutinous with a layer of viscid and translucent material when wet, nearly equal mostly but usually tapering at base.

Basidiospores 7–9(11) × 5–6.5(7.5)  $\mu$ m, Q=1.25–1.7, Qm=1.48, ellipsoid, not constricted, smooth, hyaline, thin-walled. Basidia 29–39 × 7.5–10  $\mu$ m, clavate, 4-spored; sterigmata up to 5  $\mu$ m. Lamellar trama subregular, with hyphal elements 10–20  $\mu$ m wide. Pileipellis an ixotrichoderm. Clamp connections present.

Habitat and known distribution. Solitary or scattered, on moist ground in a mixed forest with mosses in North-eastern China, so far known in North America and East Asia.



**Figure 5.** Basidiomata of *Gloioxanthomyces nitidus* (**a–b** GDGM41710 **c–d** GDGM42150 **e–f** GDGM42151).

Material examined. CHINA. Jilin Province, Antu County, Changbaishan Mountains, 20 August 2012, Ming Zhang, Jiang Xu, Chao-Qun Wang (GDGM41710, GDGM42150 and GDGM42151).

# Discussion

Phylogenetically, the distinction of the three subfamilies (Lodge et al. 2014) within Hygrophoraceae has very convincing support in the multi-locus tree of this study



Figure 6. Line drawings of *Gloioxanthomyces nitidus* (GDGM41710). **a** basidiomata **b** basidiospores **c** basidia.

(Fig. 1). In addition, the establishment of the three well-defined monophyletic tribes in subfamily Hygrocyboideae is supported in this phylogenetic frame where the tribe Hygrocybeae with 73% support values and the tribe Humidicuteae with low support value are sister clades, while the tribe Chromosereae with 76% support values is located at their base. However, the cuphophylloid grade appears not to be monophyletic, thus more studies are still needed to understand the phylogenetic positions of *Ampulloclitocybe*, *Cantharocybe* H.E. Bigelow & A.H. Sm. and *Cuphophyllus* (Donk) Bon.

In the multi-gene analyses, *Sinohygrocybe* is placed together with two other genera in Chromoserae. *Chromosera* and *Gloioxanthomyces* are sister genera under the monophyletic tribe Chromosereae, while *Sinohygrocybe* is an independent generic lineage; and the distances between *Sinohygrocybe* and *Chromosera* or *Gloioxanthomyces* are further than the distance between *Chromosera* and *Gloioxanthomyces*. Such results are confirmed in the ITS phylogenetic tree (Fig. 2). According to the Blastn results, the ITS and LSU sequence identities of the new species to the known taxa are not more than 76% and 96%, respectively, with the *Chromosera* and *Gloioxanthomyces* sequences in GenBank. Thus, it is clear the new genus is independent of those two existed genera.

Beside the molecular analyses, morphological data also support its recognition within tribe Chromosereae. *Sinohygrocybe* shares a bright pileus colour and decurrent lamellae with the other genera *Chromosera* and *Gloioxanthomyces* (Table 2). However, the genus *Chromosera*, typified by *C. cyanophylla* (Fr.) Redhead, Ammirati & Norvell, differs from *Sinohygrocybe* in having omphaloid basidiomata, ephemeral dextrinoid reactions in the context, ratio of basidiospore to basidium length <5, ephemeral pigment bodies in the pileipellis and lilac pigments sometimes present (Redhead et al. 1995, Candusso 1997, Lodge et al. 2014); while *Gloioxanthomyces* differs from *Sinohygrocybe* by having weaker/ delicate basidiomata, viscid pileus and stipe surface, gelatinised lamellar edge and cheilocystidia, shorter basidia (Boertmann 1990, 2012) with a length ratio of basidium to basidiospore 4–5. *Sinohygrocybe* shares some macroscopic characters with *Hygrocybe*, typified by

Species name	Type location	Basidiospores (µm)	Basidia (µm)	Reference
Gloioxanthomyces nitidus	USA, South Carolina	6.5–9(11) × 4–6.5(7.5)	29–39 × 7.5–10	Bessette et al. 2010, this study
Gloioxanthomyces vitellinus	Sweden	(6.5)7–9(9) × (5)5.5–7(7.5)	30–45 × 7–10	Boertmann 2010
Chromosera citrinopallida	USA, Washington	$7-9(10) \times 4.5-5$	10-45 × 6-8	Smith and Hesler 1954
Chromosera cyanophylla	Sweden	(6.8)7.2–8.0(8.8) × (3.2)3.6–4.4	24–28 × 5.5–6.5	Holec et al. 2015
Chromosera lilacina	northern Fennoscandia	7–8.5(10) × (4)5–6(6.5)	30–45 × 7–9	Candusso 1997
Chromosera viola	Belgium, Namur Province	6.5–10.5(11) × 5–7(7.5)	36–61 × 8–11	Candusso 1997
Chromosera xanthochroa	Scotland	$(5.5)6-8.5(10) \times (3.8)4-5.2(5.5)$	25–32 × 6.5– 7.5(8.5)	Candusso 1997
Sinohygrocybe tomentosipes	China, Sichuan & Hunan Province	8–10(10.5) × (4.5)5–7(7.5)	41–80 × 4–10	This study

Table 2. Type location, basidiospores and basidia dimensions of species of the tribe Chromosereae.

*H. conica*, including bright colour of basidiomata and the distant lamellae, but *Hygrocybe* differs from *Sinohygrocybe* by having more fragile lamellae, more glabrous stipe (at least at the upper portion), often constricted spores and shorter basidia.

*Sinohygrocybe* samples were collected in both late summer (August) and winter (November), showing that they likely have a quite long fruiting season. It should be noted, however, that they are more abundant at times with lower temperature and higher humidity. Therefore, their fruiting in summer may occur only at higher altitude (with the elevation above 1500 m).

As to the Chinese new *Gloioxanthomyces nitidus* record: 1) phylogenetically, the Chinese samples are nested in the *Gloioxanthomyces* clade as a sister branch to the North American branch (Fig. 2); 2) morphologically, it shares these characters with the North American *G. nitidus*: deep yellow basidiomata fading to whitish with age, viscid, hygrophanous surface, central concave pileus and decurrent lamellae (Bessette et al. 2012); 3) geographically, *G. nitidus* and *G. vitellinus* are distributed in North America and Asia and Europe, respectively, indicating that *Gloioxanthomyces* is a Holarctic genus. It is assumed that both North American and East Asian *G. nitidus* were separated from the same ancestor because of geographical isolation, thus they are very similar at present; however, they may continue to diverge, eventually becoming separate species in the future since they live on detached continents.

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# References

- Bessette AE, Roody WC, Sturgeon WE, Bessette AR (2012) Waxcaps mushrooms of eastern North America. Syracuse University Press, New York.
- Boertmann D (1990) The identity of *Hygrocybe vitellina* and related species. Nordic Journal of Botany 10(3): 311–317. https://doi.org/10.1111/j.1756-1051.1990.tb01775.x
- Boertmann D (2010) The genus *Hygrocybe*, 2nd revised edition (Fungi of Northern Europe vol. 1). Danish Mycological Society, Copenhagen.
- Boertmann D (2011) Relationship of *Hygrocybe vitellina* and *H. nitida*–Preliminary Report. Omphalina 2(1): 4–5.
- Boertmann D (2012) Update on Hygrocybe nitida. Omphalina 3(1): 13-15.
- Candusso M (1997) Fungi Europaei 6. Hygrophorus s.l. Libreria Basso, Alassio.
- Crous PW, Gams W, Stalpers JA, Robert V, Stegehuis G (2004) MycoBank: an online initiative to launch mycology into the 21st century. Studies in Mycology 50: 19–22.
- Dentinger BTM, Gaya E, O'Brien H, Suz LM, Lachlan R, Díaz-Valderrama JR, Koch RA, Aime MC (2016) Tales from the crypt: Genome mining from fungarium specimens improves resolution of the mushroom tree of life. Biological Journal of the Linnean Society 117(1): 11–32. https://doi.org/10.1111/bij.12553
- Gardes M, Bruns TD (1993) ITS primers with enhanced specificity for basidiomycetes application to the identification of mycorrhizae and rusts. Molecular Ecology 2: 113–118. https://doi.org/10.1111/j.1365-294X.1993.tb00005.x
- Katoh K, Rozewicki J, Yamada KD (2017) MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. Briefings in Bioinformatics: 1–7. https://doi. org/10.1093/bib/bbx108
- Kornerup A, Wanscher JH (1978) Methuen handbook of colour (3rd edn). Eyre Methuen, London, 252 pp.
- Lawrey JD, Lücking R, Sipman HJM, Chaves JL, Redhead SA, Bungartz F, Sikaroodi M, Gillevet PM (2009) High concentration of basidiolichens in a single family of agaricoid mushrooms (Basidiomycota, Agaricales, Hygrophoraceae). Mycological Research 113: 1154–1171. https://doi.org/10.1016/j.mycres.2009.07.016

- Lodge DJ, Padamsee M, Matheny PB, Aime MC, Cantrell SA, Boertmann D, Kovalenko A, Vizzini A, Dentinger BTM, Kirk PM, Ainsworth AM, Moncalvo JM, Vilgalys R, Larsson E, Lücking R, Griffith GW, Smith ME, Norvell LL, Desjardin DE, Redhead SA, Ovrebo CL, Lickey EB, Ercole E, Hughes KW, Courtecuisse R, Young A, Binder M, Minnis AM, Lindner DL, Ortiz-Santana B, Haight J, Læssøe T, Baroni TJ, Geml J, Hattori T (2014) Molecular phylogeny, morphology, pigment chemistry and ecology in Hygrophoraceae (Agaricales). Fungal Diversity 64: 1–99. https://doi.org/10.1007/s13225-013-0259-0
- Matheny PB (2005) Improving phylogenetic inference of mushrooms with RPB1 and RPB2 nucleotide sequences (*Inocybe*, Agaricales). Molecular Phylogenetics and Evolution 35: 1–20. https://doi.org/10.1016/j.ympev.2004.11.014
- Miller MA, Pfeiffer W, Schwartz T (2010) Creating the CIPRES Science Gateway for inference of large phylogenetic trees, Gateway Computing Environments Workshop (GCE), 1–8. https://doi.org/10.1109/GCE.2010.5676129
- Redhead SA, Lutzoni F, Moncalvo JM, Vilgalys R (2002) Phylogeny of agarics: partial systematics solutions for core omphalinoid genera in the Agaricales (Euagarics). Mycotaxon 83: 19–57.
- Redhead SA, Ammirati JF, Norvell LL (1995) Omphalina sensu lato in North America 3: Chromosera gen. nov. Beihefte Sydowia 10: 155–167.
- Redhead SA, Ammirati JF, Norvell LL, Vizzini A, Contu M (2012) Validation of combinations with basionyms published by Fries in 1861. Mycotaxon 118: 455–458. http://dx.doi. org/10.5248/118.455
- Smith AH, Hesler LR (1954) Additional North American Hygrophori. Sydowia 8: 304–333.
- Stamatakis A (2014) RAxML Version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30(9): 1312–1313. https://doi.org/10.1093/bioinformatics/btu033
- White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (Eds) PCR protocols: a guide to methods and applications. Academic Press, San Diego, 315–322. https://doi.org/10.1016/B978-0-12-372180-8.50042-1
- Yang ZL, Feng B, Hao YJ (2013) *Pseudoarmillariella bacillaris*, a new species with bacilliform basidiospores in Asia. Mycosystema 32: 127–132.
- Young AM (2005) Fungi of Australia: Hygrophoraceae. ABRS, Canberra; CSIRO Publishing, Melbourne.
- Young AM, Wood AE (1997) Studies on the Hygrophoraceae (Fungi: Homobasidiomycetes: Agaricales) of Australia. Australian Systematic Botany 10: 911–1030. https://doi. org/10.1071/SB96005