Multiple-marker phylogeny and morphological evidence reveal two new species in Steccherinaceae (Polyporales, Basidiomycota) from Asia

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Abstract
Two new wood-inhabiting fungi, *Mycorrhaphium subadustum* sp. nov. and *Trullella conifericola* sp. nov., are proposed and described from Asia based on ITS, nrLSU and *tef1* molecular phylogeny and morphological characteristics. *Mycorrhaphium subadustum* is characterized by a stipitate basidiocarp, velutinate pileal surface concentrically zoned, hydnoid hymenophore, a dimitic hyphal system in spine trama and monomitic in context, absence of gloeocystidia, presence of cystidioles and the non-amyloid, cylindrical to ellipsoid basidiospores. *Trullella conifericola* is characterized by a laterally stipitate basidiocarp with flabelliform to semicircular pileus, hirtellous pileal surface with appressed coarse hair and concentrically zoned and sulcate, tiny pores (10–12 per mm), a dimitic hyphal system, absence of any type of cystidia, short clavate basidia and thin-walled, smooth, cylindrical to allantoid basidiospores. Phylogenetic analyses based on a three-marker dataset were performed using maximum likelihood and Bayesian inference methods. The two new species formed isolated lineages with full support in Steccherinaceae. The distinguishing characters of the two new species as well as allied species are discussed, and a key to species of *Mycorrhaphium* is provided.

Keywords
Hydnaceous fungus, molecular phylogeny, polypores, taxonomy, wood-inhabiting fungi

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Introduction

Steccherinaceae Parmasto was typified by the genus *Steccherinum* Gray (1968). It belongs to the residual polyporoid clade of the Polyporales Gäum. (Basidiomycota). It is a distinct and well-defined group based on phylogenetic evidence (Miettinen et al. 2012; Binder et al. 2013). Steccherinaceae includes around 23 genera according to Zmitrovich (2018). The taxa in this family show highly variable morphological and anatomical features. For instance, the basidiocarps range from resupinate (e.g. *Junghuhnia* Corda.) to pileate (e.g. *Austeria* Miettinen and *Flabellaophora* G. Cunn.), and the hymenophore can be poroid (e.g. *Citripora* Miettinen) or hydnoid (e.g. *Mycorrhaphium* Maas Geest. and *Steccherinum* Gray). The hyphal system ranges from monomitic (e.g. *Caudicicola* Miettinen, M. Kulju & Kotir. and *Elaphroporia* Z.Q. Wu & C.L. Zhao), dimitic (e.g. *Antrodiella* Ryvarden & I. Johans.) to trimitic (e.g. *Metuloidea* G. Cunn.). Any type of cystidia can be absent (e.g. *Frantisekia* Spirin & Zmitr.) or take the form of gloeocystidia (e.g. *Antella* Miettinen and *Butyrea* Miettinen) or encrusted cystidia (e.g. *Flaviporus* Murrill). The basidiospores are typically cylindrical, allantoid (e.g. *Nigroporus* Murrill and *Trullella* Zmitr.) or ellipsoid (e.g. *Steccherinum* Gray). Nevertheless, the members of the family also share several characters including the white-rot nutritional mode, small pores or densely arranged spines, smooth and relatively small basidiospores, and mainly cyanophilic but inamyloid hyphae (Gray 1821; Corda 1842; Murrill 1905; Maas Geesteranus 1962; Cunningham 1965; Ryvarden and Johansen 1980; Spirin et al. 2007; Yuan and Dai 2009; Yuan and Wu 2012; Yuan et al. 2012; Yuan 2014; Miettinen and Ryvarden 2016; Kotiranta et al. 2017; Wu et al. 2018; Zmitrovich 2018).

Morphological and phylogenetic analyses have provided more accurate identification and contributed to the definition of the taxonomic status of the genera in Steccherinaceae. In recent years, phylogenetic analysis based on multi-marker data has been widely used in the taxonomy of these fungi (He and Dai 2012; Miettinen et al. 2012; Binder et al. 2013; Dai et al. 2014; Miettinen and Ryvarden 2016; Justo et al. 2017; Kotiranta et al. 2017; Westphalen et al. 2018; Yuan et al. 2018; Yuan et al. 2020).

The species of the Steccherinaceae are widely distributed all over the world. During the investigation of specimens in Steccherinaceae from Asia, several specimens which represent two undescribed species were found. The morphological and molecular features showed that they belong to the genus *Mycorrhaphium* and *Trullella*. In this study, we describe them as two new species based on morphological characteristics and three-marker phylogenetic analyses.

Material and methods

Morphological studies

The studied specimens were deposited at the herbarium of the Institute of Applied Ecology, Chinese Academy of Sciences (IFP). Microscopic procedures followed Yuan...
and Qin (2018). Microscopic observations were made on tissue sections mounted in cotton blue and Melzer's reagent to test for any amyloid and/or dextrinoid reactions (cotton blue: 0.1 mg Methyl blue (SIGMA, PCode: 1001545602) dissolved in 60 g pure lactic acid; Melzer's reagent: 1.5 g KI (potassium iodide), 0.5 g I (crystalline iodine), 22 g chloral hydrate, distilled water 20 mL). The following abbreviations are used in the text: KOH = 2.5% potassium hydroxide; CB = cotton blue; CB+/– = cyanophilous/acyanophilous; IKI = Melzer’s reagent; IKI– = neither amyloid nor dextrinoid; $L_m$ = mean spore length (arithmetic average of all spores); $W_m$ = mean spore width (arithmetic average of all spores); $Q$ = variation in the ratios of $L_m/W_m$ between specimens studied, and $n$ = total number of spores measured from a given number of specimens. Sections were studied at magnifications up to ×1000 using a Nikon Eclipse E600 microscope (Tokyo, Japan) with phase-contrast illumination, and dimensions were estimated subjectively with an accuracy of 0.1 μm. Microscopic drawings were made with the aid of a drawing tube. The spores’ measurements excluded the apiculus, and 5% of the measurements at each end of the range are given in parentheses. The spores’ measurements were made with a Nikon SMZ 645 stereomicroscope. Special colour terms are from Kornerup and Wanscher (1981).

**Molecular procedures and phylogenetic analyses**

DNA was extracted from dried herbarium specimens with a Thermo Scientific Phire Plant Direct PCR kit (Thermo Fisher Scientific, Waltham, Massachusetts, USA) according to the manufacturer’s instructions and was used for the polymerase chain reaction (PCR). Nuclear ribosomal RNA markers were used to determine the phylogenetic position of the new species. The internal transcribed spacer (ITS) was amplified with the primers ITS4 (5’ TCCTCCGCTTATTGATATGC 3’) and ITS5 (5’ GGAAGTAAAAGTACACAAGG 3’); LR0R (5’ ACCCGCTGAACTTAAGC 3’) and LR7 (5’ TACTCCACCAAGATCT 3’) for partial nrLSU; 983F (5’ GCYCCYGGHCAYCGTGAYTTYAT 3’) and 2218R (5’ ATGACACCRACRCRRGYTG 3’) for tef1 (White et al.1990; Gardes and Bruns 1993; Rehner and Buckley 2005; Matheny et al. 2007).

PCR reactions were performed in 30 μL reaction mixtures containing 15 μL of 2×Phire Plant PCR buffer, 0.6 μL Phire Hot Start II DNA Polymerase, 1.5 μL of each PCR primer (10 μM), 10.5 μL double deionized H$_2$O (ddH$_2$O), and 0.9 μL template DNA. The PCR thermal cycling program condition was set as follows: initial denaturation at 95 °C for 5 min, followed by 34 cycles at 95 °C for 30 s, the annealing temperatures were as follows: 58.9 °C for ITS4/ITS5, 47.2 °C for LR0R/LR7, 57.6 °C for 983F/2218R, then 72 °C for 20 s, and a final extension at 72 °C for 7 min. PCR amplification was confirmed on 1% agarose electrophoresis gel stained with ethidium bromide (Stöger et al. 2006) and sequenced at the Beijing Genomics Institute (BGI) with the same primers as used in PCR. The newly generated DNA sequences were assembled and manually modified with the software DNAMAN8 (Lynnon Biosoft, Quebec, Canada). The sequences quality control followed the guidelines by Nilsson...
et al. (2012). All newly obtained sequences were submitted to GenBank (Sayers et al. 2020). Sequences from allied genera were based on the studies of Miettinen et al. (2012), Yuan (2014) and Westphalen et al. (2019) or found in GenBank (http://www.ncbi.nlm.gov) using the BLAST option and downloaded (Table 1). DNA alignments were performed using the MAFFT v.7.471 online service (https://mafft.cbrc.jp/alignment/server/index.html; Katoh et al. 2019). Intron regions of tef1 as well as low-homology regions of ITS1 and ITS2 were removed before phylogenetic analyses, and the sequence datasets were combined using BioEdit v 7.2.6 (Hall 2005).

Bayesian analysis and Maximum likelihood were applied to the ITS + nrLSU + tef1 dataset. All characters were weighted, and gaps were treated as missing data. Bayesian analysis with MrBayes 3.2.7 (Ronquist et al. 2012) implemented the Markov Chain Monte Carlo (MCMC) technique. The combined dataset was divided into seven partitions: ITS1, 5.8S, ITS2, nrLSU and tef1 1st, 2nd as well as 3rd codon positions. The best-fit models selected were K80+G for ITS1, GTR+I+G for 5.8S, JC+G for ITS2, GTR+I+G for nrLSU, JC for tef1 1st, TrNef+G for tef1 2nd and GTR+I+G tef1 3rd which were determined by the jModelTest 2.1.10 (Darriba et al. 2012) based on the Corrected Akaike Information Criterion (AICc). Four simultaneous Markov chains were run with 10 million generations and starting from random trees and keeping one tree every 100th generation until the average standard deviation of split frequencies was below 0.01. The value of burn-in was set to discard 25% of trees when calculating the posterior probabilities. Bayesian posterior probabilities were obtained from the 50% majority rule consensus of the trees kept. A Maximum Likelihood (ML) analysis uses the seven-partitions’ database which is the same as Bayesian analysis and performed in RAxML v8.2.4 (Stamatakis 2014). The best tree was obtained by performing 1000 rapid bootstrap inferences followed by a thorough search for the most likely tree (Stamatakis et al. 2008). Phylogenetic trees were checked and modified in FigTree 1.4 (Rambaut 2012). The combined dataset and trees were deposited in TreeBASE (No. S27633).

**Results**

**Phylogenetic analyses**

Multiple-marker analyses provide an advantage of accurately and promptly discovering a new species or genus (Taylor et al. 2000). Therefore, we used three markers in our dataset which included 75 ITS, 68 nrLSU and 20 tef1. The combined dataset includes two species belonging to the genera *Mycorrhaphium* and *Trullella* respectively, and other 69 samples from 23 allied genera. *Climacocystis borealis* (Fr.) Kotl. & Pouzar was used as the outgroup. The data matrix comprised 163 sequences and had an aligned length of 2121 bases. Bayesian analysis resulted in an average standard deviation of split frequencies = 0.004878. The maximum likelihood and Bayesian analyses produced similar topologies and therefore, only the ML tree is shown in Figure 1.
**Table 1.** Specimens and sequences used in this study. Type specimens are indicated as superscript T and the newly generated sequences in this study are in bold.

| Species | GenBank No. | Specimen/culture voucher | Locality | References |
|---------|-------------|--------------------------|----------|------------|
| Antella americana (Ryvarden & Gilks) Ryvarden | JN710509 | JN710509 | JN710711 | KHL 11949 | Sweden | Miettinen et al. 2012 |
| A. americana | EU232186 | EU232270 | – | HHH 4100-Sp | USA | GenBank Database |
| A. chinensis (H.S. Yuan) Miettinen | JX110844 | KC485542 | – | Dai 9019\(^1\) | China | 2013 |
| A. chinensis | JX110843 | KC485541 | – | Dai 8874\(^1\) | China | 2013 |
| A. niemelaei (Vamplea & Vlasik) Miettinen | AF126876 | – | – | Renvall 3218 | Finland | Johansson et al. 2000 |
| A. niemelaei | AF126877 | – | – | Haikonen 14727 | Finland | Johansson et al. 2000 |
| A. lactea H.S. Yuan | KC485530 | KC485548 | – | Yuan 5720\(^2\) | China | 2014 |
| A. lactea | KC485532 | KC485550 | – | Yuan 5757\(^2\) | China | 2014 |
| A. semispina (Berk. & M.A. Curtis) Ryvarden | JN710521 | JN710521 | – | X242 | Canada | Miettinen et al. 2012 |
| Astrodialla sp. | JN710523 | JN710523 | – | Núñez 1040 | Japan | Miettinen et al. 2012 |
| A. stipitata H.S. Yuan & Y.C. Dai | KC485525 | KC485544 | – | Yuan 5640 | China | 2014 |
| Astraporrella neotropica Ryvarden | HQ659221 | HQ659221 | – | Miettinen XI021 | Belize | Miettinen et al. 2012 |
| Austeria citrea (Berk.) Miettinen | JN710511 | JN710511 | – | X171 | New Zealand | Miettinen et al. 2012 |
| Butyrea lutesalba (P. Karst.) Miettinen | JN710558 | JN710558 | JN710719 | isolate 5403 | Estonia | Miettinen et al. 2012 |
| B. japonica (Núñez & Ryvarden) Miettinen & Ryvarden | JN710556 | JN710556 | JN710718 | isolate 10202\(^2\) | Japan | Miettinen et al. 2012 |
| B. japonica | KC485536 | KC485553 | – | Li 1648 | China | 2014 |
| Cuhabolithia seuletii (Boudouet & Galzin) Pařek | AF141626 | AF141626 | – | FCUG 722 | Sweden | GenBank Database |
| Citropora barnaensis Miettinen | JN710526 | JN710526 | – | OMM9999\(^4\) | China | Miettinen et al. 2012 |
| Climacocystis borealis (Fr.) Kort. & Pouzar | JN710527 | JN710527 | – | KHL 13318 | Estonia | Miettinen et al. 2012 |
| Elaphroporaria ailaoshanensis Z.Q. Wu & C.L. Zhao | MG231568 | MG748854 | – | CLZhao 595\(^5\) | China | Wu et al. 2018 |
| E. ailaoshanensis | MG231572 | MG748855 | – | CLZhao 596 | China | Wu et al. 2018 |
| Esbeirodon fimbriatum (Pers.) Banker | JN710530 | JN710530 | – | KHL 11905 | Sweden | Miettinen et al. 2012 |
| Flabelliporona sp1 | JN710533 | JN710533 | – | Miettinen 14305 | Indonesia | Miettinen et al. 2012 |
| Flabelliporona sp2 | JN710534 | JN710534 | – | Miettinen 11443 | Indonesia | Miettinen et al. 2012 |
| Flabelliporona sp3 | JN710535 | JN710535 | – | Syamsi NOM677 | Indonesia | Miettinen et al. 2012 |
| Flabelliporona sp4 | JN710536 | JN710536 | – | Ryvarden 34508 | USA | Miettinen et al. 2012 |
| Flabelliporona sp. | MT269765 | MT259330 | MT793111 | isolate 12794 | China | This study |
| F. sp. | MT269766 | MT259331 | MT793112 | isolate 12796 | China | This study |
| Flaviporus brownii (Humb.) Donk | KY175008 | KY175008 | KY175022 | MWA 362/12 | Ecuador | Westphalen et al. 2018 |
| F. brownie | JN710538 | JN710538 | – | X462 | Australia | Miettinen et al. 2012 |
| F. liemanni (Fr.) Ginnis | JN710509 | JN710539 | – | X249 | China | Miettinen et al. 2012 |
| F. liemanni | KC502914 | – | – | Yuan 1766 | China | 2014 |
| Frantiseka mutschelklausenii (Pilát ex Pilát) Spirin | FJ496670 | FJ496678 | – | BRNM 710170 | Czech Republic | Tomáškovský et al. 2010 |
| F. mutschelklausenii | JN710544 | JN710544 | – | isolate 1377 | Australia | Miettinen et al. 2012 |
| F. ustoni Y.C. Dai & Niemelä | KC485526 | – | – | Dai 8249 | China | 2014 |
| F. ustoni | KC485527 | KC485545 | – | Wei 3081 | China | 2014 |
| Junghuhnia crustacea (Jungh.) Ryvarden | JN710553 | JN710553 | – | X626 | Indonesia | Miettinen et al. 2012 |
| J. micropora Spirin, Zmitr. & Malyshova | JN710559 | JN710559 | JN710720 | Spirin 2652 | Russia | Miettinen et al. 2012 |
| Lanelliporora americana | JN710567 | JN710567 | – | Lasser 10119 | Ecuador | Miettinen et al. 2012 |
| Lecanorectis fractipes (Berk. & M.A. Curtis) Jülich | KX378866 | KC378866 | – | MT 13/2012 | Brazil | Westphalen et al. 2016 |
The two new species *Mycorrhaphium subadustum* and *Trulella conifericola* were both defined with three markers and they form full-support (100% ML and 1.00 BPP) isolated lineages respectively in this study. The new species *M. subadustum*...
Two new species in Steccherinaceae

Figure 1. Maximum likelihood tree based on the combined ITS + nrLSU + tef1 sequence dataset illustrating the phylogeny of *Myccorrhaphium subadustum* and *Trullella conifericola* and related taxa in Steccherinaceae. The new species are in bold. Branches are labelled with maximum likelihood bootstrap higher than 50% and Bayesian posterior probabilities more than 0.95.

clustered together with *Myccorrhaphium* spp. and form a subclade with American *M. adustum*. In case of another new species *T. conifericola*, although the material of *T. conifericola* Cui 2851 was only provided with ITS sequences, it showed a high similarity of ITS to the other two samples (Yuan 12657 and Yuan 12655) with 99.59% and 98.77% respectively. Furthermore, the morphological and anatomical features, distribution and the coniferous-saprophytic habit suggested it represented an individual which belongs to *T. conifericola*. Three samples of *T. conifericola* get together with another six samples from the *Trullella* clade with support 92% in ML and 1.00 BPP. The phylogenetic tree obtained in this study is similar to that of Miettinen et al. (2012). All the species were divided into 23 main clades which include *Antella*, *Myccorrhaphium*, *Amelochroa*, *Trullella* and *Antella*.
Antrodiella, Atraporiella, Austeria, Butyrea, Cabalodontia, Citripora, Elaphroporia, Etheirodon, Flabellophora, Flaviporus, Frantisekia, ‘Glaesia’, Jungbuhnia, Lamellocarps, Laweomyces, Metuloidae, Mycorrhaphium, Nigroporus, ‘Scetum’, Steccherinum, Trullella and Xanthoporus. It is notable that the genera Austeria, Flabellophora, Mycorrhaphium, Nigroporus and Trullella formed a large clade in Steccherinaceae with a strong support (85% ML and 1.00 BPP).

Taxonomy

*Mycorrhaphium subadustum* T. Cao & H.S. Yuan, sp. nov.
MycoBank No: 838509
Figures 2, 3

**Diagnosis.** Basidiocarps stipitate; pileus semicircular to dimidiate; pileal surface velutinate, concentrically zonate, pileal margin yellowish white; hymenophore hydnoid. Hyphal system dimitic in spine trama and monomitic in context; generative hyphae with clamp connections; cystidia and gloeocystidia absent, cystidiols present. Basidiospores cylindrical to allantoid, CB–, IKI–.

**Holotype.** China. Liaoning Province, Huanren County, Laotudingzi Nature Reserve, on fallen branch of angiosperm, 4.VIII.2018, Yuan 12976 (holotype IFP 019374).

**Etymology.** Subadustum (Lat.), referring to the affinity with *M. adustum*.

**Description.** Basidiocarps annual, stipitate, solitary or imbricate, corky to soft fibrous, without odor and taste when fresh, light in weight when dry. *Pilei* semicircular to dimidiate, 2.5–4.5 cm wide and 0.3 cm thick. *Pileal surface* velutinate, smooth, concentrically zonate, yellowish white to greyish orange (4A2–5B4); margin acute, yellowish white (4A2). Hymenophore hydnoid; spines crowded, evenly distributed, greyish orange (5B4), fibrous, subulate to terete, straight to somewhat flexuous, solitary or confluent, up to 1 mm long, 5–7 per mm; sterile margin smooth, yellowish grey (4B2), up to 2 mm wide. Context yellowish white (3A2), leathery, azonate, homogeneous, up to 0.5 mm thick. Stipe up to 3 cm long, 1 cm wide, straight and base inflated, surface tomentum eventually glabrous, brownish orange (5C4).

**Hyphal structure.** Hyphal system monomitic in context, dimitic in spine trama; generative hyphae often with clamp connections and simple septate occasionally present; skeletal hyphae thick-walled to subsolid, CB+, IKI–; tissues pale yellow in KOH.

**Context.** Generative hyphae with clamp connections, colorless, thin- to slightly thick-walled, frequently branched, 3–5 μm diam; skeletal hyphae absent.

**Spines.** Generative hyphae often with clamp connections, simple-septate occasionally present, colorless, thin- to slightly thick-walled, moderately branched, 2.5–4 μm diam; skeletal hyphae thick-walled to subsolid, unbranched, subparallel along the spine, 3–5 μm diam. Gloeocystidia absent; cystidiols present among the basidia, fusiform, 8–12 × 1.5–3 μm. Basidia clavate, with a basal clamp and four sterigmata, 8–13.5 × 2–3.5 μm; basidioles in shape similar to basidia, but slightly smaller.
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Figure 2. Basidiocarps of *Mycorrhaphium subadustum* (IFP 019374, holotype). Scale bar: 10 mm.

*Basidiospores* cylindrical to ellipsoid, colorless, thin-walled, smooth, CB—, IKI—, (3.5—)3.8–4.0(4.2) × (1.5–)1.8–1.9(–2.0) μm, \( L_m = 3.89 \mu m \), \( W_m = 1.83 \mu m \), \( Q = 2.13–2.17 \) (n = 60/2).

**Type of rot.** White rot.

**Distribution.** In temperate zones.

**Additional specimen examined.** CHINA. Jilin Province, Antu Country, Changbai Mountain Nature Reserve, Huangsongpu, on fallen branch of angiosperm, 2.VIII.2008, *Dai 10173* (IFP 008336).

*Trullella conifericola* T. Cao & H.S. Yuan, sp. nov.

MycoBank No: 836287

Figures 4, 5

**Diagnosis.** Basidiocarps annual, sessile or laterally stipitate; pileus flabelliform to semicircular; pileal surface hirtellous, with appressed coarse hair, concentrically zonate and sulcate; pores round to angular. Hyphal system dimitic; generative hyphae with clamp connections; skeletal hyphae CB+, IKI—. Basidiospores cylindrical to allantoid, thin-walled.
Figure 3. Microscopic structures of *Mycorrhaphium subadustum* (IFP 019374, holotype) a Basidiospores b Basidia and basidioles c Cystidioles d Hyphae from spine trama e Hyphae from context.

**Holotype.** **VIETNAM.** Lam dong Province, Lac Duong District, Lac Duong District, Bidoup Nui Ba National Park, on fallen branch of *Pinus kesiya*, 15.X.2017, Yuan 12655 (holotype IFP 019372).

**Etymology.** *Conifericola* (Lat.), referring to growth on the coniferous substrate.

**Description.** *Basidiocarps* annual, sessile or laterally stipitate, solitary to imbricate, without special odor or taste, leathery when fresh, shrinking, hard corky and
light in weight upon drying. *Pileus* flabelliform to semi-circular, applanate, projecting 4–10 cm and 1 cm thick at the base; pileal surface hirtellous, with appressed coarse hair, concentrically zonate and sulcate, alternating white and greyish orange (6A1–6B3) when fresh, yellowish white (2A2/3A2/4A2) and nearly azonate when dry; margin acute, drying involute and wavy. *Pore surface* light orange (5A4), shiny; pores round to angular, tiny, 10–12 per mm, hardly visible to the naked eye; dissepiments entire; sterile margin ca. 1 mm wide. *Context* color paler than pores and upper surface, yellowish white (2A2–3A2), soft corky, azonate, 0.5–1.5 mm thick. *Tubes* non-stratified, concolorous with pore surface, dense, ca. 1.5 mm thick when dry. *Stipe* round, glabrous and smooth, light yellow to greyish yellow (4A4–4B5), 0.5–2 cm long and 2–4 mm in diam, dense and homogenous.

*Hyphal structure.* *Hyphal system* dimitic: generative hyphae bearing clamp connections, skeletal hyphae CB+, IKI–; tissues unchanged in KOH.

*Context.* Dominated by generative hyphae, interwoven; generative hyphae hyaline, thin- to slightly thick-walled, clamp connections abundant, frequently branched, 2.5–5.5 μm diam; skeletal hyphae hyaline, thick-walled with a wide lumen, unbranched, 1.5–5 μm diam.
**Figure 5.** Microscopic structures of *Trullella conifericola* (IFP 019372, holotype) a basidiospores b basidia and basidioles c hyphae from trama d hyphae from context.

**Tubes.** Dominated by skeletal hyphae, interwoven; generative hyphae hyaline, thin- to slightly thick-walled, moderately branched, 2–4 μm diam; skeletal hyphae hyaline, thick-walled to semisolid, straight to flexuous, unbranched, 1.5–3.5 μm diam. Cystidia or other sterile hymenial elements absent. Basidia short 8–15 × 4–5.5 μm,
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clavate, 4-sterigmata of 0.5–1 μm in length, with a clamp connection at base; basidi-oles similar to basidia in shape, but slightly smaller.

**Basidiospores.** Cylindrical to allantoid, slightly curved, hyaline, thin-walled, smooth, CB–, IKI–, (4.0–)4.1–5.5(–5.8) × (1.6–)1.8–2.3(–2.5) μm, $L_m = 4.94$ μm, $W_m = 2.09$ μm, $Q = 2.36–2.45$ (n = 60/2).

**Ecology.** On fallen gymnosperm branch, causing a white rot.

**Distribution.** In high altitude area of subtropical to tropical zones.

**Additional specimens examined.** CHINA. Fujian Prov., Wuyishan Forest Park, on fallen trunk of *Pinus kesiya*, 16.IX.2005, *Cui 2851* (IFP 000645). VIETNAM. Lam dong Province, Lac Duong District, Bidoup Nui Ba National Park, on fallen branch of *Pinus kesiya*, 15.X.2017, *Yuan 12657* (IFP 019373).

**Discussion**

The phylogenetic profiling showed that the new species *Mycorrhaphium subadustum* as well as *Trullella conifericola* are nested in the Steccherinaceae which belongs to the residual polyporoid clade (Miettinen et al. 2012; Binder et al. 2013; Zmitrovich 2018; Westphalen et al. 2019) where they emerge robustly supported isolated lineages. Furthermore, these lineages are supported by morphological characteristics.

*Mycorrhaphium* was recommended by Maas Geesteranus (1962) and typified by *M. adustum*. The two samples of the new species *M. subadustum* (Yuan 12976 and Dai 10173) clustered in *Mycorrhaphium* clade, were both collected on fallen branches of angiosperm from northeast of China. The similarity of ITS and nrLSU sequences between the two samples of *M. subadustum* are 99.00% as well as 99.64% respectively, and they form a full-support isolated lineage which is closely related to *M. adustum*, the type species of the genus. The type material of *M. subadustum* Yuan 12976 have a 95.56% similarity of ITS sequences with the American *M. adustum* KHL12255. Morphologically, *M. subadustum* is similar to *M. adustum* in having the velutinate and concentrically zonate pileal surface, presence of clamps and simple septa, a dimitic hyphae system in spine trama and monomitic in context, absence of cystidia as well as gloeocystidia and the non-amyloid basidiospores. However, *M. adustum* often have a dark-colored pileal margin, which is distinctly different from the yellowish white ones of *M. subadustum*. Anatomically, the new species can be differentiated from *M. adustum* by the slender generative hyphae in context (3–5 μm vs. 4–6.3 μm), cyanophilous hyphae and presence of cystidiols (Maas Geesteranus 1962; Ryvarden 1989; Westphalen et al. 2019).

*Mycorrhaphium* embraced nine species (http://www.indexfungorum.org, 2020) and among which there are others two species described from Asia: *Mycorrhaphium sessile* H.S. Yuan & Y.C and *M. stereoides* Maas Geest. *M. sessile* is a species described from China, but the characteristics such as the sessile basidiocarps and presence of gloeocystidia can differentiate it from *M. subadustum* (Yuan and Dai 2009). *Mycorrhaphium stereoides* is related to *M. subadustum* in having stipitate basidiocarps, hyd-
noid hymenophore, a monomitic hyphal system in context and dimitic in spines, but differs from it by the presence of gloecystidia and the larger basidiospores (4–6.3 × 2.5–3.8 μm) (Maas Geesteranus 1971). The North Europe Mycorrhapheium pusillum (Brot.) Maas Geest. is closely related to M. subadustum in having the stipitate basidiocarps as well as pale colored and zonate pileal surface, but differs it by the presence of gloecystidia, absence of clamps and the broader basidiospores (Q = 1.52 in M. pusillum vs. 2.13–2.17 in M. subadustum) (Tervonen et al. 2015). Mycorrhapheium ursinum Decock & Ryvarden is a new species from African; its habit of growing on the soil can be distinguished from M. subadustum. Ryvarden (1989) as well as Mossebo and Ryvarden (2003) have provided keys to a part of species in Mycorrhapheium and after which several new taxa have been described. We provide a new key to the whole described species (except M. ursinum) of the genus in this study.

In the phylogenetic tree, nine samples of Trullella species which include the new species T. conifericola form the clade with strong support (92% ML and 1.00 BPP). Trullella is a genus which was originally proposed as ‘Trulla’ by Miettinen and Ryvarden (2016) and renamed by Zmitrovich (2018). Trullella conifericola is quite an extraordinary species in the genus because of its coniferous-saprophytic habit. The type species of Trullella, T. dentipora (Ryvarden & Iturr.) Zmitr., was described from South America. Trullella dentipora, together with the other species of the genus, inhabits dead angiosperm trees (e.g. Quercus and Cecropia peltata) (Patouillard 1902; Murrill 1907; Miettinen and Ryvarden 2016). Morphologically and anatomically, T. conifericola resembles others Trullella spp. in having sessile or laterally stipitate basidiocarps, mostly small and regular pores, a dimitic hyphal structure, nearly monomitic in the context, and curved cylindrical spores. However, the new species can be distinctly differentiated from other species by having a hirtellous pileal surface with appressed coarse hair, larger spores than those of previous Trullella species (Lm = 4.94 μm and Wm = 2.09 μm in T. conifericola vs Lm = 4.00–4.77 μm and Wm = 1.39–1.91 μm in others Trullella spp.), and inhabiting fallen gymnosperm branches. Trullella composed of six species as of now, and the key to these species was provided by Miettinen and Ryvarden (2016).

Besides, the genera Mycorrhapheium and Trullella together with Austeria, Flabellophora and Nigroporus form a large clade in the phylogenetic tree with strong support (85% ML and 1.00 BPP), and share similar morphological features, including zonate or sulcate pileal surfaces, tiny pores or dense spines and a context that is entirely or almost monomitic. They form a distinct subgroup in the Steccherinaceae.

Key to species of worldwide Mycorrhapheium

1  Hymenophore hydnoid .............................................................................................................2
– Hymenophore poroid..............................................M. hispidum Westph. & Miettinen
2  Spores less than 3.5 μm long..................................................................................................3
– Spores more than 3.5 μm long...............................................................................................4
3  Stipe present, spines less than 2 mm long....M. adustulum (Banker) Ryvarden
– Stipe absent, spines up to 4 mm long.....................................................................................M. sessile
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| Step | Description | Species |
|------|-------------|---------|
| 4    | Spines less than 5 mm long, spores less than 5 \( \mu \)m long | ........................................ 5 |
|      | Spines up to 10 mm long, spores up to 6.3 \( \mu \)m long | \( M. \) stereoides |
| 5    | Pileal less than 2 cm wide, gloeocystidia present | ........................................ \( M. \) pusillum (Brot.) Maas Geest. |
|      | Pileal more than 2 cm wide, gloeocystidia absent | ........................................ 6 |
| 6    | Habit on the ground | ........................................ 7 |
| 7    | Spines more than 3 mm long | \( M. \) africanum (Mossebo & Ryvarden) |
|      | Spines less than 3 mm long | \( M. \) citrinum (Ryvarden) |
| 8    | Pileal margin black, hyphae acyanophilous | \( M. \) adustum |
|      | Pileal margin yellowish white, hyphae cyanophilous | \( M. \) subadustum |

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