A new species of bird’s nest fungi: characterisation of *Cyathus subglobisporus* sp. nov. based on morphological and molecular data

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Key words

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Abstract Recent collections of bird’s nest fungi (i.e. *Crucibulum*, *Cyathus*, *Mycoecia*, *Nidula*, and *Nidularia* species) in northern Thailand resulted in the discovery of a new species of *Cyathus*, herein described as *C. subglobisporus*. This species is distinct by a combination of ivory-coloured fruiting bodies covered with shaggy hairs, plications on the inner surface of the peridium and subglobose basidiospores. Phylogenetic analyses based on ITS and LSU ribosomal DNA sequences using neighbour-joining, maximum likelihood and weighted maximum parsimony support *Cyathus subglobisporus* as a distinct species and sister to a clade containing *C. annulatus*, *C. renweii* and *C. stercoraceus* in the Striatum group.

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INTRODUCTION

The genus *Cyathus* along with the genera *Crucibulum*, *Mycoecia*, *Nidula*, and *Nidularia* are known as the bird’s nest fungi because of their small vase-shaped or nest-like fruiting bodies containing lentil-shaped or egg-like peridioles. *Cyathus* is the most speciose genus in the family Nidulariaceae (Agaricales). *Cyathus* is distinguished from the other three genera in the Nidulariaceae based on grey to black peridioles with funicular cords and peridia composed of three layers of tissues (Brodie 1975). Historically, *Cyathus* was monographed by Lloyd (1906) and Brodie (1975, 1984), and their species concepts, especially those of Brodie (1975), are followed by most mycologists (Liu & Li 1989, Ren & Zhou 1992, Yang et al. 2002, Chen et al. 2003, Zhou et al. 2004). Recognition of *Cyathus* species is based on morphological characters such as fruiting body shape, coverings and plications of peridia, anatomy of peridioles, and the size and shape of basidiospores (Brodie 1975).

Molecular phylogenetic studies based on rDNA sequence data including several *Cyathus* species and other gasteromycetous fungi (e.g., *C. striatus* in Hibbett et al. 1997, Hibbett & Thorn 2001 and *C. stercoraceus* in Moncalvo et al. 2002) showed that *Cyathus* nested within the euagarics clade. The most recent treatment of the Agaricales by Matheny et al. (2006) based on sequence analyses of six loci included *Crucibulum laeve* and *Cyathus striatus* as representatives of the Nidulariaceae. Their phylogenetic reconstruction indicated that the Nidulariaceae was sister to the Cystodermataceae (represented by *Cystodermatopsis amianthinum*). Together these two clades appear sister to the Agaricaeae s.l. but without bootstrap support. A phylogenetic study of the genus *Cyathus* by Zhao et al. (2007) using ITS and LSU ribosomal DNA sequence datasets, and based primarily on type and authentic specimens of 22 taxa of *Cyathus*, indicated that the genus was monophyletic and included three infrageneric groups recognisable by morphological characters.

In Brodie’s monographs (Brodie 1975, 1984) eight species were known from southeast Asia: *C. cheliensis* and *C. Olivaceobrunneus* from China; *C. crispus*, *C. ellipsoides*, and *C. Griseocarpus* from India; *C. elmeri* and *C. gracilis* from the Philippines, and *C. tripex* from the West Indies, the Philippines, and Thailand. Within the last 20 years, seven new taxa have been described from south China: *C. africans* var. *latisporus* (Chen et al. 2003), *C. comucopoides* (Ren & Zhou 1992), *C. lijiangensis* (Zhou et al. 2004), *C. luxiensis* (Chen et al. 2003), *C. mega* *sporos* (Ren & Zhou 1992), *C. renweii* (Zhou et al. 2004), and *C. yunnanensis* (Liu & Li 1989). Based on morphological analyses of 48 *Cyathus* taxa, including 30 type specimens in Zhao et al. (2006), three *Cyathus* species were found to represent synonyms of existing species. *Cyathus cheliensis*, *C. gan-suensis* (Yang et al. 2002), and *C. mega* *sporos* were accepted as synonyms of *C. limbatu*, *C. pygmaeus*, and *C. poepipigii*, respectively.

There has been a recent interest in studies of basidiomycetes in Thailand, particularly around the area of the Mushroom Research Centre (Le et al. 2007a–c, Wannathes et al. 2007). Some *Cyathus* species have been reported previously from Thailand (Brodie 1975, Ellingsen 1982, Soytong 1994, Desjardins et al. 2004). However, no new species have been described from Thailand prior to this paper. In this study *Cyathus subglobisporus* sp. nov. is described and its phylogenetic position is investigated based on ITS and LSU rDNA sequence data.
MATERIALS AND METHODS

Morphological studies

Macromorphological characters of freshly collected material were documented as follows. Colour terms follow Kornerup & Wanscher (1978). Peridioles were sectioned by hand, mounted in distilled water, and examined using a light microscope. Spore statistics include average dimensions ± SD; Q, the quotient of spore length and spore width in any one spore; and Qcr, the mean of Q-values ± SD. Duplicates are deposited in the BIO-TEC Bangkok Herbarium (BBH), Bangkok, Thailand, the H.D. Thiers Herbarium (SFSU) at San Francisco State University, San Francisco, California, USA, and the herbarium of the Mushroom Room Research Centre (MRC), Chiang Mai, Thailand.

Molecular phylogenetic studies

DNA extractions were made from gleba using a commercial DNA extraction kit (E.Z.N.A. Forensic Kit, D3591-01, Omega Bio-Tek). PCR reactions performed with primer pairs LROR and LR5 and ITS4 and ITS5. Sequencing protocols follow those of Zhao et al. (2007).

Newly generated sequences (ITS and LSU sequences from the new species), and those retrieved from GenBank (20 ITS and 19 LSU sequences; Table 1) were initially aligned using Clustal X with default settings (Thomson et al. 1997). Manual adjustments were made in BioEdit v. 7.0.4 and gaps were introduced to improve alignments. The ITS and LSU alignments were separately submitted to TreeBASE (accession number: SN3455). All sequences used in this study were derived from type or paratype specimens or from authentic material determined by us or by H.J. Brodie (cf. Zhao et al. 2007).

Phylogenetic analyses were performed using PAUP v. 4.0b10 (Swofford 2003). Heuristic searches of the ITS, LSU, and ITS+LSU datasets were performed separately under three optimality criteria: weighed parsimony (WP), maximum likelihood (ML), and neighbour-joining (NJ). Unordered characters, random taxon addition sequences, gaps treated as missing data, and tree bisection-reconnection (TBR) branch swapping were used in all analyses. For weighted maximum parsimony, maxtrees was limited to 5 000 trees with 1 000 replications. The weighted parameters were produced using MrModeltest v. 2.2 (Nylander 2004). Bootstrap values (BS) were obtained from 1 000 replicates. Unconstrained trees (WP, ML, and NJ trees) were compared in PAUP using Kishino-Hasegawa and Lutzoni & Stefan Zoller, Duke University) as described in Miadlikowski et al. (2002). The best nucleotide substitution models for maximum likelihood were chosen by using MrModeltest v. 2.2 (Nylander 2004). Bootstrap support (BS) values above 50 % are shown.

![Fig. 1 Phylogenetic relationship of Cyathus inferred by maximum likelihood analysis of ITS rDNA sequences. Bootstrap support (BS) values above 50 % are shown.](image-url)
Shimodaira-Hasegawa tests (Kishino & Hasegawa 1989). Trees were viewed in TreeView v. 1.6.6 (Page 1996) and exported to graphics programmes.

The informal infrageneric group names Ollum, Pallidum, and Striatum in Cyathus follow the phylogenetic nomenclature established by Zhao et al. (2007). Although these names constitute improper Latin and do not match the specific epithets olla, pallidus, and striatus, they were established by Zhao et al. (2007) to distinguish the clades from similarly named infrageneric groups used by Brodie (1975), viz., Striatus, Pallidus, and Olla, that contain different subsets of species.

RESULTS

DNA alignment and phylogeny

The ITS dataset consisted of 776 characters of which 371 characters were constant, 109 variable characters were parsimony-uninformative, and 171 characters were parsimony-informative. One hundred and twenty four characters were ambiguous and were excluded. The sequences represent 16 Cyathus species (18 strains), Crucibulum laeve, and Nidula niveotomentosa, while Cystoderma amianthinum was used as the outgroup for rooting purposes based on the previous result that C. amianthinum (Cystodermateae) is sister to Nidulariaceae (Matheny et al. 2006). In all phylogenies under different optimality criteria (NJ, ML, and WP), the genus Cyathus is monophyletic with 100 % bootstrap and all trees have similar topologies. ITS results indicate that Cyathus species were partitioned into three main clades as shown by Zhao et al. (2007). Our new taxon, Cyathus subglobisporus, belongs to the Striatum group, albeit with weak statistical support (Fig. 1).

The LSU dataset consisted of 797 characters, of which 661 characters were constant, 56 variable characters parsimony-uninformative, and 70 characters parsimony-informative. Ten characters were excluded. The LSU dataset includes 16 Cyathus species (17 strains), Crucibulum laeve, and Nidula niveotomentosa. Cystoderma amianthinum was chosen as the outgroup for rooting purposes. Phylogenies (NJ, ML, and WP) show that Cyathus is monophyletic with 100 % bootstrap support, and that C. subglobisporus clusters with C. setosus, and together they are sister to the Pallidum group but this relationship was not statistically supported (Fig. 2).

The combined ITS and LSU sequence dataset consisted of 1573 characters of which 1045 characters were constant; 171 variable characters were parsimony-uninformative, 223 charac-

![Fig. 2](image_url)  
Phylogenetic relationship of Cyathus inferred by maximum likelihood analysis of LSU rDNA sequences. Bootstrap support (BS) values above 50 % are shown.

![Fig. 3](image_url)  
Phylogenetic relationships of Cyathus inferred by maximum likelihood analysis of combined ITS and LSU rDNA sequences. Bootstrap support (BS) values above 50 % are shown.
ters were parsimony-informative, and 134 characters were excluded. This dataset represents 10 Cyathus species (11 strains; all taxa for which both ITS and LSU data were available), Crucibulum laeve, and Nidula niv potentomata. Kishino-Hasegawa and Shimodaira-Hasegawa tests among NJ, ML, and WP indicated that the ML tree was the best tree (Fig. 3). Cyathus is monophyletic with 100% bootstrap support, and C. subglobisporus is sister to C. annulatus, C. renwelli, and C. stercoreus in the Striatum group with 67% bootstrap support.

**Taxonomy**

*Cyathus subglobisporus* R.L. Zhao, Desjardin, K. Sotyong & K.D. Hyde, sp. nov. — MycoBank MB512024; Fig. 4

Peridium obconicum, crassum, 7–10 mm altum, ore 5–8 mm lato, extra pale lilac flavum vel pallide brunneum, pilis resupinatis et fasciculatis obtectum, intus griseum, argenteum, tenuter sed distincte striatum; labium minute fimbriatum; epiphragma albidum, tenue peridiole 1.5–2 mm diam, lentiformia, tunicam peripallide fuscam ferentia; cortex simplex; sporae subglobosae vel ellipsoidiae, 13–18 μm longae, 12–16 μm latae.

Etymology: Refers to its subglobose spores.

**Fruiting** bodies clavate when young, then opening, extending and becoming obconic to infundibuliform, with relatively straight sides in side view; 7–10 mm high, 5–8 mm wide at the top, with the quotient of height by width 1–1.4; external peridium covered by hairs aggregated into shaggy or hirsute clusters, ivory, pale yellow or buff when young, sometimes with a hint of pale orange, then darkening to pale brown with age and the hairs remaining pallid. Inner peridium surface grey to brownish grey, darkening with age, distinctly plicate when young, becoming striate to smooth with age. Epiphragma membranous, white, covered by buff to pale orange-white hairs similar to those on the external surface of the peridium, cracking irregularly during maturation and disappearing or leaving a minutely fimbriate lip along the top edge of the peridium. Base of the fruiting bodies narrower than the rest of the fruiting body but lacking a distinct stipe. Peridioles 1.5–2 mm diam, lenticular, greyish brown to pale brown; peridiole covering composed of two layers: a black inner cortex layer, 15–25 μm thick, and a yellowish brown or dark brown outer tunica layer, 25–50 μm thick. Basidiospores 13–18 × 12–16 μm (av. = 15.8 ± 2.8 × 14.1 ± 2.1, Q = 1–1.31, Qexp = 1.12 ± 0.19, n = 50), subglobe or rarely broadly ellipsoid, hyaline, smooth, thick-walled (1.5–2–(–3) μm). Basidia not observed. Clamp connections present.

Habitat — On rotten bamboo stems in moist forest.


**DISCUSSION**

At first glance the proposed new species looks like *Cyathus griseo carpus* (Brodie 1984), which is commonly encountered in northern Thailand. Both species share features of pale yellow or pale brown fruiting bodies, an external peridium covered by pale yellow hairs that aggregate into conic mounds, an inner peridium surface that is striate to plicate, and grey-toned peridioles. The subglobe basidiospores and pale brown peridioles of *C. subglobisporus*, however, can differentiate it from *C. griseo carpus*. The latter species possesses much smaller, more ellipsoid basidiospores (av. = 7.3 ± 0.7 × 5.3 ± 1.3 μm, Q = 1.4 ± 0.6, from holotype) and pale grey peridioles. The LSU tree shows that *C. subglobisporus* is distinct from the Ollum group and is distantly related to *C. subglobisporus*.

Species of *Cyathus* whose fruiting bodies are pale yellow or pale brown and have a distinctly plicate inner peridium include *C. annulatus*, *C. berkeleyanus*, *C. bulleri*, *C. cornucopioides*, *C. crispus*, *C. duros*, *C. guandishanensis*, *C. helenae*, *C. pallidus*, *C. setosus*, *C. tianshanensis*, and *C. yunnanensis*. Only *C. bulleri*, *C. guandishanensis*, and *C. yunnanensis*, however, possess subglobe or globose spores. After comparison of their spore size, *C. subglobisporus* is distinct in possessing larger spores (av. = 15.8 ± 2.8 × 14.1 ± 2.1, Q = 1.12 ± 0.19) than those of *C. bulleri* (av. = 7.3 ± 1.7 × 6.8 ± 1.8 μm, Q = 1.08 ± 0.15, from isotype) and *C. guandishanensis* (av. = 11.6 ± 2.15 × 8.6 ± 1.4 μm, Q = 1.37 ± 0.46, from holotype), and smaller spores than those of *C. yunnanensis* (av. = 22.38 ± 3.6 × 18 ± 3, Q = 1.25 ± 0.42, from holotype). Of the latter three species sequence data was obtained successfully only from *C. guandishanensis*. The LSU tree shows that *C. guandishanensis* belongs to the Ollum group and is distantly related to *C. subglobisporus*.

The spores of *Cyathus olivaceobrunneus* (named after its olive-brown peridium) are similar to those of *C. subglobisporus* in shape and size, and the former species has been suspected to be a synonym of *C. poeppigii* (Brodie 1975). Examination of the type specimens of *C. olivaceobrunneus* showed the colour of its fruiting bodies to be much darker and to have longer spores (av. = 17.88 ± 8.75 × 13.2 ± 2, Q = 1.36 ± 0.34, from holotype) than those of *C. subglobisporus*. We were unable to generate quality sequence data from available material of *C. olivaceobrunneus* for comparison with *C. subglobisporus*.

The genus *Cyathus* was originally subdivided into seven groups based on morphological characters (Brodie 1975, 1984). Brodie’s classification system is not supported by phylogenetic analysis of molecular data, and the recognition of only three infrageneric groups (Ollum, Pallidum, and Striatum) was established based on morphological and molecular data (Zhao et al. 2007). In this study, the combined ITS and LSU phylogenies indicate that *C. subglobisporus* belongs to the Striatum group.
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REFERENCES


Lloyd CG. 1906. The Nidulariaceae. In: Index of the Mycological Writings of C.G. Lloyd vol. II. Cincinnati, Ohio, USA.


Nylander JAA. 2004. MrModeltest 2.2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University.


