A new clitocyboid genus Spodocybe and a new subfamily Cuphophylloideae in the family Hygrophoraceae (Agaricales)

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Abstract

Phylogenetically, the genera Cuphophyllus, Ampulloclitocybe and Cantharocybe are treated as basal in the family Hygrophoraceae, despite weak support. However, the exact phylogenetic positions of the three genera have remained unresolved, and taxa related to these genera are poorly known. In this study, a new clitocyboid genus Spodocybe was proposed based on multigenic phylogenetic inference datasets and morphological evidence. The analyses of ITS as well as two combined datasets ITS-nrLSU-rpb2 and ITS-nrLSU-rpb1-rpb2-tef1-a-atp6 supported that (1) Spodocybe formed a well-supported monophyletic clade; and (2) sisters Spodocybe and Ampulloclitocybe, along with Cantharocybe and Cuphophyllus also formed a monophyletic lineage, as sister to the rest of the Hygrophoraceae. Meanwhile, two new species, namely S. rugosiceps and S. bispora, from southwestern China, were documented and illustrated. These results support the new proposed genus Spodocybe, and that Spodocybe, Ampulloclitocybe, Cantharocybe and Cuphophyllus should be retained in the Hygrophoraceae as a new subfamily Cuphophylloideae.

Keywords

Ampulloclitocybe, Cantharocybe, Cuphophyllus, morphological characters, phylogenetic analysis, taxonomy
Introduction

The widespread genus *Clitocybe* (Fr.) Staude currently encompasses large numbers of species with clitocyboid habit, sharing the features of saprophytic nutrition, funnel-shaped pileus, decurrent lamellae, a usually white, cream or pale colored spore-deposit and smooth and inamyloid spores (Singer 1986; Breitenbach and Kraenzlin 1991; Læssøe and Petersen 2019). As a consequence of the poor, broad and unrepresentative morphological characteristics, the genus appeared heterogeneous and was subsequently proven to be polyphyletic based on the phylogenetic analysis (Moncalvo et al. 2002; Harmaja 2003).

Based on phylogenetic analyses over the past 20 years, (i) many new genera within the Tricholomatoid clade were proposed to accommodate previous *Clitocybe* species deviating from the core Clitocybeae clade (Matheny et al. 2006), such as *Cleistocybe* Ammirati, A.D. Parker & Matheny (Ammirati et al. 2007), *Trichocybe* Vizzini (Vizzini et al. 2010), *Atractosporocybe* P. Alvarado, G. Moreno & Vizzini, *Leucocybe* Vizzini, P. Alvarado, G. Moreno & Consiglio and *Rhizocybe* Vizzini, G. Moreno, P. Alvarado & Consiglio (Alvarado et al. 2015); (ii) Several clitocyboid groups were reconfirmed as independent genera, for instance, *Singerocybe* Harmaja (Qin et al. 2014) and *Infundibulicybe* Harmaja (Binder et al. 2010); and (iii) some others were even transferred to the Hygrophoroid clade (Binder et al. 2010), such as *Ampulloclitocybe* Redhead, Lutzoni, Moncalvo & Vilgalys (Redhead et al. 2002) and *Cantharocybe* H.E. Bigelow & A.H. Sm. (Hosen et al. 2016). However, many clitocyboid taxa remain to be reclassified.

The molecular phylogenetic relationships among members of the Hygrophoraceae Lotsy were well studied by Lodge et al. (2014). In their work, the family was divided into subfamily Hygrophorideae E. Larss., Lodge, Vizzini, Norvell & S.A. Redhead, Hygrocyboideae Padamsee & Lodge, Lichenomphalioideae Lücking & Redhead and Cuphophylloid grade. Meanwhile, the Cuphophylloid grade was retained in the Hygrophoraceae as the base comprising the genera *Cuphophyllus* (Donk) Bon, *Ampulloclitocybe* and *Cantharocybe*, despite weak phylogenetic support (Matheny et al. 2006; Binder et al. 2010; Lodge et al. 2014). Consequently, the taxonomic problem of the three genera on whether to be included or excluded in the Hygrophoraceae has remained unresolved.

Recently, some collections were shown to be closely related to *Clitocybe trulliformis* (Fr.) P. Karst. based on ITS-BLAST searches while at the same time they were surprisingly related to taxa of the genus *Cuphophyllus* based on nrLSU-BLAST searches. As far as we know, *C. trulliformis* and allied species were lacking taxonomic revision, especially regarding their molecular phylogenetic status. Furthermore, the phylogenetic delimitation of the Hygrophoraceae was ambiguous due to the uncertain positions of *Cuphophyllus, Ampulloclitocybe* and *Cantharocybe*. Hence, the aims of this study were (a) to propose and describe a new genus of the Hygrophoraceae for species related to *C. trulliformis* based on morphological and molecular analyses and (b) to reconstruct the phylogeny of the Hygrophoraceae for determining the exact phylogenetic placements of *Cuphophyllus, Ampulloclitocybe* and *Cantharocybe* with multi-gene data.
Materials and methods

Specimens

Twenty-three specimens of species similar to *C. trulliformis* and related species were collected from southwestern and northeastern China and western Germany, during 2007 to 2020. The fresh fruitbodies were dried using heat or silica gel. Voucher specimens were deposited in the Herbarium of Kunming Institute of Botany, Chinese Academy of Sciences (KUN-HKAS). Detail information of these specimens is given in Table 1.

Morphological observation

Macroscopic characters of species were described based on the raw field record data and photographs. Colors used in description referred to Kornerup and Wanscher (1978). For the microscopic structure observation, tissue sections of dried specimens were mounted in 5% KOH solution or distilled water and structures of lamellar trama, pileipellis and stipitipellis, basidia and basidiospores were observed with a light microscopy. For the description of lamellar trama structure, seven types, including regular, subregular, divergent, pachypodial, bidirectional, tri-directional and interwoven, were used following Lodge et al. (2014). Besides, Melzer's reagent was applied to test the amyloidity of the basidiospores. In the description of basidiospores, the abbreviation \([n/m/p]\) represent that the measurements were made on \(n\) basidiospores from \(m\) basidiomes of \(p\) collections. The range notation \((a)b–c(d)\) stands for the dimensions of basidiospores in which \(b–c\) contains a minimum of 90% of the measured values while \(a\) and \(d\) in the brackets stand for the extreme values. In addition, a Q value show the length/width ratio of basidiospores and a Qm value for average Q ± standard deviation. All microstructures were illustrated by hand drawing.

DNA extraction, PCR and sequencing

Total genomic DNA was extracted using the Ezup Column Fungi Genomic DNA Purificaton Kit (Sangon Biotech, Shanghai, China) according to the manual. For the PCR amplification, (1) Primers ITS5 and ITS4 (White et al. 1990) were used for the internal transcribed spacer (ITS); (2) LROR and LR5 (Vilgalys and Hester 1990) for the nuclear ribosomal large subunit (nrLSU); (3) EF1-983F and EF1-1953R (Matheny et al. 2007), designed primers SPO-TEF1-F (5’-ATTGCYGGYGGTACYGGTGA-3’) and SPO-TEF1-R (5’-TCVAGDGATTTACCTGTHCGRC-3’) or another pair of designed primers HYG-TEF1-F (5’-CTTCGCTTYACTCTYTGGYGTCC-3’) and HYG-TEF1-R (5’-GCCAACCTTTGCAATGTG-3’) for the translation elongation factor 1-α (tef1-α); (4) RPB1-Af and RPB1-Cr (Matheny et al. 2002) or designed primers SPO-RPB1-F (5’-ACGAGGTTGTYGTGGGTAAT-3’) and SPO-RPB1-R (5’-GGAGGNGDACHGGCATATG-3’) for the DNA-directed RNA polymerase II second largest subunit 1 (rpbl); (5) RPB2-6F and RPB2-7.1R (Matheny 2005) for the
Table 1. Specimens used in phylogenetic analysis and their GenBank accession numbers. The newly generated sequences are shown in bold.

| Species                              | Voucher | Locality   | GenBank accession number                      |
|--------------------------------------|---------|------------|-----------------------------------------------|
|                                      |         |            | ITS   | nrLSU | rpb2  | rpb1  | rpe1-α | atp6  |
| Acantholichen pannaroides            | MDF352  | Costa Rica | KT429795 | KT429807 | KT429817 |
| Acantholichen campestris             | DIC595b | Brazil     | KT429798 | KT429810 | KT429818 |
| Acantholichen galapagoensis          | MDF058  | Ecuador    | KT429785 | KT429800 | KT429812 |
| Ampulloclitocybe clavipes            | KUN-HKAS| China: Jilin| MW616462 | MW600481 | MW656467 |
|                                       |         |            |        | MW656467 | MW656461 | MW656478 |
| Arthelia auriscalpium                | TUB 011588 | Germany | DQ071732 |
| Arthelia acerosa                     | Lueck2  | Germany    | KP065766 | KP065784 |
| Cantharellula unboundata             | CBS 398.79 | France | MH861222 | MH872990 |
| Cantharellula gruberi                | AFTOL-ID 1017 | USA | DQ200927 | DQ234540 | DQ358879 | DQ435808 | DQ059045 |
| Cantharellula brunneovolutina        | AH42539 | Spain      | JN006422 | JN006420 |
| Cantharellula virosa                 | TENN63483 | Bangladesh | KK52405 | KK52403 | KK503143 |
| Chromosera cyanophylla               | AFTOL-ID 1684 | USA | DQ486688 | DQ457655 | DQ81509 |
| Chromosera ambigua                   | GE18008-1 | France | MK645573 | MK645587 | MK645593 |
| Chromosera tenebrosa                 | GE18035  | Canada     | MK645577 | MK645591 | MK645597 |
| Chromosera xanthochroa                | GE18033  | Canada     | MK645576 | MK645590 | MK645596 |
| Chrysomphalina chrysophylla          | AFTOL-ID 1523 | USA | DQ192180 | DQ457656 |
| Chrysomphalina grossula               | OSC 113683 | Bangladesh | EU644704 | EU652373 |
| Clitocybe aff. costata                | DJL06TN80 | USA | FJ596913 |
| Clitocybe herbarum                   | G0171    | Hungary    | MK277719 |
| Clitocybe trulliformis               | 14562    | Italy      | JP07809 |
|                                       | 4804     | Russia     | MH930178 |
| Clitocybe cf. trulliformis           | G0460    | Hungary    | MK277728 |
| Clitocybe sp.                        | NAMA 2015-206 | USA | MH910535 |
| Clitocybe sp.                        | NAMA 2015-318 | USA | MH910563 |
| Clitocybe sp. Observer 302917        | Mushroom Observer 302917 | USA | MK607556 |
| Corella brasiliensis                 | MDF017   | Bolivia    | KY780569 | KY867172 |
| Corella aff. Mclinti                | MDF200   | Brazil     | KY780569 | KY867172 |
| Cyphellostereum galapagoense         | Lueck7   | Germany    | KP065771 | KP065789 |
| Cyphellostereum imperfectum          | DJL-Scol-8 | UK | KF291097 | KF291058 |
| Cyphellostereum aff. prantius        | CFMR PR-6601 | Puerto Rico | KF291099 | KF291100 | KF291102 |
| Cyphellostereum prantius             | AFTOL-ID 1682 | USA | DQ486683 | DQ457650 | DQ435804 |
| Cyphellostereum sp.                  | KUN-HKAS 105671 | China: Tibet | MW762875 | MW763000 | MW789179 | MW789163 |
| Gloeocethomyces nitidus              | GDGM41710 | China: Jilin | MG712283 | MG712282 | MG711911 |
A new genus, a new subfamily and phylogeny of the family Hygrophoraceae

| Species                          | Voucher                  | Locality         | GenBank accession number |
|----------------------------------|--------------------------|------------------|-------------------------|
|                                  |                          |                  | ITS         | nrLSU | rpb2 | rpb1 | rpb1-α | atp6 |
| **Hyasiella splendidissima**     | Herb. Roux n. 4044       | France           | JN944400 | JN944401 |      |      |        |      |
|                                  | Herb. Roux n. 3666       | Moldova          | JN944398 | JN944399 |      |      |        |      |
| **Hyasiella venustissima**       | A. Gminder 971488        | Italy            | KF291092 | KF291093 |      |      |        |      |
|                                  | E. C. 08191              | Italy            | JN944393 | JN944394 |      |      |        |      |
| **Humidicatia marginata**        | AFTOL-ID 1727            | USA              | DQ490624 | DQ457672 | DQ472702 | DQ447906 |
| **Humidicatia auratocephalus**   | QCAM M6000               | Ecuador          | KY689661 | KY780120 |      |      |        |      |
| **Humidicatia sp.**              | CFMR BZ-3923             | Belize           | KF291110 | KF291111 |      |      |        |      |
| **Hygaster noduliporus**         | AFTOL-ID 2020            | USA              | EF561625 |          |      |      |        |      |
| **Hygaster albellus**            | AFTOL-ID 1997            | Puerto Rico      | KF381521 | E551314  |      |      |        |      |
| **Hygocybe conica**              | FO 4674                  |                  | DQ97139  |          |      |      |        |      |
| **Hygocybe cf. acutoceps**       | CFMR NC-256              | USA              | KF291117 | KF291118 | KF291120 |          |
| **Hygocybe coccina**             | AFTOL-ID 1715            | USA              | DQ490629 | DQ457676 | DQ472723 | DQ447910 | GU187705 |
| **Hygocybe aff. conica**         | AFTOL-ID 729             |                  | AY854074 | AY684167 | AY803747 |          |
| **Hygrophorus chrysodon**        | US97/138                 | Germany          | AF430279 |          |      |      |        |      |
| **Hygrophorus flavodiscus**      | KUN-HKAS 82501           | China: Tibet     | MW616463 | MW600482 | MW656472 | MW656462 | MW656479 |
| **Hygrophorus gliocyclus**       | KUN-HKAS 112569          | China: Tibet     | MW762876 | MW763001 | MW789180 | MW789164 | MW773440 | MW789195 |
| **Hygrophorus hypotrichus**      | KUN-HKAS 68013           | Yunnan           | MW616464 | MW600483 | MW656473 | MW656468 | MW656463 | MW656480 |
| **Hygrophorus pudorinus**        | KUN-HKAS 55043           | Yunnan           | MW616465 | MW600484 | MW656474 | MW656469 | MW656464 | MW656481 |
| **Hygrophorus sp. 1**            | KUN-HKAS 79992           | China: Tibet     | MW616466 | MW600485 | MW656475 | MW656465 | MW656482 |
| **Hygrophorus sp. 2**            | KUN-HKAS 112569          | China: Jilin      | MW616468 | MW600487 | MW656477 | MW656466 | MW656484 |
| **Hygrophorus sp. 3**            | KUN-HKAS 87261           | Yunnan           | MW616469 |           |        |        |        |      |
| **Hygrophorus sp. 4**            | KUN-HKAS 112567          | China: Tibet     | MW762878 | MW763003 | MW789182 | MW789166 | MW773442 | MW789197 |
| **Lichenomphalia budonioides**   | GAL18249                 | USA              | JQ65873 | JQ65875 |        |        |        |      |
| **Lichenomphalia meridionalis**  | S-270-FB1                | Japan            | LC428308 | LC428307 |        |        |        |      |
| Neohygrocybe ovina               | GWG H. ovina Rhosiaif (ABS) |               | KF291233 | KF291234 | KF291236 |          |        |      |
| Neohygrocybe grieneumgi           | GDGM 44492               | China            | MG779451 | MG786565 |        |        |        |      |
| Neohygrocybe ingrata             | DJL05TN62 (TENN)         | USA              | KF381525 | KF381558 | KF381516 |        |        |      |
| Neohygrocybe rubovina            | GRSM 77065               | China            | KF291140 | KF291141 |        |        |        |      |
| Spodocybe bispora                | KUN-HKAS 73310           | Yunnan           | MW672880 | MW763005 | MW789184 | MW789168 | MW773444 | MW789199 |
| Spodocybe bispora                | KUN-HKAS 73332           | Yunnan           | MW672881 | MW763006 | MW789185 | MW789169 | MW773445 | MW789200 |
| Spodocybe rugosiceps            | KUN-HKAS 112564          | Yunnan           | MW672882 | MW763007 | MW789186 | MW789170 | MW773446 | MW789201 |
| Spodocybe rugosiceps            | KUN-HKAS 112561          | Yunnan           | MW672883 | MW763008 | MW789187 | MW789171 | MW773447 | MW789202 |
| Spodocybe rugosiceps            | KUN-HKAS 81981           | Yunnan           | MW672884 | MW763009 | MW789188 | MW789172 | MW789203 |      |
| Spodocybe rugosiceps            | KUN-HKAS 69830           | Yunnan           | MW672885 | MW763010 | MW789189 | MW789173 | MW773448 | MW789204 |
| Species                      | Voucher       | Locality | GenBank accession number    |
|------------------------------|---------------|----------|-----------------------------|
| Spodocybe rugosiceps        | KUN-HKAS 71071 | China: Yunnan | MW762886, MW763011, MW789190, MW789197, MW773449, MW789205 |
| Spodocybe sp. 1             | KUN-HKAS 112560 | China: Jilin | MW762890, MW763015, MW789194, MW789178, MW789162, MW789209 |
| Spodocybe sp. 2             | KUN-HKAS 112565 | China: Yunnan | MW762889, MW763014, MW789193, MW789177, MW789161, MW789208 |
| Pseudomycillia exotropides  | AFTOL-ID 1557 | USA      | DQ192175, DQ154111, DQ474127, DQ516076, GU187735 |
| Pseudomycillia basilicaris  | KUN-HKAS 76377 | China     | KC222315, KC222316 |
| Sinogrocybe tomentosipes     | GDGM 50075    | China: Hunan | MG685873, MG696902, MG696906 |
| Amylocorticium cebennense   | CFMR HHB-2808 | USA       | GU187505, GU187561, GU187770, GU187439, GU187675 |
| Asperotricha olera           | DAOM 265047   | Canada    | KF381518, KF381541 |
| Macrotyphula fistulosa      | IO. 14. 214   | Spain     | MT232352, KY224088, MT242317, MT242354 |
| Macrotyphula juncea         | IO. 14. 177   | Sweden    | MT232353, MT232306, MT242337, MT242355 |
| Macrotyphula phacorrhiza    | IO. 14. 167   | France    | MT232364, MT232315, MT242348, MT242367 |
| Phyllostix niglandus        | IO. 14. 196   | Spain     | MT232308, MT242338, MT242319, MT242557 |
| Phyllostix sp.              | AFTOL-ID 773  | USA       | MQ404382, MT232305, MT232309, MT242339 |
| Pleurocybella corrugata     | UPS F-611882  | Sweden    | MT242352, MT232309, MT242339 |
| Plicaturopsis crispa        | AFTOL-ID 1924 | USA       | MQ404686, MQ470820, GU187816 |
| Pterulicium echinatum       | ZRL.20151311  | China     | LT716065, KY418881, KY419026, KY419079, KY419076 |
| Petrichium gracile          | IO. 14. 142   | Sweden    | MT232356, MT232310, MT242358 |
| Saccospora oreadina         | AFTOL-ID 536  | Sweden    | DQ494695, AY691887, DQ598992, DQ479398, GU187754 |
| Serpalomycetes borealis     | CFMR L-8014   | USA       | GU187512, GU187570, GU187782, GU187686 |
| Tricholomopsis decora      | AFTOL-ID 537  | USA       | DQ403848, AY691888, DQ408112, DQ479493, DQ299195 |
| Tricholomopsis siliquroides | ZRL.20151760  | USA       | LT716068, KY418884, KY419029, KY419079 |
| Tiphula capitata            | IO. 15. 122   | Spain     | MT232357, MT232312, MT242341, MT242321, MT242360 |
| Tiphula incarnata           | IO. 14. 92    | Sweden    | MT232362, MT232313, MT242346, MT242325 |
| Tiphula micans              | IO. 14. 165   | Sweden    | MT232361, KY224102, MT242345, MT242324, MT242364 |

DNA-directed RNA polymerase II second largest subunit 2 (\(rpb2\)); and (6) ATP6-3 and ATP-6 (Kretzer and Bruns 1999) for ATP synthase subunit 6 (\(atp6\)).

The PCR mixtures contained 1× PCR buffer, 1.5 mM MgCl\(_2\), 0.2 mM dNTPs, each primer at 0.4 μM, 1.25U of Taq polymerase (Sangon Biotech, Shanghai, China), and 1 μL of DNA template in a total volume of 25 μL. Reactions were performed with the following program: initial denaturation at 94 °C for 5 min, 35 cycles at 94 °C for 30 s, 50 °C (\(atp6\)), 52 °C (nrLSU, tefl-\(\alpha\), \(rpb1\) and \(rpb2\)) or 54 °C (ITS) for 30 s, and 72 °C for 30 s (ITS and \(atp6\), 50 s (nrLSU and \(rpb2\)) or 75 s (tefl-\(\alpha\) and \(rpb1\)), and for terminal elongation, the reaction batches were incubated at 72 °C for 10 min. All PCR products were detected by 2% agarose gel electrophoresis and then sent to the Kunming branch of Tsingke Biological Technology Co., Ltd. (Beijing, China) for sequencing.
Phylogenetic tree construction

Sequences used for phylogenetic analysis (presented in Table 1) were aligned by using MAFFT v7.471 (Katoh and Standley 2016) and then manually adjusted by using BI-OEDIT v7.2.5 (Hall 1999). The intron regions of tef1-a, rpb2 and rpb1 were excluded except the conserved rpb1-intron2. Three datasets of ITS-nrLSU-rpb2, ITS-nrLSU-rpb1-rpb2-ef1-a-atp6 and ITS (Suppl. materials 1, 2 and 3) were used to construct phylogenetic trees. The two multi-gene matrixes were generated by SEQUENCEMATRIX 1.7.8 (Vaidya et al. 2011). GTR + I + G was inferred as the best-fit model for the three matrixes selected according to the AIC in MRMODELTEST v2.4 (Nylander 2004). Maximum likelihood (ML) trees with 1000 bootstrap replicates and Bayesian inferences were generated with RAXML v8.0.20 (Stamatakis 2006) and MRBAYES v3.2.7 (Ronquist and Huelsenbeck 2003), respectively.

Results

Molecular phylogenetic analysis

As shown in Table 1, a total of 393 sequences (109 ITS, 110 nrLSU, 40 tef1-a, 38 rpb1, 74 rpb2 and 22 atp6) from 118 samples were used in the phylogenetic analyses, 131 (23 ITS, 23 nrLSU, 20 tef1-a, 20 rpb1, 23 rpb2 and 22 atp6) of which were newly generated in the present study.

The combined dataset ITS-nrLSU-rpb2 comprised 221 sequences from 88 samples with a total of 3135 positions. In the three-gene tree (Fig. 1), 11 specimens from four novel Spodocybe species collected in this study, C. cf. trulliformis and C. herbarum formed a strongly supported monophyletic clade (BP = 100%, PP = 1.0), as sister to Ampulloclitocybe (BP = 63%, PP = 0.98). The phylogenetic analysis showed that the new proposed genus Spodocybe should be placed within the Hygrophoraceae, although intergeneric branched orders among Spodocybe, Ampulloclitocybe, Cantharocybe and Cuphophyllus were unstable with low support values.

In order to accurately determine the position of Spodocybe in the family Hygrophoraceae and better clarify the phylogenetic relationships of Spodocybe, Ampulloclitocybe, Cantharocybe and Cuphophyllus, a further six-gene matrix ITS-nrLSU-rpb1-rpb2-ef1-a-atp6 composed of 179 sequences from 54 samples with 5405 positions was used to rebuild the Hygrophoraceae tree. As revealed by the six-gene phylogenetic analysis (Fig. 2), the branch support level of the six-gene tree was obviously improved, compared with that of the previous three-gene tree. The monophyly of Spodocybe clade was strongly supported (BP = 100%, PP = 1.00), including Spodocybe rugosiceps (BP = 100%, PP = 1.00), S. bispora (BP = 100%, PP = 1.00) and two unnamed Spodocybe species. Spodocybe and Ampulloclitocybe were sister clades (BP = 78%, PP = 0.99), then further clustered with Cantharocybe (BP = 59%, PP = 0.97) and finally together with Cuphophyllus formed an independ-
Figure 1. ML analysis of Hygrophoraceae combined ITS, nrLSU and rpb2 sequence data, with *Macrotyphula juncea*, *Macrotyphula phacorrhiza* and *Phyllotopsis* sp. as outgroups. Bootstrap values (BP) ≥ 50% from ML analysis and Bayesian posterior probabilities (PP) ≥ 0.90 are shown at nodes. The newly generated sequences are shown in bold.
A new genus, a new subfamily and phylogeny of the family Hygrophoraceae

Figure 2. ML analysis of Hygrophoraceae combined ITS, nrLSU, rpb1, rpb2, tef1-α and atp6 sequence data with representatives of Amylocorticiaceae, Pterulaceae and the Hygrophoroid clade (Aphroditeola, Macrotyphula, Phyllotopsis, Pleurocybella, Sarcomyxa, Tricholomopsis and Typhula) as outgroups. Bootstrap values (BP) ≥ 75% from ML analysis and Bayesian posterior probabilities (PP) ≥ 0.95 are shown at nodes. Branches with BP ≥ 75% and PP ≥ 0.95 are bolded. The newly generated sequences are shown in bold. Lamellar trama types of specimens collected in this study were identified by ourselves and others referred to Lodge et al. (2014) and Hosen et al. (2016).

In addition, an ITS dataset (23 sequences; 1053 positions) was applied to phylogenetic analysis for displaying the relationships among Spocybe species from this study and species of Clitocybe treated from GenBank. In the ITS tree (Fig. 3), Spocybe
species formed a highly supported monophyletic clade with *C. trulliformis* and related species (BP = 100%, PP = 1.00), which was also a sister clade to *Ampulloclitocybe* with strong support (BP = 91%, PP = 0.99).

**Taxonomy**

*Cuphophylloideae* Z. M. He & Zhu L. Yang, subf. nov.
MycoBank No: 839377

**Diagnosis.** Characterized generally by clitocyboid basidiomes, convex to funnel-shaped pileus, decurrent lamellae, absence of veils, inamyloid basidiospores and presence of clamps.

**Etymology.** From the type genus *Cuphophyllus*.

**Type genus.** *Cuphophyllus* (Donk) Bon.

**Description.** Basidiomes small, medium-sized to large, mostly clitocyboid, rarely omphalinoid or mycenoid; veils absent. Pileus convex, planate to funnel-shaped; surface usually dry, smooth, lubricous or rarely viscid. Lamellae decurrent to deeply
decurrent. Basidiospores ellipsoid, oblong or subglobose, thin-walled and inamyloid. Pileipellis usually a cutis, sometimes ixocutis or trichoderm. Lamellar trama regular, subregular, interwoven or bidirectional. Clamp connections present.

**Habitat, ecology and distribution.** Usually gregarious or caespitose on ground, rarely on wood; widespread in temperate and tropical regions.

The genera *Ampulloclitocybe*, *Cantharocybe*, *Cuphophyllus* and *Spodocybe* are included in the subfamily Cuphophylloideae, which is in correspondence with Cuphophylloid grade of Lodge et al. (2014) plus *Spodocybe*.

**Spodocybe** Z. M. He & Zhu L. Yang, gen. nov.
MycoBank No: 839050

**Diagnosis.** Differs from *Ampulloclitocybe* by its small basidiomes and subregular lamellar trama rather than medium-sized basidiomes and bidirectional lamellar trama. Differs from *Cuphophyllus* in the ratio of basidia to basidiospore length less than 5, and lamellar trama subregular rather than interwoven. Differs from *Cantharocybe* in its absence of cheilo- and caulocystidia, having small basidiomes rather than large ones and having subregular lamellar trama rather than regular one.

**Etymology.** *Spodo-* refers to grey; *-cybe* refers to head; that is a *Clitocybe*-like genus with grey pileus.

**Type species.** *Spodocybe rugosiceps* Z. M. He & Zhu L. Yang.

**Description.** Basidiomes small, clitocyboid. Pileus convex, applanate to infundibuliform; surface dry, greyish (2B1), grey-brown (5C4) to dark grey-brown (5E4); center depressed with age. Lamellae decurrent to deeply decurrent, white (1A1) to cream (1A2), thin, moderately crowded, sometimes furcate and interveined. Stipe central, subcylindrical, concolorous with pileus. Basidiospores ellipsoid, oblong to cylindrical, colourless, hyaline, smooth, thin-walled, inamyloid; ratio of basidia to basidiospore length less than 5. Pileipellis and stipitipellis a cutis. Lamellar trama subregular. Clamp connections abundant, present in all parts of basidiome.

**Habitat, ecology and distribution.** Saprophytic, usually gregarious or caespitose on the ground of coniferous or coniferous and broad-leaved mixed forest; distributed in the temperate and subtropical zones from June to November.

**Spodocybe rugosiceps** Z. M. He & Zhu L. Yang, sp. nov.
MycoBank No: 839052
Figs 4A, B, 5

**Diagnosis.** Differs from *S. bispora* in having a rugose pileus, smaller basidiospores and 4-spored rather than 2-spored basidia. Differs from *C. trulliformis* in having smaller basidiospores and a rugose rather than felty-squamulose pileus.

**Etymology.** *rugosiceps* refers to the rugose pileus.
Figure 4. Basidiomes of described Spodocybe species. **A, B** Spodocybe rugosiceps (KUN-HKAS 112563, KUN-HKAS 112562, respectively) **C, D** Spodocybe bipora (KUN-HKAS 73332, KUN-HKAS 112562, respectively). Scale bars: 1 cm.

**Type.** China. Yunnan Province: Kunming City, near Yeya Lake, at 25.136658°N, 102.873027°E, alt. 2000 m, 11 Aug 2020, Z. M. He 72 (KUN-HKAS 112563, holotype).

**Description.** Basidiomes small, clitocyboid. Pileus 0.5–2 cm in diam, at first nearly applanate, then concave; surface dry and rugose, gray-brown (5E2-4) to gray-black (4F2-4) in the center and gray-brown (5C2-4) or gray (5B1-2) towards margin; center often slightly umbonate; margin straight and undulating; context thin and white (1A1) to cream (1A2). Lamellae deeply decurrent, white (1A1) to cream (1A2), thin (up to 2 mm high), crowded, sometimes forked and intervenose. Stipe 2.5–6 × 0.2–0.4 cm, central, narrowly cylindrical to subcylindrical, sometimes flexuous, hollow; surface dry and nearly smooth, concolorous with pileus; context white (1A1).

Basidiospores \[60/3/3\] 5–6 (6.5) × (2.5)3–3.5(4) μm, Q = (1.38)1.55–1.95(2), Qm = 1.73 ± 0.14, elongate, colorless, hyaline, smooth, thin-walled, inamyloid. Basidia 20–24 × 5–6 μm, clavate, 4-spored, colorless, hyaline, thin-walled; sterigmata up to 4 μm long; ratio of basidia to basidiospore length values about 3–5. Cystidia absent. Lamellar trama subregular; hyphae colorless, hyaline, cylindrical, thin-walled, 3–10 μm wide. Pileipellis a cutis, but in places upright or trichodermal in appearance, made up with thin-walled cylindrical hyphae 3–9 μm wide. Stipitipellis a cutis, composed of
thin-walled cylindrical hyphae 3–10 μm wide. Clamp connections present in all parts of basidiome.

**Habitat, ecology and distribution.** Gregarious or caespitose, growing saprotrophically in forest litter, often under conifers, on the ground, known from subtropical zone of Yunnan, China; from July to October.

**Additional specimens examined.** CHINA. Yunnan Province: Dali Bai Autonomous Prefecture, Yunlong Country, Tianchi National Nature Reserve, at 25.850365°N, 99.274236°E, alt. 2509 m, 28 Sep 2019, X. H. Wang 7471 (KUN-HKAS 112561); Kunming City, Fangwang Tree Farm, at 25.063737°N, 102.870690°E, alt. 2262 m, 22 Sep 2011, Z. L. Yang 5586 (KUN-HKAS 71071); Kunming City, Kunming Institute
of Botany, at 25.147081°N, 102.748855°E, alt. 1990 m, 24 Aug 2020, Z. L. Yang 6391 (KUN-HKAS 112562); Kunming City, Qiongzhu Temple, at 25.071304°N, 102.630934°E, alt. 1900 m, 28 Jul 2013, T. Guo 779 (KUN-HKAS 81981); Yulong Country, Lashi Village, at 26.883902°N, 100.234594°E, alt. 2655 m, 31 Jul 2011, L. P. Tang 1369 (KUN-HKAS 69830).

**Spodocybe bispora** Z. M. He & Zhu L. Yang, sp. nov.
MycoBank No: 839054
Figs 4C, D, 6

**Diagnosis.** Differs from *S. rugosiceps* in having a nearly smooth pileus, larger basidiospores and 2-spored rather than 4-spored basidia. Differs from *C. trulliformis* in having a nearly smooth rather than felty-squamulose pileus.

**Eymology.** *Bispora* refers to 2-spored.

**Type.** China. Yunnan Province: Baoshan City, Longyang District, Shuizhai Village, at 25.273967°N, 99.306216°E, alt. 2400 m, 12 Aug 2011, J. Qin 324 (KUN-HKAS 73310, holotype).

**Description.** Basidiomes small, clitocyboid. Pileus 1.5–3 cm in diam, plano-convex to funnel-shaped; surface dry and nearly smooth, greyish-brown (4B2-3) to grey-brown (4E3-5); center depressed, usually with a low umbo, somewhat darker; margin generally straight and undulating, incurved when old; context thin and white (1A1). Lamellae deeply decurrent, white (1A1) to cream (1A2), thin, 1–2 mm high, relatively crowded, sometimes forked and intervenose. Stipe 1–3 × 0.2–0.4 cm, central, sub-cylindrical, hollow; surface dry and nearly smooth, concolorous with pileus; context white (1A1).

Basidiospores [60/3/3] (7)7.5–10.5(11.5) × 3–4 μm, Q = (2.05)2.11–3(3.33), Qm = 2.56 ± 0.3, cylindrical, colorless, hyaline, smooth, thin-walled, inamyloid. Basidia 20–30 × 4–5.5 μm, clavate, 2-spored, colorless, hyaline, thin-walled; sterigmata up to 10 μm long; ratio of basidia to basidiospore length less than 5 (about 2–4). Cystidia absent. Lamellar trama subregular, colorless, hyaline, made up of thin-walled cylindrical hyphae with 3–10 μm wide. Pileipellis a cutis, composed of thin-walled cylindrical hyphae 3–11 μm wide. Stipitpellis a cutis, composed of thin-walled cylindrical hyphae 3–10 μm wide. Clamp connections in all parts of basidiomes.

**Habitat, ecology and distribution.** Saprophytic, usually gregarious on the ground of coniferous or coniferous and broad-leaved mixed forest, known from Yunnan, China; July to September.

**Additional specimens examined.** China. Yunnan Province: Kunming City, Qipan Mountain, at 26.060020°N, 102.576823°E, alt. 1900 m, 25 Jul 2020, Z. M. He 35 (KUN-HKAS 112564); Nujiang City, Lanping Country, No. 311 Provincial Highway, at 26.636613°N, 99.557809°E, alt. 2660 m, 14 Aug 2011, J. Qin 346 (KUN-HKAS 73332).
A new genus, a new subfamily and phylogeny of the family Hygrophoraceae

Figure 6. Microscopic features of *Spodocybe bispora* (KUN-HKAS 73310, holotype) a basidiospores b basidia c pileipellis. Scale bars: 10 μm.

**Discussion**

**The new genus *Spodocybe***

In our current study, the new clitocyboid species were clustered into a monophyletic lineage (BP = 100%, PP = 1.00) in the Hygrophoraceae according to the multi-gene phylogenetic analysis (Figs 1, 2). As a result, the new generic name *Spodocybe* is proposed here to accommodate the new lineage, which is irrelevant to Clitocybeae of the Tricholomatoid clade (Matheny et al. 2006; Alvarado et al. 2015). The three-gene tree of the Hygrophoraceae (Fig. 1) in this study presented basically consistent topological structure with Lodge et al. (2014), and showed that *Spodocybe* was a sister to *Ampulloclitocybe* located within the family Hygrophoraceae and further confirmed by a six-gene tree (Fig. 2).
Besides the molecular analyses, morphological data also support its separation from the relative genera. *Spodocybe* shares clitocyboid basidiomes, decurrent lamellae, inamyloid basidiospores and the presence of clamps with the other genera *Ampulloclitocybe*, *Cuphophyllus* and *Cantharocybe*. However, the genus *Ampulloclitocybe*, typified by *A. clavipes*, differs from *Spodocybe* in having medium-sized basidiomes and bidirectional lamellar trama (Harmaja 2002; Lodge et al. 2014). Afterwards, *Cuphophyllus* differs from *Spodocybe* in having long basidia, typically 7–8 (rarely 5–6) times the length of the basidiospores, highly interwoven lamellar trama, rarely subregular (Voitk et al. 2020). Finally, *Cantharocybe* differs from *Spodocybe* in having large basidiomes, broad lamellae, cheilo- and caulocystidia, clamps but not on all hyphal septa or at the base of every basidium and more regular lamellar trama (Ovrebo 2011; Hosen et al. 2016). In view of the four genera above with different structures in lamellar trama (Fig. 2), the type of lamellar trama can become a good distinguishing microscopic character for them.

For a long time, *C. trulliformis* has been placed in the genus *Clitocybe* based on the clitocyboid feature and habit since 1879 (Karsten 1879). However, *C. trulliformis* shares many morphological characteristics with *Spodocybe*, such as the small basidioma with applanate to infundibuliform pileus, grey-brown pileus and stipe, decurrent and whitish lamellae, and smooth and inamyloid basidiospores (Bas et al. 1995). Besides, the ITS phylogenetic analysis in our study (Fig. 3) showed that *C. trulliformis* and related *Clitocybe* species were involved in the *Spodocybe* clade as well, indicating that *C. trulliformis* and related species should be placed with *Spodocybe*. In consequence, it is foreseeable that *C. trulliformis* and other related clitocyboid species will eventually be moved to *Spodocybe*. Accordingly, more taxonomic work is needed in future.

The placements of *Spodocybe*, *Cuphophyllus*, *Ampulloclitocybe* and *Cantharocybe*

In previous studies, *Cuphophyllus*, *Ampulloclitocybe* and *Cantharocybe* were treated as basal in Hygrophoraceae (Lodge et al. 2014), but their phylogenetic placements were not resolved. In a six-gene phylogenetic analysis by Binder et al. (2010) and a three-gene analysis by Wang et al. (2018), *Ampulloclitocybe* and *Cantharocybe* were located between *Cuphophyllus* and the rest of the Hygrophoraceae, but without support. While two four-gene analyses by Lodge et al. (2014) showed that *Ampulloclitocybe* and *Cantharocybe* were sister clades as basal to *Cuphophyllus* along with the rest of the Hygrophoraceae with weak support. However, in our six-gene analysis (Fig. 2), the new proposed genus *Spodocybe* and *Ampulloclitocybe* were sisters (BP = 78%, PP = 0.99) and they clustered with *Cantharocybe* followed by *Cuphophyllus*, forming a supported monophyletic sister clade to the rest of the Hygrophoraceae (BP = 83%, PP = 1.00). Hence, *Spodocybe, Ampulloclitocybe, Cantharocybe* and *Cuphophyllus* should be retained in Hygrophoraceae, and a new subfamily, *Cuphophylloideae*, is proposed to accommodate the lineage.
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**Supplementary material I**

**Alignment of ITS-LSU-RPB2 dataset used in the three-gene phylogenetic analysis**

Authors: Zheng-Mi He, Zhu L. Yang

Data type: fasta file

Explanation note: ITS: 1-1380, LSU: 1381–2356, RPB2: 2357–3135.

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Link: https://doi.org/10.3897/mycokeys.79.66302.suppl1
**Supplementary material 2**

**Alignment of ITS-LSU-RPB1-RPB2-TEF1-ATP6 dataset used in the six-gene phylogenetic analysis**
Authors: Zheng-Mi He, Zhu L. Yang  
Data type: fasta file  
Explanation note: ITS: 1–1217, LSU: 1218–2158, RPB1: 2159–3358, RPB2: 3359–4089, TEF1: 4090–4967, ATP6: 4968–5405  
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Link: https://doi.org/10.3897/mycokeys.79.66302.suppl2

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**Supplementary material 3**

**Alignment of ITS dataset used in the single-gene phylogenetic analysis**
Authors: Zheng-Mi He, Zhu L. Yang  
Data type: fasta file  
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